# EXHIBIT 37

## SUBMITTED UNDER SEAL

# EXHIBIT 38

## SUBMITTED UNDER SEAL

# EXHIBIT 39

REDACTED

IN THE DISTRICT COURT OF THE UNITED STATES
FOR THE MIDDLE DISTRICT OF ALABAMA
NORTHERN DIVISION

BRIANNA BOE, et al,
Plaintiffs,
and
UNITED STATES OF AMERICA,
Intervenor Plaintiff,
vs. Civil Case No. 2:22-cv-184-LCB
HON. STEVE MARSHALL, in his
official capacity as Attorney General
of the State of Alabama, et al,
Defendants.
_____

The Remote Zoom Videoconference Deposition of
DANIEL SHUMER, M.D.,
Taken at 211 West Fort Street, Room 2330,
Detroit, Michigan,
Commencing at 9:11 a.m.,
Tuesday, April 2, 2024,
Before Leisa M. Pastor, CSR-3500, RPR, CRR.

APPEARANCES:

RENEE M. WILLIAMS
COTY MONTAG
U.S. Department of Justice - Civil Rights Division
950 Pennsylvania Avenue NW
Room 7.1817
Washington, D.C. 20530-0001
(202) 307-2222
renee.williams3@usdog.gov
coty.montag@usdog.gov
Appearing on behalf of Plaintiff.

CHRISTOPHER MILLS
Spero Law, LLC
557 East Bay Street
Suite 22251
Charleston, D.C. 29413
(843) 606-0640
cmills@spero.law
Appearing on behalf of Defendants.

ALSO PRESENT:
George Plow



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EXHIBITS

Detroit, Michigan
Tuesday, April 2, 2024
9:11 a.m.

DANIEL SHUMER, M.D.,
was thereupon called as a witness herein, and after having first been duly sworn to testify to the truth, the whole truth and nothing but the truth, was examined and testified as follows:

MS. WILLIAMS: Renee Williams, United States.

MS. MONTAG: Coty Montag, United States.

EXAMINATION

BY MR. MILLS:

Q. Good morning, Dr. Shumer. Thanks for coming today. You've given deposition testimony before, right?

A. Yes.

MS. WILLIAMS: Oh, sorry, just before we get started, we would like the -- to be able to reserve and to read and sign, if that's okay.

MR. MILLS: Sounds good.

MS. WILLIAMS: All right.

MR. MILLS: Anything else we need to cover?

MS. WILLIAMS: I don't think so.

MR. MILLS: Okay. If we discuss any sealed



material, we'll designate those parts as sealed, but we can get to that when we get there.

BY MR. MILLS:

Q. So yeah, of course if you don't understand a question, please free to ask for me to clarify. If you need a break, just let me know. We'll aim to take regular breaks, but also know that people would like to get home, so I'll try and balance those things.

If you could remember to answer verbally so the transcription can happen, that would be great.

Did you meet with anyone to prepare for today's deposition?

A. I met with Renee and Coty here.

Q. Did you discuss the deposition with anyone other than your counsel?

A. No.

Q. And did you review any documents in preparation for today's deposition?

A. Yes. I reviewed my expert report and rebuttal report and the defendant expert reports and -- yeah.

Q. Okay. Is it fair to say that you think the Endocrine Society is a reputable organization?

A. Yes.

Q. Do you generally follow the Endocrine Society's guidelines?



A. Yes.

Q. Is it fair to say you agree with or follow the Endocrine Society's approach to medical gender transition of minors?

MS. WILLIAMS: Objection.

A. Yes.

MARKED FOR IDENTIFICATION:
EXHIBIT 1
9:13 a.m.

BY MR. MILLS:

Q. I'm going to show you what I'm marking as Exhibit 1. Do you recognize this article?

A. Yes.

Q. This is an article you coauthored; is that right?

A. That's correct.

Q. And you were the lead author on this article?

A. Yes.

Q. And it was published in the Journal of Advanced Pediatrics; is that --

(Knock at the door.)

MS. WILLIAMS: Can we go off?

MR. MILLS: Sure.

(Off the record at 9:14 a.m.)

(On the record at 9:14 a.m.)

BY MR. MILLS:



Q. Okay. We can go back on, sorry.

So this was published in Advanced Pediatrics?

A. I'm not sure if that's the title of the article. I think it might be Advances in Pediatrics.

Q. Okay. Okay, I think you're right. Okay, perfect. Thanks.

If you could look at page 2 with me just under the heading "Definitions." It says the first sentence, "Gender identity describes one's internal feeling of gender, for example, boy or girl, man or woman, agender (identifying as having no gender), or a nonbinary understanding of one's gender."

Do you still agree with that definition?

A. Yes.

MARKED FOR IDENTIFICATION:
EXHIBIT 2
9:15 a.m.

BY MR. MILLS:

Q. Okay. I wanted to show you -- this was -- I'm handing you what I'm marking as Exhibit 2. This is a question and answer you did with -- through the University of Michigan Medical School; is that right?

A. Yes.

Q. Could you look at page 1 under the bold "What is the

Page 10

difference between sex and gender," the second sentence, "Gender identity is something you can't measure with a blood test or x-ray. It's only something a person can tell you about themselves from their lived experience."

Do you still agree with that description?

A. Yes.

Q. You can go back to the first document again under "Definitions." This is the next sentence after the one we already read.

"This is in contrast to biologic sex which describes the chromosomal, hormonal, and anatomic determinants which result in characterizing people as male or female."

Do you still agree with that?

A. Yes, but I would add that due to the biologic underpinnings of gender dysphoria, I would include gender dysphoria as a component of sex.

Q. So you don't think that gender identity is in contrast to biologic sex any more?

A. So I think that the -- the definition of gender identity is -- is an internal sense of one's self as outlined here, boy, girl, man, or woman, agender or nonbinary.

If I were writing this paragraph again, I

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don't think I would use the words "in contrast," and I would include gender identity as a component of biologic sex.

Q. Has something changed since you wrote this in 2017 that would lead you to change that description?

A. Yes, my understanding of gender identity as -- as having biologic underpinnings.

Q. And what is the basis for that change in understanding?

A. Research outlining those biologic relationships between gender identity, and research outlining the biologic underpinnings of gender identity, including twin studies, studies related to children with disorders of sex development, studies related to population-based brain anatomic differences.

Q. And which studies in particular have come out since this was published in August 2017 that would support that description?

A. I think many of the studies that are related to those topics that I outlined came out before 2016; however, my thinking through these topics and understanding how gender identity and sex are related and intertwined has changed.

Q. Okay. I'm going to show you now what I'm marking as Exhibit 3.

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MARKED FOR IDENTIFICATION:
EXHIBIT 3
9:19 a.m.

BY MR. MILLS:

Q. This is a scientific statement from the Endocrine Society.

Endocrinology is your specialty, right?

A. Yes.

Q. And we've already talked about the Endocrine Society. Do you recognize the names, any of the names who coauthored this statement?

A. I'm familiar with a couple of the names.

Q. If you could look at page 2 with me the first paragraph under the line kind of in the middle of the page. Yeah, page 2.

It says, "Sex is an important biological variable that must be considered in the design and analysis of human and animal research. The terms sex and gender should not be used interchangeably. Sex is dichotomous with sex determination in the fertilized zygote stemming from unequal expression of sex" --

COURT REPORTER: Can you slow down just a hair, please?

MR. MILLS: Sure.

COURT REPORTER: You lost me at zygote.

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BY MR. MILLS:

Q. "Sex is dichotomous with sex determination in the fertilized zygote stemming from unequal expression of sex chromosomal genes."

Did I read that correctly?

A. Yes.

Q. What does dichotomous mean?

A. I would -- I would say dichotomous means two and separate.

Q. And do you agree that sex is determined in the fertilized zygote?

A. I think that they're referring to chromosomal sex, and if they're -- if my assumption is correct, then I would agree with that.

Q. So you agree that sex is dichotomous?

A. I don't -- I don't know that I agree with -- with that specifically. I agree with it when talking about chromosomal sex being XX or XY predominantly, but I think saying that sex is dichotomous misses some of the nuance of how sex can be more complicated.

Q. If you could flip to page 10 of this document. On the second column, the last paragraph starts with, "Sex is an essential part of vertebrate biology, but gender is a human phenomenon. Sex often influences gender, but gender cannot influence sex."

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Would you agree with that statement?
A. Well, there's a lot of parts of that, so let me try to break it down.
Gender is a human phenomenon. I agree that humans have gender identity. I'm not sure if other animals have gender identity, so I think that I would agree with that.
Sex often influences gender. I think that makes sense to me.
Gender cannot influence sex, I think that -- to me that means that someone's gender identity doesn't influence the other components of sex, so in that way I would agree, but I would also put forward that my definition of sex includes gender identity as a component.
Q. So you would say this statement is wrong because it just says outright gender cannot influence sex?
A. No, that's not what I said. I don't think that gender identity can influence the other components of sex so I wouldn't disagree with that.
Q. But you would agree this statement doesn't say "other components," it just says "sex"?
A. I agree that it doesn't say "other components."
Q. So you wouldn't have written this like it's written?
A. I don't think I would have.

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Q. If you could flip to page 8, near the top of the first column, the second sentence, "Gender identity is a psychological concept that refers to an individual's self perception."
Do you agree with that statement?
A. Yes.
Q. I wanted to go back to Exhibit 1, which was your article in the Advances in Pediatrics. This is on page 5. At the end of the second to last paragraph the last sentence says, "Yet, the vast majority of transgender persons do not have an identified DSD or endocrinopathy."
Did I say that right?
A. You did.
Q. A DSD refers to a disorder of sexual development?
A. That's correct.
Q. And what does endocrinopathy mean?
A. An endocrine disorder.
Q. And so do you agree with this statement that the vast majority of transgender persons do not have either one?
A. Yes.
Q. So when you treat transgender persons with gender dysphoria, you are not typically treating for a DSD, correct?

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A. That's correct.
Q. And gender dysphoria is not a DSD?
A. That's correct.
Q. Transgender status is not a DSD, correct?
A. That's correct.
Q. And when you treat transgender patients with gender dysphoria, you are not treating an endocrine disorder; is that right?
A. That's correct. Well, I would say that I'm treating a disorder with hormones. So whether we call that an endocrine disorder or not, they don't have -- typically they don't have an abnormality in their sex hormone production as it relates to their sex assigned at birth.
Q. But transgender status is not an endocrine disorder, correct?
MS. WILLIAMS: Objection.
A. I think that -- that the semantics there are hard for me to parse out. You know, I think it's a disorder that endocrinologists treat. We treat it with hormonal interventions, so whether it's called an endocrine disorder or not, you know, I think is not important.
BY MR. MILLS:
Q. But in 2017, you wrote the vast majority of

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transgender persons do not have an endocrinopathy, or as you said, an endocrine disorder, so are you changing your view on that since 2017?
A. No, I'm saying in this article that we're not treating hormonal perturbation or a hormone problem. An endocrinologist is treating transgender people with hormones, so whether we call that an endocrine problem or not, I think that could be open for debate.
Dismissing that transgender status is an endocrine problem out of hand I think misses the larger point that endocrinologists treat transgender people with gender dysphoria.
Q. And gender dysphoria is not an endocrine disorder?
A. No.
Q. The Endocrine Society's statement we looked at a minute ago refer to different levels of sex steroids.
What is the typical level of testosterone in an adult male?
A. Typical level of testosterone in an adult male is roughly 200 to 900 nanograms per deciliter.
Q. What about the typical level of estrogen in an adult male?
A. It's low, less than 30 picograms per deciliter, if I'm getting my units correct.
Q. And what is the typical level of estrogen in an adult

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female?
A. The typical level of estrogen in an adult female varies through the month, but it can be between 50 and 300 picograms per deciliter.
Q. And what is the typical level of testosterone in an adult female?
A. Generally I would say less than 40 nanograms per deciliter.
Q. And do these levels that you've just said assume any medical treatments?
A. These are typical normal ranges for biologic men and women not on medical treatments.
Q. So assuming no medical treatment, still is the typical testosterone level of an adult transgender woman the same as an adult natal male?
A. It likely would be.
Q. Is that also true of estrogen?
A. Yes.
Q. And is the typical estrogen level of an adult transgender male the same as an adult natal female?
A. I would expect it to be.
Q. And that's also true of testosterone?
A. Yes.
Q. So those typical levels are manifestations of the person's biological sex; is that right?

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A. Yes.
Q. Is there a typical level of those two sex steroids, testosterone and estrogen, in transgender adults?
A. So did we just answer that for untreated transgender adults?
Q. Mm-hmm.
A. Yes.
Q. So the -- I'll ask it a different way.
The typical level of those two sex steroids in transgender adults would depend on whether they've been treated with hormones; is that fair to say?
A. The goal of treatment in someone being treated with hormones for gender dysphoria would be to bring their hormone levels in line with that which is typical of other people of that sex.
Q. Okay. I'm going to show you what I'm marking as Exhibit 4.
MARKED FOR IDENTIFICATION:
EXHIBIT 4
9:31 a.m.
BY MR. MILLS:
Q. This is an article you published with some others, is that right, concerning transgender youth?
A. Yes.
Q. If you could flip to page 2 at the top with me. This

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is about the third sentence down.
"The term transgender typically refers to those individuals for whom genotype and phenotype are mismatched, therefore, biologically male children may self-identify as female and vice versa, or youth may not fit neatly into either category."
Do you understand the term transgender to include youth who, as you sit here, do not fit neatly into either category?
A. I think generally transgender is an umbrella term to define someone whose gender identity does not match their sex assigned at birth.
Q. So a person who considers them self nonbinary could be transgender; is that right?
A. Yes.
Q. And a person who considers them self agender could be transgender?
A. Yes.
Q. And a person who considers themselves gender queer could be transgender?
A. Yes.
Q. So if you want to flip to page 8 in that same document with me.
COURT REPORTER: If you could hold on for one second, somebody rang in here.

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It's okay.
BY MR. MILLS:
Q. So we're on page 8 just before the heading toward the bottom, this is the second to last sentence before the "Challenges and Dilemma" heading.
"We also want to ensure that the child adolescent who may be gender variant does not feel compelled to choose a gender male/female when in actuality they may not fit into a typically recognized gender identity."
So some youth with divergent gender identities may not have the opposite identity as their biological sex; is that right?
A. Although most patients that I see do identify as the other sex, there are some individuals that identify somewhere -- somewhere else on a gender spectrum.
Q. How many gender identities would you say there are?
A. I don't think of gender identity in that way to count gender identities. Gender identity is a concept of knowing oneself and one's gender.
Q. If you could flip back to the Endocrine Society's scientific statement, this is what we marked as Exhibit 3, with me, and I'm going to page 9 of this document. This is the "Endocrine Society considering sex as a biological variable," page 9, and looking at

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the start of the first full paragraph in the first column.

"Although gender is strongly influenced by environmental and cultural forces, it is unknown if the choice to function in society in male/female or other roles is also affected by biological factors."

Do you agree that gender is strongly influenced by environmental and cultural forces?

A. So I'm not sure if they're referring to gender identity here or gender as a concept. So if you're asking me to agree with this sentence, I'm not sure that I -- that I can based on -- on -- on that, but I would say that -- that I don't believe gender identity to be strongly influenced by environmental or cultural forces.

Q. Do you think gender identity is influenced at all by environmental and cultural forces?

MS. WILLIAMS: Objection.

A. I think that individuals likely have an innate gender identity, and the understanding of that gender identity can be influenced by the world around us.

BY MR. MILLS:

Q. Do you agree that it is unknown if the choice to function in society in male/female or other roles is also affected by biological factors?

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A. I presented data to support the notion that gender identity is impacted by biologic factors. The choice to function in society as male/female or other roles, I'm not sure what that -- what that means exactly in this sentence, but I -- I presented data to support the notion that gender identity itself has biologic foundation.

Q. If we could flip back to Exhibit 1. This is your article in the Advances in Pediatrics, page 5 in the middle of the page. This is at the end of the second full paragraph.

You say, "Studies have failed to firmly establish causative gains." And then if we could flip back to the Endocrine Society's statement that's Exhibit 3 that we were just looking at, back to page 8. This is in the second sentence in the first column on page 8 starting halfway through the sentence.

"While associations between gender identity, neuroanatomic, genetic and hormone levels exist, a clear causative biologic underpinning of gender identify remains to be demonstrated."

Do you agree that a clear causative underpinning of gender identity remains to be demonstrated?

A. I agree that we don't have biologic variable that

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clearly causes a certain change in gender identity, yes.

That the associations that I presented are not intended to demonstrate that a certain gene is causing a change in gender identity or a particular exposure, a particular hormonal exposure is causing gender identity, but simply that there's relationship between these biologic variables and gender identity.

Q. But you don't disagree with the way this scientific statement words the absence of a clear causative biological underpinning, correct?

A. I'm reading that to say -- to mean that exactly how I just presented it, that there's not a clear cause for -- there wouldn't be a situation where you can measure something like a genetic variable or a hormonal exposure and then be able to predict one's gender identity, so in that way I would agree.

Q. And along the same lines, so you don't know with certainty what causes gender identity; is that right?

A. Correct.

Q. I'm going to show you now what I'm marking as Exhibit 5, which is an article you published with some others called "Autistic traits in mothers and children associated with children gender nonconformity."

MARKED FOR IDENTIFICATION:

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EXHIBIT 5

9:40 a.m.

BY MR. MILLS:

Q. Do you recall this article?

A. Yes.

Q. If you could just flip to page 2 of the article, and this is near the end, the second to last sentence of the big paragraph toward the bottom of the page.

You wrote, "Postnatal" -- "In addition, postnatal and environmental factors such as the social relationship between the parent and infant and cognitive learning about parental expectations and societal norms may influence gender development."

Do you still agree that postnatal environmental factors may influence gender identity?

A. Well, I said development, so I think I would agree with that.

Q. And could you explain what the difference between gender development and gender identity is?

A. Sure. So I -- I think that we've -- we've defined gender identity as an internal sense of one's self as boy, girl, man, woman, and I would describe gender development as the -- the progress of understanding gender as one grows from infancy to toddlerhood to childhood to adolescence to adulthood.

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Q. So a factor that influences gender development would necessarily influence gender identity; is that right?
A. I don't know. I think the -- the point here is that gender identity is a really complicated human characteristic that probably has lots of different inputs and factors.
The -- the factors here, the relationship between parent and infant, cognitive learning, parental expectations and societal norms, may they influence gender identity? I think it's possible. I think that we have a -- a -- we have a really complicated human characteristic here that -- that is incompletely understood, but -- but the assertion that there's biologic factors that are related to it remains -- remains clear.
Q. If the postnatal environment is important in gender development, do you agree that it is desirable to structure that environment in such a way that a child becomes comfortable with their natal sex so they don't have to undergo medical gender transition?
MS. WILLIAMS: Objection.
A. I think in the best case scenario a child would understand that whatever their gender identity is would be met with love and support.
BY MR. MILLS:

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Q. I'm going to show you what I'm marking as Exhibit 6, which is an article you coauthored entitled "Transgender and gender nonconforming adolescent care, psychosocial and medical considerations."
MARKED FOR IDENTIFICATION:
EXHIBIT 6
9:43 a.m.
BY MR. MILLS:
Q. This was an article you coauthored; is that right, Dr. Shumer?
A. Yes.
Q. If you could look at page 2, the second paragraph under "Gender Identity," the second paragraph there, the second sentence.
"For example, a prepubertal child who is gender nonconforming or has apparent gender dysphoria may or may not identify as transgender later in life."
Would you still agree with that statement?
A. Yes.
Q. So some children with gender dysphoria will identify with their biological sex later in life?
A. Yes.
Q. Sorry, I'm just getting back to where we are.
If we could flip back to the Endocrine Society scientific statement, this is Exhibit 3, and

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if you'd go to page 9, the bottom of the first column.
The very bottom of the first column says, "Attempts to identify specific genes governing gender identity have been plagued by small numbers of subjects and low statistical significance."
Do you agree with that statement?
A. I would -- I would just back up for a second and put this in context because the sentence before says genetics may play a role in gender identity.
Monozygotic twins have a 39 percent concordance for gender dysphoria, which I think references one of the articles that I included in my expert report. So the following sentence that you read I would agree is that those studies that -- that highlight that point are relatively small, and so further study to help understand the genetics of gender identify would certainly be helpful.
Q. And if it were purely genetic, monozygotic twins would have a 100 percent concordance for gender dysphoria; is that right?
A. Yeah, I think I tried to explain this in more detail in my rebuttal report, but there are certain medical conditions that we would call Mendelian traits which involve a specific gene, and one -- one gene when -- when mutated, for example, or -- or when there's a

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certain allele will 100 percent of the time express that condition.
So, for example, Huntington's disease is a Mendelian trait where you have that gene 100 percent of the time you'll have Huntington's disease, but many human characteristics while there is a genetic link are not 100 percent, you know, gene equals outcome.
Q. Sure. So the next sentence here is, "No specific gene has been reproducibly identified."
Would you agree with that?
A. Correct. There's not a specific gene when mutated a certain way or when a certain allele is present would be 100 percent predictive of a certain difference or lack of difference in gender identity.
Q. So if we go up to the second sentence in the big paragraph in the first column on page 9 it says, "A general issue is that the association of sex, gender or sexual orientation with specific brain structures or with other biological variables does not establish whether the biological variables are causes or consequences or noncausal correlates of the behavioral contribution or function of the individuals studied."
Do you agree that that issue remains sort of an open question in the studies you discussed?
A. So that's a complicated question, so let me just try

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to -- to go through that with you.
So a general issue is the -- that the association of sex, gender and sexual orientation with specific brain structures or with other biologic variables does not establish whether the biological variables are causes or consequences or noncausal correlates of the behavioral characteristic or function of the individuals studied to me is pointing out that you could have a, let's say, a biologic difference that exists in transgender people, and the question is, is that biologic difference the cause of the gender identity or is the gender identity somehow causing that biologic difference or in something to that effect. So I think with each study you have to think about the plausibility of that and think about whether that could be true.
I think for the monozygotic twin studies, it's harder for me to understand how the gender identity could impact the genetic differences. I think, you know, when we're talking about other studies that -- that I referenced in my report, I think each time we'd have to think about how that could be and not discount it out of hand that -- that the cause and effect could be one way versus the other.

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So if we -- if we take individual studies, we could try to answer that question more -- more specifically.
Q. But you agree that this could be an issue with specifically the brain studies?
A. So I think this comes up a lot in -- in -- in brain studies where, let's say, there's a difference in a brain structure in someone with a certain characteristic, is that -- is there something that caused that difference that is also attributed to the condition we're talking about, or is -- is the causation the other way around. And so that could be something that you would need to think about with brain studies.
And -- and so, you know, when we're thinking about gender identity as this variable, you know, I think, you know, whether or not the difference occurred after hormone exposure or before, those sorts of questions would be important to think through when you're trying to understand the importance of the study in answering your question.
MARKED FOR IDENTIFICATION:
EXHIBIT 7
9:51 p.m.
BY MR. MILLS:

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Q. I'm showing you what I'm marking as Exhibit 7, which is an article by a professor of psychology Kristina Olson.
Are you familiar with her work?
A. Yes.
Q. Sorry, I may have given you two copies; just ignore one of them.
Is she generally a knowledgeable person in this field of gender identity and gender dysphoria?
A. I don't know what area we're going to be talking about.
Q. And how are you familiar with her?
A. She -- she presented -- she published studies related to gender identity outcomes, I believe, related to social transition and comparing children with their peers and other unrelated -- unrelated age-matched controls, and that's how I'm most familiar with her work.
Q. I'm -- if you want to flip to page 6 of the page numbers that are at the bottom here, the first full paragraph the end of the paragraph says, "Whereas, the topic" -- sorry, I'll go back.
So this paragraph is talking about neuroscience studies about the brain structures of trans people. The end of the paragraph says,

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"Definitive conclusions about genetic and neural correlates of gender identity remain elusive."
Would you agree with that statement?
A. If you don't mind --
Q. Sure.
A. -- I'd just like to read the whole paragraph to myself --
Q. Of course.
A. -- for a second.
Yes, I think the whole paragraph nicely summarizes sort of a lot of the topics we've been talking about, how we have these differences that we've measured in the brains of transgender people, that forming a causative link is difficult in these types of studies, and so I certainly I don't disagree with the sentence that you read, and I would just add that, you know, by presenting -- bringing the study data in my expert report, I'm certainly not purporting a causative link to a certain size nuclei equals a certain gender identity, but rather using that to expand on the -- or to include it in the data that helps to demonstrate this biologic origin of gender identity.
Q. Do you agree that the brain studies you cited in your report analyzing gender identity did not control for

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sexual orientation?
A. I think that it would be helpful to look at them in detail, but I don't remember them controlling for sexual orientation.
Q. Sure, we can come back to that.
If you could flip to what I marked as Exhibit 2, which was the question and answers you gave with the Michigan --
A. Oh, I have two of these ones.
Q. Sorry about that; that was my fault.
Page 2 the second paragraph under the heading "What is gender-affirming care." This is the second paragraph under that heading.
"Not everyone with the difference in gender identity should be considered as having a medical problem or needing to see a doctor."
Do you still agree with that statement?
A. Yes.
Q. So a difference in gender identity would include an individual who's -- who is transgender, right?
A. Yes.
Q. So some transgender individuals should not be considered as having a medical problem or needing to see a doctor?
A. Yes.

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Q. I'd like to show you now what I'm going to mark as Exhibit 8 --
MARKED FOR IDENTIFICATION:
EXHIBIT 8
9:56 a.m.
BY MR. MILLS:
Q. -- which is a chapter that you wrote in a book entitled Transgender Medicine.
And do you recall this chapter?
A. Yes.
Q. Sorry, there's two pages of preliminary material, but then Chapter -- it looks like you were a coauthor of Chapter 9, entitled "Endocrine care of transgender children in adolescence"; is that right?
A. Yes.
Q. If you could flip to -- sorry, the pages are a little conflicting here -- page 166, which is the second page of your chapter; it just skips ahead to your chapter. There we go.
A. 166?
Q. That's right. And this is in the middle of the page you're defining the term transgender.
You wrote, "An umbrella term describing individuals who identify with a gender that is different from gender assigned at birth may or may not

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connote gender dysphoria or desire to seek an intervention."
So is it correct to say that some transgender persons do not have gender dysphoria?
A. Yes.
Q. And for transgender persons without gender dysphoria, medical gender transition would not be proper; is that right?
A. That's correct.
Q. Even for some transgender persons with gender dysphoria, medical gender transition might not be proper; is that right?
A. Sorry, can you way that one more time?
Q. Sure. So I'm talking about transgender persons with gender dysphoria, medical gender transition in the sense of puberty blockers and cross X hormones would not necessarily be the proper course of treatment; is that right?
A. In assessing anyone with gender dysphoria, medical transition would be considered as an option and may or may not be appropriate.
Q. Can individuals who do not identify as transgender have gender dysphoria?
A. Well, you said in an individual who does not identify as transgender, so I think to me that means that that

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person them self is applying that term transgender to their identity, so there may be -- may be a person that identifies as a sex different from their assigned sex at birth that eschews the term transgender and, therefore, wouldn't themselves state that they identify as transgender that have gender dysphoria, but in my definition of transgender, which is a person whose gender identity is different than their sex assigned at birth, then, no, someone would need to fit that definition to have gender dysphoria.
I'm not sure if I explained that.
Q. I think I understand. Thanks.
A. Yeah.
Q. So you would potentially treat an individual who does not identify as transgender but has gender dysphoria if you considered them to be transgender?
A. I don't think that -- I don't think of transgender as a medical term, so I'm really as a pediatric endocrinologist more interested if they have gender dysphoria.
Q. Do you diagnosis gender dysphoria under the DSM-5 without the input of a psychiatrist or psychologist or other mental health professional?
MS. WILLIAMS: Objection.
A. So there's a couple parts to that question. I

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certainly can and do diagnose gender dysphoria. The DSM is very clear on how one may -- can diagnose it, but in my clinical practice, I work as part of a multidisciplinary team where patients are also seeing a mental health professional, and that mental health professional is considering the diagnosis of gender dysphoria as well.

BY MR. MILLS:

Q. So have you ever diagnosed gender dysphoria and started medical treatment without the input of a mental health professional?

A. No, that's not how our clinic is set up to function.

Q. I'm going to show you what I'm marking as Exhibit 9.

MARKED FOR IDENTIFICATION:
EXHIBIT 9
10:01 a.m.

BY MR. MILLS:

Q. This is an article you coauthored entitled "Evaluation of Asperger's syndrome in youth presenting to a gender dysphoria clinic."

Do you recall this article?

A. Yes.

Q. And you were an author of it?

A. Yes.

Q. If you could just flip to page 389 of the article, and

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this is under "Discussion" in the first column, the second sentence.

"23 percent of patients presenting with gender dysphoria had possible likely or very likely Asperger's syndrome as measured by the ASDS," and then you say, "That is consistent with growing evidence of increased prevalence of ASD in gender dysphoric children."

ASD is Autism Spectrum Disorder; is that right?

A. Yes.

Q. And do you still agree that there's an increased prevalence of ASD in gender dysphoric children?

A. Yes.

Q. Near the bottom of that first column in the middle of the last paragraph you wrote, "The psychological evaluation performed" -- sorry, I'll start -- the first sentence of that last paragraph says, it talks about the evaluation and treatment of children and adolescents with gender dysphoria. You say it's guided by professional guidelines or standards of care, and then in the middle of the paragraph you say, "The psychological evaluation performed is not standardized with different clinics performing diverse batteries of psychological screening."

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Would you still agree that the psychological evaluation for gender dysphoria is not standardized?

A. Just to clarify, the end of that sentence was "testing." You said "screening."

Q. Oh, yes, sorry, sorry. Yes, you're right.

A. So I think that in general, pediatric -- pediatric patients with gender dysphoria are in our country generally treated in pediatric gender clinics which consist of a mental health component and assessment.

The -- the assessment performed in these clinics is all based on the premise that a diagnosis of gender dysphoria should be evaluated for, and that a biopsychosocial assessment, understanding of the child's gender history, the parent's perception of that gender journey, the child's social and educational history, developmental history. These are all important components of that assessment, in my opinion, and how that assessment is structured may look different depending on the resources of each clinic or the -- the tools that a mental health professional may employ to answer those questions.

Q. So just to go back, would you agree that the psychological evaluation you performed is not standardized?

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A. I would agree that there's not a cookie-cutter approach that every pediatric gender clinic follows to make this assessment, but the function of what's important, the important outcome of that assessment is similar across all gender clinics.

Q. If the evaluation is different, then the same child could be diagnosed with gender dysphoria in one place and not in another; is that right?

A. I wouldn't expect that to be the case, no.

Q. But it's possible?

A. So I think that every child is a unique individual with oftentimes a complicated story to tell, that -- that the -- the criteria outlined by the DSM for gender dysphoria are pretty clear, and so I don't think that it's likely that a patient would be diagnosed with gender dysphoria by one individual with expertise in this field and not by another, but there are certainly cases where the diagnosis is complicated or unclear, and in those situations oftentimes time can be useful in the diagnostic journey, you know, if a patient is -- is maybe partially or borderline meeting criteria for gender dysphoria, then continuing to see where that patient's gender identity half progresses over time would be a helpful tool.

Q. But to go back to my question, it is possible that the

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same child would be -- could be diagnosed with gender dysphoria in one -- by one provider and not by another provider?
A. I don't think that's very likely. I think that it's hard to say that that would be impossible, but the -- the DSM pretty clearly outlines how to make this diagnosis so I wouldn't expect that to happen.
Q. You said that children in this country are generally treated in pediatric gender clinics. What is the basis of that statement?
A. As someone that works in the field, I -- I have knowledge of the options for pediatric patients and where they're able to receive the care that they need.
Q. Do you know what percentage of children with gender dysphoria who are undergoing medical transition are treated in pediatric gender clinics?
A. I don't know a percentage, but I expect it to be very high.
Q. You're not aware of a survey of children with gender dysphoria being medically transitioned as to in what context they're being treated?
A. If there's a survey, I don't recall it.
Q. And you're not aware of what percentage of children in Alabama are treated at a pediatric gender clinic there?

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A. No.
Q. Are you aware of any pediatric gender clinics in Alabama?
A. I don't -- I'm not intimately familiar with any pediatric gender clinics in Alabama, although I have an awareness that there is one in Birmingham.
Q. And you're not familiar with any others?
A. No.
Q. Do you know of any way of gathering data on children who are treated outside of pediatric gender clinics in terms of how many children are treated that way?
A. No.
Q. So to go back to this paper, in the second column in about the middle of that big paragraph you say, "Some items on the ASDS may be naturally observed in non-ASD gender dysphoric youth" --
A. My apology, I'm not following you yet. Where are we?
Q. Sure, sure. So the second column on 389, and we're in the one, two, three, fourth sentence. You say, "For example."
A. "For example," gotcha, yeah.
Q. "For example, some items on the ASDS may be naturally observed in non-ASD gender dysphoric youth, specifically an item on the cognitive subscale, "Appears to be aware that he or she is different from

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others," "and an item on the maladaptive subscale, "Does not change behavior to match the environment," "might capture expected observations in the gender dysphoria child.
"Thus, scrupulous attention to symptomology during ASD diagnostic evaluation of gender nonconforming youth is essential to minimize any risk of misclassifying gender dysphoric youth with high functioning ASD due to symptom overlap."
And then the next sentence, "Importantly, certain symptoms may be associated with both diagnoses, but stem from vastly different origins."
Do you still agree with that discussion?
A. Yes.
Q. And so would you agree that there's also risk of misclassifying high-functioning ASD youth as gender dysphoric?
A. Give me one second.
Q. Yeah.
A. It's a complicated paragraph, so let me just reread it.
So the paragraph that we read was talking about how patients with gender dysphoria may be over classified as ASD simply because of some of these examples on the ASDS.

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So your question is a reverse, correct? Could patients with gender dysphoria be misclassified and really have ASD?
Q. (Shakes head in the positive.)
A. I think that's harder for me to explain. So I'm not -- I'm not sure that that's what this paragraph would support.
Q. So why would the symptom overlap only lead to a risk of error in one direction?
A. Because these questions appear -- appears to be aware that he or she is different from others and does not change behavior to match environment. These are questions that are trying to diagnose autism spectrum disorder, but they're not questions that you would use to diagnose gender dysphoria.
Q. You don't think those questions could be relevant under the DSM-5?
A. Pertaining to the diagnosis of gender dysphoria?
Q. That's right.
A. Not without context including discussion of gender identity, no.
Q. I'm showing you what I'm marking as Exhibit 10, which was an article you coauthored, "Mental health of transgender youth in care at an adolescent urban community health center."

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Do you recognize this article?
A. Yes.
MARKED FOR IDENTIFICATION:
EXHIBIT 10
10:12 a.m.
BY MR. MILLS:
Q. If we could just go to page 8 of the article under "Conclusion" the first paragraph. This is the last two sentences of that first paragraph under "Conclusion."
"Patients with a transgender identity or history should be recognized as having higher risk for mental health concerns and should be carefully screened and evaluated. Patients identified with cooccurring transgender identity and mental health concerns should be seen by a mental health provider who is qualified to provide evidenced-based care with sensitivity to the diversity of gender identity and expression."
Why do you think this is important?
A. I think the first sentence is important to point out that the pediatric transgender population is vulnerable from a mental health standpoint and having extra mental health support in place when managing gender dysphoria is critical.

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I think the second sentence is important because if someone has gender dysphoria and you're treating that gender dysphoria, but they have unmet -- other unmet psychiatric needs, like depression or anxiety that are unrelated to their gender dysphoria, that by not managing those things, you're not maximizing that child's health and potential.
Q. Do you think this screening and evaluation should occur before any medical interventions?
A. I do think that assessment of a patient's overall mental health is important prior to proceeding with a medical intervention, yes.
Q. So if a patient is not seen by a qualified mental health provider before medical intervention, you would say that would be a substandard quality of care?
MS. WILLIAMS: Objection.
A. My -- if we think about the, you know, WPATH Standards of Care, the recommendation is to involve a multidisciplinary team when providing care to gender dysphoric youth, so there are certainly many ways to do that, and so the composition of that team could look different in different places, but having -- having some sort of evaluation of a child's mental health by a person that is competent in performing that evaluation is something that I believe to be

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important.
BY MR. MILLS:
Q. And you would agree that the WPATH standards call for a comprehensive psychosocial assessment by a qualified mental health provider, right?
A. I'm not sure if those are the exact words, but something to that effect is something that I would support.
Q. So if that doesn't happen, you would say that the patient has not received the standard suggested by WPATH?
A. If they haven't received the care as outlined by WPATH Standards of Care, then they haven't received the standard of care as outlined by WPATH by definition.
Q. And would you say that would then be a substandard quality of care?
MS. WILLIAMS: Objection.
A. I don't know if there's a specific definition for substandard quality of care, but it wouldn't be the type of care that I would support or suggest.
BY MR. MILLS:
Q. In the context of medical gender transition, should the treating endocrinologist be aware of cooccurring psychiatric conditions the patient may have?
A. Sorry, can you repeat that once more?

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Q. Sure. So within medical gender transition for patients with gender dysphoria, should the treating endocrinologist be aware of cooccurring psychiatric conditions the patient may have?
A. Yes.
Q. And should the treating endocrinologist be aware of other issues that may affect gender dysphoric treatment such as a past history of sexual trauma?
A. That one's a little bit harder for me to answer. I think that it -- it -- if that history of sexual trauma was important in the narration of that child's gender identity, then -- then yes, but not -- I wouldn't suggest that all sexual trauma would impact one's gender identity, so it's -- so I'm not sure.
Q. In your experience, is it common for the sexual trauma to not affect gender identity?
A. Yes.
Q. Would you agree that the mental health provider working as part of an interdisciplinary team should still know about issues that may affect gender dysphoria treatment such as a past history of sexual trauma?
A. So we're in that question assuming that past history of sexual trauma does impact one's gender identity, so I -- I'm not sure that I can answer that question

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without first validating that statement.
Q. Do you think it would be significant in the diagnosis of gender dysphoria to know whether there is a past history of sexual trauma?
A. I think that that's an important component of any mental health evaluation if you're taking a complete biopsychosocial assessment, and then in talking through that sexual trauma if present, the professional can work -- work with the -- the patient or client on how their understanding of their gender identity was or was not impacted by that event.
Q. So if a comprehensive assessment happened, then someone on the interdisciplinary team should know about the history of sexual trauma even if it's not directly tied to gender dysphoria?
A. I think -- I'm not sure that I'm the right person to ask this question. I think that a mental health professional who takes -- does a biopsychosocial assessment, I'm not sure whether asking about sexual trauma is a component of all psychosocial assessments. I assume it is, but to be honest, I'm not 100 percent sure.
Q. Sure. Do you know the error rate of diagnosing gender dysphoria?
A. Well, I would say that -- that because there's

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specific criteria that -- that you use to diagnose gender dysphoria, the -- the clinician that's using those criteria wouldn't have the ability to have an error in making the diagnosis if using that criteria.
I think what you're asking is does that diagnosis of gender dysphoria and the subsequent treatment is that the correct treatment for that particular person. So I'm not sure I've explained that right, so let me -- let me try again.
You know, if a person is sitting in front of me, they either meet the criteria for gender dysphoria or they don't. So in that time and place there wouldn't be an error rate, but that's not the question that's relevant, right? The question is what do we do with that information.
Q. So you said wouldn't have the ability to make an error. Are you saying that someone applying the DSM-5 criteria could not make an error in diagnosing gender dysphoria?
A. I'm saying that if you're sitting with a patient and you're going through the criteria for gender dysphoria, it's you either meet each criteria or you don't, and then as a sum, you either do have the diagnosis of gender dysphoria or you don't in that interview that day and time.

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So, you know, if you're applying the DSM criteria, it's not the subjective. It would be either you do or you don't meet that clinical criteria. So that's why I'm having a hard time answering the question about an error rate.
Q. So on -- take a particular patient on that day, every mental health professional in the country would come to the same conclusion about whether that patient had gender dysphoria?
A. Well, if that's the goal of the DSM, right, because it's pretty clearly outlining how to make these diagnosis for mental health professionals that are using it.
Q. And you think that that is not just the goal, but the reality that 100 percent of the diagnoses of gender dysphoria are correct?
A. As I've explained it, right, you know, I think that, you know, if you're a mental health professional that's not asking the questions and just making assumptions, then I suppose you could be making an error, so perhaps not 100 percent.
But I -- I -- I would -- I would posit that, you know, when I'm -- when I'm thinking about your question clinically and I'm the endocrinologist seeing a patient, you know, the fact that they meet

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criteria for gender dysphoria is only one component of -- of the decisionmaking. That -- that much more important to me is the richness of that psychosocial assessment.
So -- so I think we're missing the boat if we're focused on meeting the -- you know, what the error rate of gender dysphoria is. Someone could have or not have gender dysphoria, but that -- what's more important to me as the clinician is understanding what their -- how their gender identity impacts their life and whether or not, you know, they require any medical intervention.
Q. Would you treat a patient who does not have gender dysphoria with medical gender transition?
A. They wouldn't require it because there's not distress associated with their gender identity difference.
Q. So it does matter to your treatment whether they have gender dysphoria?
A. Right. That would be the basic low bar that would qualify someone to consider treatment, but certainly not sufficient.
Q. By low bar, what do you mean?
A. If you don't have gender dysphoria, you don't require a medical intervention.
Q. Is it possible to misdiagnose gender dysphoria?

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A. I think that I tried to answer that question already.
Q. I'm going to mark as Exhibit 11 a deposition you gave in another case, Casey versus individual members of medical licensing board.
MARKED FOR IDENTIFICATION:
EXHIBIT 11
10:25 a.m.
BY MR. MILLS:
Q. If you could flip to page 41 -- and these are just excerpts because it was quite long. So this is the small page 41.
A. Oh, gotcha.
Q. Under line 15 to 16 you said, "I don't know what the error rate of diagnosis of gender dysphoria is."
Did I read that correctly?
A. You did.
Q. And is that what you said in this deposition?
A. Yes.
Q. And do you still agree with that statement?
A. So if we're talking about patients that are presenting to gender clinic and either meeting or not meeting the criteria for gender dysphoria, I would expect the error rate to be extremely small. And so do I know what the error rate is? No, but I would posit what I've said before, that meeting the diagnostic criteria

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for gender dysphoria is -- is objective, and -- and as a treating clinician on -- I'm interested to know that the -- whether or not the child meets those clinical criteria, but --
Q. So --
A. -- it's not a yes/no, treat if yes scenario. It's - if the patient doesn't have gender dysphoria, then they don't even need to see me.
Q. So just to go back to my question, would you say it is possible or not possible to misdiagnose gender dysphoria?
A. I think it's possible. You know, a patient may appear to meet the criteria, but -- or may -- I guess the answers a patient or client makes to the mental health professional may be misinterpreted, but I find that challenging to -- to expect to happen on an even remotely frequent basis.
Q. Would you expect that to be more frequent if the diagnosis is made by a nonmental health provider?
A. Not if that person is experienced in making the diagnosis of gender dysphoria.
Q. If they were not experienced in making the diagnosis, would you expect their rate to be higher?
A. I don't -- I don't know that the error rate would be high for anyone that's familiar with how to use the

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DSM, and if someone isn't familiar with using the DSM, then they probably wouldn't be making the diagnosis in the first place, so the question seems a bit abstract.
Q. You would say a person not familiar with the DSM should not be making the diagnosis of gender dysphoria, correct?
A. Correct.
Q. Do patients ever lie?
A. About anything?
Q. Mm-hmm.
A. Sure.
Q. Do adolescent patients ever lie?
A. Sure.
Q. Just a few more questions and then we can take a break, if that works for everyone.
So you are not a mental health professional; is that right?
A. That's correct.
Q. You're not a psychiatrist or a psychologist?
A. No.
Q. And you're not offering your opinion here as a mental health expert, correct?
A. Correct.
Q. You don't have a residency or fellowship in psychiatry?

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A. No.
Q. You don't have a degree in child and adolescent development and psychology?
A. No.
Q. Do you consider yourself trained and professionally competent in using the DSM-5 to make child and adolescent mental illness or psychiatric diagnoses generally beyond gender dysphoria?
MS. WILLIAMS: Objection.
A. As a general pediatrician, I'm comfortable making -- as a person that has gone through general pediatrics residency, I do feel comfortable making certain diagnoses like major depression -- major depressive disorder, generalized anxiety disorder, and then certainly other more complex psychiatric conditions I do not feel competent in making those diagnoses.
BY MR. MILLS:
Q. Sure. And you are not an epidemiologist, correct?
A. Correct.
Q. You don't claim to be an expert in statistical analysis; is that right?
A. I do have a master's of public health, and as part of that degree I was trained in epidemiology and statistics, but that's not my career.
Q. And when you coauthor papers involving statistical

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analysis, does another researcher typically perform that statistical analysis?
A. In -- for the most part, we -- I work with statisticians when I'm writing papers, although during fellowship one of the tasks is to do the statistics on your own, so I have participated in those -- those endeavors, but love having a good statistician on the team.
Q. So the articles that you've published that, you know, may be referenced in your report involving statistical analysis, you know, someone else did that analysis generally, is that fair to say, in terms of the number crunching, p-values?
A. I guess we could look at a particular article and I could recall.
Q. Sure. Have you ever conducted a systematic review of the literature on medical gender transition in minors?
A. No.
Q. Have you -- sorry, scratch that.
You're not a neuroscientist, correct?
A. Correct.
Q. You don't have any training in -- specialized training in brain studies; is that right?
A. Correct.
Q. You don't conduct brain studies?

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A. I don't.
Q. You don't interpret brain imaging in your practice?
A. I do.
Q. Have you ever used brain imaging to treat gender dysphoria in your clinic?
A. No.
Q. You haven't written any articles on neuroscience, have you?
A. No.
Q. Have you ever peer reviewed a neuroscience journal article?
A. Not to my memory.
Q. You're not a genetic researcher, correct?
A. Genetic researcher. No.
Q. You don't have any formal training in genetics research?
A. Not above and what is required in medical school and residency and fellowship for pediatric endocrinologists.
Q. Sure. You haven't published any articles on genetics?
A. No.
Q. You don't typically use genetics to treat gender dysphoria in your clinic?
A. No.
Q. You've never conducted a survey about the parameters

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of gender-affirming care provided by other practitioners, correct?
A. Correct.
Q. So you don't have any personal knowledge of how many other practitioners follow the WPATH Standards of Care 8, right?
A. I have personal knowledge as it relates to me knowing many of the providers across the country, interacting with them academically, so that in that respect I do have knowledge of how -- how other -- how gender care is provided across the country.
Q. But you wouldn't be able to venture a number with confidence as to how many other providers in the United States follow WPATH Standards of 8 -- Standards of Care 8 in treating minors with gender dysphoria?
A. I would posit that it's a very high percentage, but beyond that I don't have a number to offer.
Q. And you don't have a number to offer if -- on the same question looking at providers in Alabama; is that right?
A. Correct.
Q. And you also don't know what percentage of providers in the United States follow the Endocrine Society's guidelines to treating gender dysphoria in minors?
A. You know, similarly to all areas of medicine there's

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guidelines and standards of care, and as an endocrinologist I could be asked the same question about diabetes and I would have the same answer, that we have guidelines and recommendations set because these conditions are -- are common and treated by endocrinologists across the country and across the world.
So I would say that the vast majority of endocrinologists would treat patients with diabetes according to the American Diabetes Association diabetes standards or care, and couldn't offer a percentage of people that are practicing outside of those guidelines. In a similar way, I would have a similar answer to the treatment of gender dysphoria.
Q. So you have no knowledge of whether most minors with gender dysphoria are treated through a multidisciplinary care model, do you?
A. To the existent that I'm familiar with the options for youth across the country, I would say that that type of model is by far the most common model and the percentage would be very higher.
Q. You have no knowledge of whether most gender dysphoric minors in Alabama are treated through a multidisciplinary care model, correct?
A. I have no particular knowledge of them outside of my

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answer to the previous question.
Q. And you're only aware of a single multidisciplinary care model being provided in Alabama; is that right?
A. That's the clinic that I'm aware of. I'm not aware of others, but don't claim to know all of the gender clinics across the country.
Q. You have no knowledge of how many minors nationwide are prescribed medical gender transition interventions, do you?
A. A number, no.
Q. Your earliest publication or presentation on a topic related to transgender medicine was in 2013; is that right?
A. That sounds correct.
Q. And when did you begin treating minors with gender dysphoria?
A. I was involved with the gender clinic at Boston's Children Hospital as a fellow, so I was seeing patients under supervision and completed my training in 2015 at which point I began practicing independently.
Q. And have you -- do you have any knowledge of how the -- of what has happened subsequently with the patients you were treating at Boston Children's while you were a fellow?

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A. So I -- all -- certainly not all of the patients that I've been treating are enrolled in a longitudinal study and have interval follow-up in their twenties and thirties. So similarly to patients that I saw in fellowship for any other condition, I don't have a mechanism for longitudinal follow-up for all of those parents.
Q. So in 2015, if the oldest patient you saw that was a minor was age 18, that would mean the oldest minors who you helped treat with medical gender transition interventions would be around 27 now; is that right?
A. The math seems to check.
Q. So you aren't aware of any follow-up with your patients beyond the age of 27?
A. Correct.
Q. How did you come to be involved in this case?
A. I believe the legal representation for the -- the US reached out to me directly.
Q. How often does your clinic see patients for gender dysphoria? Well, sorry, minor patients for gender dysphoria?
A. So there's several physicians that work in the clinic and several mental health professionals, so every day someone is seeing patients. I see patients two half days a week.

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Q. So about how many patients would you see a month of minors considering medical gender transition?
A. Are you asking minors -- are you asking how many patients under 18 that I see are considering, or we're assessing for, or are being seen that are already on, or what is your more precise question?
Q. Sure, sure. That you see that are either considering or are already on medical gender transition interventions?
A. Oh, okay. So probably about 60. Per month you asked?
Q. Yes.
A. Yeah.
MR. MILLS: I think it's a good time for a break, if that's okay with everyone.
All right, we can go off the record.
(Recess taken at 10:40 a.m.)
(On the record at 10:48 a.m.)
BY MR. MILLS:
Q. Would you agree that puberty is a sexually dimorphic process?
A. Puberty means -- puberty is a stage in life where a child's body becomes an adult's body and typically that goes one of two directions according to the hormonal sex of the individual.
Of course there can be variability. You

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know, female body people with PCOS can have higher androgen levels. There can be other endocrine differences, but generally there's a masculinizing and a feminizing puberty as the -- if we're dichotomizing.
Q. So would you agree with this definition: Puberty is the process of physical maturation where an adult -- sorry, I'll start over.
Pubertal is the process of physical maturation where an adolescent reaches sexual maturity and becomes capable of reproduction?
A. I think that captures some of what I was talking about. And, you know, I would -- I would say that there's more elements to puberty than simply contained in that one sentence.
Q. Would you agree that developing reproductive capacity is a fundamental purpose of puberty?
A. It's something that occurs during puberty. I'm not sure that you can say that a stage has a purpose. That, you know, sort of to me implies that puberty is an entity itself that has a particular purpose in mind, but reproductive potential -- the development of reproductive potential is something that occurs during a stage in life that we're talking about which is puberty.
Q. Would you say it is the central aspect of puberty?

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A. I don't know how I would respond to that. I think there's lots of different elements of puberty, so to say that gaining reproductive potential is the central aspect, no, I'm not sure that I would agree with that.
Q. So evolutionarily do you think there are other purposes of puberty?
A. Sure.
Q. What would those be?
A. Increasing height and strength. Those are a couple examples.
Q. When does puberty typically begin?
A. On average between ages 10 and 12.
Q. And does it vary in males and females?
A. To some extent, yes.
Q. So female puberty could start as early as 8 to 9; is that typical?
A. It would be considered precocious puberty or abnormally early puberty if female puberty started prior to age 8. So 8 is a reasonable cutoff for what would be considered normal, and then can be also normal to not start puberty until 12.
Q. And what about for boys; what would be the cutoff for precocious puberty?
A. Generally the ages that pediatric endocrinologists think about would be 9. Starting male puberty younger

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than age 9 would be precocious, and absence of puberty by age 14 would be delayed.
Q. So a 10-year-old boy who was starting puberty -- sorry. Would you consider a 10-year-old boy starting puberty to have precocious puberty?
A. No.
Q. Physical changes associated with puberty often cause anxiety or distress regardless of gender identity; is that right?
A. I'm not sure how frequently that's true. Is there a source that I could refer to?
Q. I just was curious in your experience, you know, do you find that adolescents starting puberty are worried about their physical changes?
A. Some may be.
Q. Do you think that's -- in your experience is that common?
A. I don't hear other patients that I take care of expressing anxiety about puberty in my practice, but I'm sure that some patients are anxious about puberty.
Q. When thinking about the dividing line between children and adolescents, would you consider puberty to be the dividing line starting puberty?
A. I -- I think that I'm not sure that I hold significance to children versus adolescents in that

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particular way, but I think that's a reasonable way to think about it.
Q. Can puberty cause adolescents' view of their own gender identity to evolve?
A. Could you say that again, please?
Q. Yeah. Can puberty cause adolescents' view of their own gender identity to evolve?
A. The experience that I hear from adolescents is that, you know, their -- an adolescent may describe that they had a particular feeling, that they were uncertain what that feeling was, and then as puberty progressed and they started to tangibly see the development of secondary sex characteristics, they had a better understanding of that feeling as a difference in gender identity, so in that way, yes.
Q. Does sexual attraction usually emerge during puberty?
A. I don't -- I don't think that I know the answer to that question specifically. I think that -- that as a pediatric endocrinologist I hate to posit an expert response on that.
I think there are certainly children that are prepubertal that have attractionality, either same sex or opposite sex attraction, so the evolution of sexual orientation is something that I -- I hesitate to speak on further.

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Q. But would you agree generally that puberty can lead to an increase in feelings of sexual attraction?
A. I would agree with that.
Q. Can the emergence of sexual attraction or the development of sexual attraction -- I'll start over.
Can the development of sexual attraction during puberty cause adolescents' view of their own gender identity to evolve?
A. That's not something that I heard from patients that -- that explain their gender identity to me that they're talking about sexual orientation and attractionality as a different concept than their gender identity, so I don't think that I would agree with that statement.
Q. If you could go back to Exhibit 1. This was your Advances in Pediatrics article. I'm sorry, I know you have a stack in front of you.
A. Advances in Pediatrics.
Q. Mm-hmm. So this is on page 6 in the middle of the page. The second full paragraph is talking about children who will persist in their gender identity during adolescence and adulthood versus those who will desist.
On the one, two, three, fourth sentence you say, "Important factors in early adolescence included

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the social environment, feelings toward pubertal changes, and the emergence of sexual attraction."

So you would agree that in the study you're talking about here emergence of sexual attraction was considered an important factor in identifying persistent gender dysphoria?

A. Could you tell me what the start of that sentence was?

Q. Yeah. So you're talking about one of the Dutch studies here about persistent. So I question was, this study that you talked about in your report found that the emergence of sexual attraction was an important factor in earlier adolescence for the persistence of gender dysphoria, right?

A. Yeah, so I think what I'm saying here is that when you're a prepubertal child and you're having -- you're exploring concepts like gender and attractionality, those concepts can -- can be confusing and sometimes conflated, but that the emergence of -- as puberty begins and you have the development of secondary sex characteristics and you're thinking about attractionality and gender in more tangible ways, that the ability to disconflate, if that's a word, gender identity from attractionality becomes easier.

Q. So your report says that, "Persistence or intensification of gender dysphoria as puberty begins

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is used as a helpful diagnostic tool as it becomes more predictive of gender identity persistence into adolescence and adulthood."

Do you still agree with that statement?

A. Yes.

Q. And that's why you don't give puberty blockers before Tanner stage 2; is that right?

A. That's one reason, another being that you don't need to block something that doesn't exist.

Q. If you gave puberty blockers before Tanner stage 2, it would deprive you of what you described as a helpful diagnostic tool, correct?

A. Correct.

Q. If you gave puberty blockers before Tanner stage 2, it would block even the Tanner stage 2 development of secondary sex characteristics, correct?

A. It would.

Q. And so you allowed those secondary sex characteristics to begin development up to Tanner stage 2 before providing puberty blockers?

A. Correct.

Q. By the same token, persistence or intensification of gender dysphoria as puberty progresses could be a helpful diagnostic tool; is that right?

A. Yes.

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Q. Would you agree that a 19-year-old will have a better sense of their gender identity than an 11-year-old?

A. No. I think everyone has an equal sense of their gender identity at that time. The question is how predictive is that gender identity of their future gender identity.

Q. And so would you agree that a 19-year-old will have -- will be able to provide a better prediction of their future gender identity than an 11-year-old?

A. If that 11-year-old has started to develop secondary sex characteristics and is having distress associated with them, then I would think that that 11-year-old's assessment of their gender identity would be quite predictive of their future gender identity similarly to a 19-year-old.

Q. Would you still say that the 19-year-old's assessment would be more accurate?

A. Accurate of what?

Q. Their future gender identity.

A. I would. That's why we use pubertal suppression to buy additional time and processing and understanding; that's why we don't treat 11-year-olds with gender-affirming hormones.

Q. So would you say a diagnosis of gender dysphoria -- sorry, scratch that.

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Would you also agree then that a 19-year-old will have a better sense of their future gender identity than a nine-year-old who is before Tanner stage 2?

A. Again, you're asking if their -- because everyone's gender identity at that time is a -- is -- you're asking is a 19-year-old's gender identity currently more predictive of their gender identity when they're, say, 29 compared to a nine-year-old's?

Q. That's right.

A. I would agree.

Q. And just to confirm, you said in your clinic you don't treat with cross-sex hormones at age 11; is that right?

A. I don't.

Q. And is that true even if someone started puberty blockers, a girl, say, started puberty blockers at Tanner stage 2 at age 9?

A. I have a hard time stating that I would have a hard and fast age cutoff for something that I consider more of a developmental decisionmaking process with patients and families, but it's not my practice. I haven't had patients at age 11 that I have felt comfortable starting gender-affirming hormones.

Q. So to go back to the line of questions we were just

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talking about in terms of future gender identity, you would agree that an 11-year-old -- sorry, scratch that. We can move on from that.

I have an article that I'm marking as Exhibit 11, which is entitled "Criminalization of gender-affirming care interfering with central treatment for transgender children." Oh, sorry, this is 12. I'm just going to change that number.

A. Oh, yeah.

Q. I lost track here.

MARKED FOR IDENTIFICATION:
EXHIBIT 12
11:05 a.m.

BY MR. MILLS:

Q. This is Exhibit 12, "Criminalization of gender-affirming care." This is an article you coauthored; is that right?

A. Yes.

Q. And it was published in the New England Journal of Medicine; is that right?

A. Yes.

Q. Okay. If you could go to page -- the first page of -- 579 is the first page. The start of the last paragraph here in the third column you say, "Gender dysphoria can be treated with both nonmedical and

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medical intervention."

Do you still agree with that?

A. Yes.

Q. So sometimes medical interventions for gender dysphoria are not warranted?

A. Correct.

Q. And sometimes nonmedical interventions would satisfactorily resolve any gender dysphoria?

A. It's possible.

Q. If you could flip back to Exhibit 4, which is your article "Serving Transgender Youth." And I'm on page 5 in the middle of the page, kind of right in the middle of the long paragraph on the page, the sentence that starts with, "Further," looks like the fourth sentence, "Further, we have found psychotherapy exceedingly helpful for treating cooccurring mental health issues and for exploring the child and/or adolescent's thought processes, family functioning strength and support systems."

Do you still agree with that statement I just read?

A. Yes.

Q. So psychotherapy can be exceedingly helpful for treating cooccurring mental health issues?

A. Certainly.

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Q. And treating those issues can be necessary for a child's health; is that right?

A. Yes.

Q. So continuing on it says, "In addition, psychotherapy enables a deeper exploration of the child's gender dysphoria, the range of gender expression and gender identity questioning, and whether the subjective experience fits more into a model of binary identity, e.g. male/female versus a fluidity of gender and gender nonconformity."

Do you still agree with that statement?

A. Yes.

Q. Page 7 the start of the second paragraph, really the first full paragraph, the paragraph right above "medical intervention," the first sentence, "Continuing psychotherapy for youth is typically recommended by our protocol."

Is that still true in your clinic?

A. I think that every adolescent could benefit from therapy, especially adolescents that are in -- undergoing gender transition.

A patient that is not experiencing any mental health problems at all may not require therapy and wouldn't be required to be in therapy to continue treatment, but I as a -- as a pediatrician, I find

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that therapy is of value for most adolescents.

Q. But patients with gender dysphoria are experiencing mental health -- a mental health issue, correct?

A. Gender dysphoria is a mental health condition outlined in the DSM with the treatment being the medical interventions that we -- that we have been reviewing.

So if a patient has -- is being treated for gender dysphoria and has -- has no other mental health problems, while therapy wouldn't be required, I think that it's always helpful to have someone in your corner that you can bounce things off of because adolescence is an unpredictable and challenging time for everybody.

Q. So just to go back to the sentence, "Continuing psychotherapy with youth with gender dysphoria is typically recommended by our protocol," is that still true in your clinic?

A. Yes.

Q. And would you consider that continuing psychotherapy part of the standard of care?

A. Well, I don't know that the standard of care outlines that every person that's receiving gender-affirming hormonal care requires psychotherapy, but the fact that it's typically recommended by me and by our clinic is true.

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Q. And sometimes do you treat patients, minor patients with gender dysphoria with psychotherapy alone?
A. If that helps to address their gender dysphoria or if they otherwise are unable to receive hormonal interventions.
Q. And some minor patients see their gender dysphoria resolved with psychotherapy and without additional medical interventions?
A. So I think that generally a patient that is receiving psychotherapy as treatment for their gender dysphoria is exploring in that psychotherapy how they can express their gender identity in a way that alleviates their gender dysphoria, so that psychotherapy could involve figuring out safe ways to make a social transition or whether social transition is safe for that patient, you know, exploring things like that.
So it's -- it's not that the psychotherapy is being used to say, you know, despite the fact that you have this difference in gender identity, you know, you're going to, you know, learn to forget about that gender identity and accept the sex that you were assigned at birth. It's more, you know, what nonmedical approaches can we use to -- to help you cope with this disconnect that you have between your body and your gender identity.

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Q. And sometimes the psychotherapy plus nonmedical approaches are sufficient to resolve the gender dysphoria; is that right?
A. It could be.
Q. And this psychotherapy that you're describing would not be conversion therapy; is that right?
A. Correct.
Q. If you could look at Exhibit No. 1, this is back to your Advances in Pediatrics article. This on page -- let's see here what page are we on. This is on page 4, the paragraph just before the "Development of Gender Identity" heading, this is the second sentence.
"Prior to the late 1990s, treatment of children or adolescents with gender dysphoria was not considered."
Do you still agree with that statement?
A. In the ways that we're describing today with hormonal interventions, that's correct.
Q. Right. So this is referring basically to puberty blockers or cross-sex hormones?
A. Correct.
Q. To go back a page to page 3, the first sentence under "Historical Perspectives: Prior" -- sorry, I'll wait for you.
This is the first sentence under

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"Historical Perspectives: Prior to the isolation of sex hormones their development into an injectable or oral compound to be administered in development of surgical techniques, there was no options -- there were no options to change one's secondary sex characteristics."
Do you still agree with that statement?
A. Yes.
Q. And then flipping to page 9 of the same article, in the middle, this is the third sentence under "Overview of Medical Management."
"Primary goals of sexual interventions include 1) prevention of" --
A. "Of medical."
Q. Oh, sorry. "Primary goals of medical interventions include 1) prevention of the development of unwanted secondary sex characteristics of the biologic sex; and 2) promotion of the development of desired secondary sex characteristics of the affirmed gender."
So the purpose of puberty blockers is what you said in number 1 there, prevent the development of unwanted sex characteristics of the biologic sex, right?
A. That would be one of the goals of pubertal blockade.
Q. And the purpose of cross-sex hormone therapy is to

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change the appearance of one's secondary sex characteristics?
A. Ultimately the purpose of both of these medications is to treat gender dysphoria and improve quality of life, but more proximally, yes, the gender-affirming hormones would promote the development of the desired secondary sex characteristics.
Q. And so these two purposes which, as you said, both go to the ultimate treating gender dysphoria, these purposes are the same regardless of the patient's biologic sex, right?
A. Correct.
Q. And these treatments do not change the chromosomal sex; is that right?
A. That's correct.
Q. They don't change the genetic sex?
A. I would think of that as the same as chromosomal sex.
Q. Okay. And they do not change the gonadal sex, correct?
A. Correct.
Q. If we could flip back to Exhibit 8, which was the chapter in the book, and we are going to the bottom of page 171. In looking at Figure 9.1 here, so this figure shows when you would typically start medical interventions to treat gender dysphoria, right?

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A. Yes.
Q. Okay. And we talked a little bit about this, but it shows puberty blockers being started around age 10 or at Tanner stage 2, right?
A. Right. It says Tanner stage 2 with this karat type symbol implying that that could be a variety of different ages --
Q. Sure.
A. -- centered around -- around 10, 10 and a half, 11.
Q. Right, yeah, and we discussed that earlier. So let's see. Sorry.
And that use of puberty blockers around age 10 or at Tanner stage 2 is consistent with WPATH and Endocrine Society guidelines?
A. Yes.
Q. You wouldn't consider a 10-year-old to be an older adolescent, would you?
A. No.
Q. So it would not be correct to say that under the existing guidelines medical interventions for gender dysphoria are reserved for older adolescents, correct?
A. No. I would -- I would -- I would use hormonal interventions such as testosterone, estrogen in place of medical to make that sentence accurate.
Q. Okay. Because puberty blockers are not reserved for

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older adolescents?
A. Correct.
Q. If you'd turn to 169 of this same document at the very top of the page, "The current hormonal management of transgender youth involved from strategies first described by Delemarre van de Waal and Cohen-Kettenis at the Amsterdam gender clinic in 2006."
Do you agree with that statement, other than my butchering of the Dutch names?
A. Yes.
Q. And did the use of puberty blockers to treat precocious puberty originate before 2006?
A. Yes.
Q. Does the standard course of treatment for precocious puberty present significant risks to fertility?
MS. WILLIAMS: Objection.
A. No.
BY MR. MILLS:
Q. So if you go back to 172 of this document at the top, the second sentence, "The goals of supervision include i. Prevention of development of unwanted secondary sex characteristics, ii, mitigation of the accompanying dysphoria associated with puberty; and iii, The ability to delay decisions around gender-affirming hormone treatment."

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This refers to puberty blockers, right?
A. Yes.
Q. And when you use puberty blockers to treat precocious puberty, you are trying to prevent the premature development of secondary sex characteristics, right?
A. Yes.
Q. You are not trying to prevent the development of sex characteristics entirely, correct?
A. Eventually that person will develop secondary sex characteristics upon discontinuation of the GnRH agonists, so you're delaying the development of those secondary sex characteristics. You're allowing for full height potential and other goals of care when you're treating precocious puberty.
Q. Right, but a goal is not to prevent the development of sex characteristics entirely forever?
A. Correct.
Q. And when you -- when you use puberty blockers to treat precocious puberty, you are not trying to mitigate gender dysphoria?
A. Correct.
Q. And you're not trying to delay decisions around gender-affirming hormone treatment when you're using them in the context of precocious puberty?
A. That's correct.

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Q. So these goals of using puberty blockers to treat gender dysphoria are different from the goals of using puberty blockers to treat precocious puberty, right?
A. Correct.
Q. If you could look at the bottom of page 172. This is at the end of the paragraph that's almost at the bottom. "The majority of patients presenting to care may not present at Tanner -- sorry, I'll start over.
MS. WILLIAMS: I'm sorry, where -- just a minute. Where are you exactly?
MR. MILLS: This is the last full paragraph on 172, the end of the paragraph, the last two sentences.
MS. WILLIAMS: Great.
BY MR. MILLS:
Q. "The majority of patients presenting to care may not present at Tanner stage 2. In our clinical practice, about two-thirds of adolescent patients present to care at a more advanced pubertal stage. In these cases, the decision regarding whether to consider GnRH agonist treatment is more complex."
So you're saying for most patients in your clinic when you're thinking about using puberty blockers, puberty has already progressed past Tanner stage 2, right?

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A. Well, it's a little complicated because the majority of patients that are presenting postpubertal, you know, we are not considering GnRH agonists, and I would say that even for patients that present mid-puberty, GnRH agonists may or may not meet our treatment goals.

So, for example, a transgender young man who is midway through puberty and has started the menstrual cycle, you could theoretically give that patient GnRH agonists and stop the menstrual cycle and prevent progression of breast development, but you could just as easily use other medications to stop the menstrual cycle. The breast development has already happened, so the advantage of using GnRH agonists in that situation wouldn't be very high. A transgender girl who is partially into puberty, if she hasn't developed masculine facial features, then perhaps GnRH agonists would be more helpful.

In both of those situations, you know, I'm explaining an example that we wouldn't be yet considering hormones, but whether or not the GnRH agonists would be helpful or not really depends on the clinical scenario and may or may not be helpful later in puberty.

Q. Sure. So go to the bottom of the page here.

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"The following factors should be considered when discussing GnRH agonist use for the transgender adolescent presenting at a pubertal stage more advanced than Tanner stage 2." And then there's a couple things, and flip over to page number 4, "Is the patient male or female."

So when you're thinking about whether to use puberty blockers in those post-Tanner stage 2 patients, that discussion might vary based on the patient's sex, right, biological sex?

A. Yeah. For the example --

Q. Right.

A. -- that I just demonstrated to you.

Q. Right. Because the -- and that's just to try and explain what you said, and that's because the -- the secondary sex characteristics of males and females differ in their development?

A. Correct. A mid-pubertal trans boy may be most concerned about their menstrual cycle. Breast development progressing slightly might not be as big of a concern. Whereas, trans girl would be -- could be most concerned about facial masculinization, and GnRH agonists would be a useful tool to stop further facial masculinization, but there are simpler ways to treat the menstrual dysphoria.

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Q. Sure. So just to go back to what we read a minute ago, the majority of patients presenting to you for gender dysphoria are past Tanner stage 2; is that right?

A. Correct.

Q. And is that different from the patients you treat for precocious puberty?

A. That's hard to say. I think that patients with precocious puberty are also a variable group. Some patients are presenting for -- to medical attention at the very first sign of pubertal changes, where others are late to be picked up and may be further progressed into puberty before presenting to care.

Q. But would you say that most of the patients you see for precocious puberty are still at Tanner stage 2?

A. I'm not sure I could say that.

Q. The risk of delaying a normally timed growth spurt is present when using puberty blockers for gender dysphoria; is that right?

A. Say that one more time, please.

Q. The risk of delaying the normally timed growth spurt is present when using puberty blockers for gender dysphoria?

MS. WILLIAMS: Objection.

A. So when you're using pubertal suppression for gender

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dysphoria, you're delaying the pubertal growth spurt, yes.

BY MR. MILLS:

Q. When you use puberty blockers to treat precocious puberty, is the goal that the growth spurt will occur at the same time as it would have in a patient without precocious puberty?

A. Yes.

Q. You would agree that puberty blockers are not approved by the FDA to treat youth with gender dysphoria?

A. Right, gender dysphoria is not an indication for use.

Q. And that's because the FDA has not received satisfactory data demonstrating safety and efficacy?

A. I do believe that would be what would be required to obtain that indication.

Q. So if we go back to the book chapter we've been looking at page 174, and again this is Exhibit 8, this is the third sentence on 174 at the very top, "Unlike estrogen monotherapy, testosterone monotherapy is more effective at suppressing further development of female secondary sex characteristics, and the additional benefit of concurrent use of GnRH agonists is likely minimal."

Is that one reason why it matters whether the patient is a male or a female?

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A. Well, yes. If you're -- if you're -- we're talking about two different -- two different types of patients when we're talking about trans young men and trans young women.

When you're treating with testosterone, testosterone by itself typically serves the purpose of raising the testosterone level up into the normal male range and suppressing the estrogen level into the normal male range. Whereas, estrogen by itself for a trans woman typically can raise the estrogen level up into the normal female range, but by itself oftentimes does not lower the testosterone level into the normal female range.

Q. So this is going to sound like a dumb question, but so your use of the cross-sex hormone testosterone or estrogen would depend on the individual's biological sex?

A. Yes.

Q. If we go back to Exhibit 1, which was the Advances in Pediatrics article and go to page 24, which is the last page, there's a table.

MS. WILLIAMS: Just a second.

MR. MILLS: Sure. Yeah, the back cover.

BY MR. MILLS:

Q. Table 2 is entitled "Medications used in the treatment

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of transgender adolescents."

So this is -- these are treatments for gender dysphoria that you're listing here, correct?

A. Yes.

Q. And this table is not listing treatments for other conditions, correct?

A. Well, these medications can be used for other conditions, but this is a table specifically talking about the treatment of gender dysphoria.

Q. Sure. So the second -- the second half of the table says, "Promotion of the development of desired secondary sex characteristics."

So the point of the cross-sex hormone therapy is to develop secondary sex characteristics that would not otherwise be present based on the biological sex; is that right?

A. Yes.

Q. All right. You list two medications for use here, and we've been talking about them already.

You agree you would use testosterone in biological females for treatment of gender dysphoria, right?

A. Yes.

Q. And according to Table 2, the mechanism of that treatment is activation of androgen receptors, right?

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A. Yes.

Q. Does testosterone have antidepressant effects in biological males?

A. I would say potentially there's -- there's men with low testosterone can have low energy and lower mood, so treating low testosterone can improve mood. I wouldn't say that -- I wouldn't think of testosterone as a treatment for depression, but depression that's concurrent with low testosterone in a cisgender man could improve with treatment.

Q. Would testosterone have the same mood-elevating effects in biological females?

A. It's possible.

Q. So the other treatment here is -- I'm really going to butcher this -- estradiol?

A. Yeah. Estradiol is just a medical term for estrogen.

Q. Okay. So according to the table, the mechanism of that treatment is activation of estrogen receptors, right?

A. Yes.

Q. And so you would agree you would use this medication estrogen in biological males for treatment of gender dysphoria, right?

A. Yes.

Q. In using estrogen or testosterone to treat gender

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dysphoria is also an off-label use, correct?

A. Correct.

Q. And that means that the FDA has never approved it for that indication?

A. That's correct.

Q. And that means that the FDA has not reviewed satisfactory clinical trial data establishing the safety and efficacy of these interventions for that indication?

A. Yes, that means that would be necessary to gain that indication.

Q. These hormone therapies, estrogen and testosterone, must be continued indefinitely into adulthood as long as the person wishes to continue medical gender transition; is that right?

A. Yes.

Q. Do you advise your patients that are going through the process of hormone therapy that this will be a treatment that they will have to undertake for a long period of time?

A. No, that's not how I frame it. I -- I talk to patients about the fact that every time we get together we're going to be talking about whether continuing the medical intervention is something that still feels like the right approach.

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Q. So you don't tell them that the therapies would have to be continued indefinitely as long as they wish to continue gender transition?
A. Yes, I both tell them that they would continue the medication so long as they would like to promote the development and maintenance of those secondary sex characteristics, but also that at every visit we would be reevaluating their goals and need for treatment.
Q. You wouldn't use testosterone for treatment of gender dysphoria in biological males, correct?
A. No.
Q. Because that would not treat a biological male with gender dysphoria, right?
A. Correct.
Q. Would it be in your view malpractice to prescribe testosterone to a biological male for treatment of gender dysphoria?
MS. WILLIAMS: Objection.
A. I can think of scenarios that you might prescribe testosterone to a biological male with gender dysphoria, but it wouldn't be treating their gender dysphoria.
So, for example, a biological male who is having suppression of testosterone and subsequent erectile dysfunction may be treated with a small

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amount of testosterone to treat the erectile dysfunction, but that would be treating the erectile dysfunction and not the gender dysphoria.
BY MR. MILLS:
Q. And by the same token, you would not use estrogen in biological females for treatment of gender dysphoria, correct?
A. Correct.
Q. So let's assume a patient with appropriately diagnosed gender dysphoria came into your office and was ready to start sex hormone therapy. What other information would you need to know to decide what to prescribe?
A. I would need -- sorry, could you say that one more time?
Q. Sure, I'll rephrase the question.
So again, take a patient with appropriately diagnosed gender dysphoria; they came in your office, they were ready to start on sex hormones. Would you need to know their biological sex to know what to prescribe?
A. I would need to know their anatomical hormonal sex. If that's the term we're using for biological sex, then yes.
Q. Okay. And do you test existing levels of estrogen or testosterone before starting treatment with cross-sex

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hormones for gender dysphoria?
A. I do like to maintain baseline hormone levels before starting treatment.
Q. Okay. And why is that?
A. To compare to follow-up labs.
Q. And is that routine in your practice?
A. Yes.
Q. If we could keep looking at this same article, go to page 12 in the middle, the second full paragraph.
A. Oh, which --
Q. Oh, sorry. This -- that's right, the Advances article, and instead of 17 B estradiol, I'm just going to say estrogen if that's okay?
A. Yes.
Q. So MTF, which I understand is male-to-female individuals are treated with estrogen to induce female secondary sex characteristics. And then skipping a sentence, "These changes are more effective when testosterone production is reduced either by using GnRH agonist medication or a progestin concurrently. Higher doses of estrogen would be required to produce feminizing changes if the testosterone concentration is in the normal male range."
So your discussion here refers to a biological male whose sex hormones are in the normal

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male range, right?
A. A male body person who is transitioning with estrogen, yes, this is what I'm describing, the options for treatment to -- to result in female level of estrogen and a female level of testosterone.
Q. And the reason higher doses of estrogen would be needed if testosterone is in the normal male range would be the testosterone has to be suppressed below the normal male range for estrogen to be effective?
A. Correct.
Q. And that estrogen level would be above the normal biological male range; is that right?
A. The concern here is that if you're using estrogen by itself as monotherapy, then you would need higher than ideal amounts of estrogen to achieve that goal, so that's why we combine estrogen with other antiandrogen medications.
Q. Right. But even in combination, the estrogen level of this male-to-female individual would be significantly above the estrogen level expected in a biological male, right?
A. Yes.
Q. We have no way of knowing what estrogen or testosterone level a specific transgender girl would have arrived at if she had been born female, correct?

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A. We know what the normal range is for -- for female body people, and so we use that range as a target and also clinical information such as feminization progress. But if you're asking counterfactual if this person was born assigned female at birth what would their estrogen level be, the estrogen level would vary throughout the day, but, no, I don't have a way to know exactly what the estrogen level would be in that counterfactual.
Q. If a biological female with gender dysphoria needs hormone therapy, it doesn't matter what gender identity the patient identifies as, correct?
A. Sorry, one more time.
Q. Yeah. If a biological female with gender dysphoria needs hormone therapy to treat the gender dysphoria, it doesn't matter what gender identity the patient identifies as, correct?
MS. WILLIAMS: Objection.
A. I think it does, it does matter. If that person identifies as female, I would have a hard time understanding why they would have gender dysphoria, so that would be something that I would need to explore, that wouldn't make sense to me, so it would matter what their gender identity is.
BY MR. MILLS:

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Q. If they said -- if the biological female said she was nonbinary, you would still be willing to treat the gender dysphoria with hormone therapy?
A. I would need to better understand what that meant to that patient and how that identity resulted in gender dysphoria, and also whether masculinization would be helpful to treat that gender dysphoria in that scenario because certainly some patients, like the one you're describing, would benefit from testosterone and others would not.
Q. When you decide not to give estrogen to a biological female for treatment of gender dysphoria and to give testosterone instead, are you discriminating against that person based on their sex?
MS. WILLIAMS: Objection.
A. I don't think I understand your question.
BY MR. MILLS:
Q. So earlier you said you wouldn't give estrogen to a biological female for treatment of gender dysphoria because you would give testosterone.
When you decide to use testosterone instead of estrogen based on the person's I think you said anatomical sex, would you consider that discrimination against that person based on their sex?
MS. WILLIAMS: Objection.

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A. No, I would -- I would call it appropriate medical management of gender dysphoria.
BY MR. MILLS:
Q. Has anyone ever accused you of discriminating based on sex for making those treatment decisions?
A. No.
Q. Have you ever been investigated by the federal government for discriminating on the basis of sex?
A. No.
Q. Would you consider yourself to have violated any law prohibiting discrimination on the basis of sex on that basis?
MS. WILLIAMS: Objection.
A. No.
BY MR. MILLS:
Q. If we have a biological female who was put on puberty blockers at Tanner stage 2 and then given testosterone as a treatment for gender dysphoria, the testosterone will not cause the female to develop reproductive capacity, correct?
A. I'm not sure that I agree with that statement completely. The patient that you're describing that's on GnRH agonists and then testosterone in the clinical scenario where now that patient is 18 and desiring fertility capacity, my advice would be to discontinue

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the testosterone and allow for endogenous puberty to occur.
Q. Sure. I'll ask it a little different way, I don't think I was clear.
So in the biological male puberty context testosterone leads to the development of reproductive capacity through spermiogenesis, right?
A. I think that's a little oversimplified, but as an endocrinologist I would say it's the LH and FSH hormones from the pituitary that is stimulating the testicles to produce testosterone and sperm cells. The testosterone is also required for the maintenance of that sperm-making organ to function properly, so in a longwinded way, I guess I'm agreeing with you.
Q. Okay. But in the biological female who was put on blockers at Tanner stage 2 and then given testosterone, that person is not going to develop sperm?
A. At the current time that person -- sorry, this is a --
Q. Biological female.
A. -- biological female on blockers and then on GnRH agonists and then starting on testosterone?
Q. Right.
A. So I would not expect that -- that person to be making follicles and ovulating. I suppose it's possible, but

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I would not expect it during treatment.
Q. And that person would also not be producing sperm?
A. Correct.
Q. Okay. Again, I'm sorry, I know that's kind of -- it seems like a silly question.
And then -- and then the same consideration, a biological male put on agonists at Tanner stage 2 and then given estrogen, that treatment -- the estrogen would not cause the male to develop female reproductive capacity in the sense of producing eggs?
A. Correct.
Q. And those doses of estrogen would also, as long as they're administered, preclude the male from developing male reproductive capacity; is that right?
A. I would expect it to be less likely that that person would have spermatogenesis while -- while not -- while on the treatment as you outlined.
Q. So relative to going through puberty without these interventions, this biological male would be less likely to develop reproductive capacity?
A. Yes. During the treatment course that you're outlining, that's correct.
Q. Have you ever prescribed testosterone to a biological male who wished to get stronger for bodybuilding?

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A. I may have prescribed testosterone to someone with low testosterone who also wanted to be stronger, but not someone with the normal male testosterone level who simply wanted to be stronger.
Q. Would you be willing to prescribe testosterone to a male who simply wanted to be stronger for bodybuilding?
A. No.
Q. Why not?
A. Because it's not recommended by any endocrine authority or medical body.
Q. So you wouldn't consider that treatment to be safe and effective; is that right?
A. It would probably be effective. I would have concerns about putting someone's testosterone level at a higher than normal level for a male. That would not be -- I would not consider that safe.
Q. And you believe you can opine on that safety even though you don't use this treatment for that indication?
A. In order to achieve the goals that you're describing, I think that you're implying that the testosterone level would be supratherapeutic or the testosterone level in this person would be higher than normal for a typical male, and in that situation based on my

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knowledge of -- of how testosterone works in the body, I would expect that person to be at higher risk for other problems such as polycythemia and hypertension, for example.
Q. And you can come to that conclusion even though you have not prescribed it before to someone who simply wanted to get stronger?
A. Correct.
Q. Have you ever prescribed estrogen to arrest growth in a biological female without gender identity issues who presented with complaints of tall stature?
A. I don't believe so. This was something that was more common several decades ago when -- when tall stature was a more common complaint for women, and the use of estrogen for tall stature in otherwise healthy woman is no longer recommended.
There are some tall stature conditions that you might consider using estrogen to close growth plates, some genetic tall stature disorders where it could be useful. I'm not sure that I've ever seen a patient that met those criteria, but if I did, then I would be comfortable doing that.
Q. Sorry, you would be or wouldn't be?
A. I would be if a female patient had a tall stature disorder and was going to be exceedingly tall and that

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would be interfering with her health, then estrogen could be considered as a treatment modality to arrest the growth plates.
Q. Have you conducted any clinical trials related to gender dysphoria?
A. No.
Q. I'm handing you an article you cited in I think your rebuttal report I'm marking as Exhibit 13, "Transgenderism and Reproduction."
Do you recognize this article?
MARKED FOR IDENTIFICATION:
EXHIBIT 13
11:51 a.m.
A. I believe so.
BY MR. MILLS:
Q. If you could turn to page 576, which is the second page, that key points box in the top left, the third point in that box it says, "Reproductive options for all trans persons are not equal because not only the gametes are of importance, but also the sex of the future partner."
Do you agree that statement?
A. I think it's a little bit of an odd statement, to be honest. I think what it's saying is that, you know, fertility may or may not be valued the same for every

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person, and they're implying that your attractionality may make your -- may make your valuation of fertility different, and while that may be true, I'm not sure that that would be universally true, so I think it's a tricky sentence to know whether I would agree or disagree; it's a complicated one.

Q. So specifically the part they say "the gametes are of importance," so would you agree that the treatments for gender dysphoria may have different effects on fertility depending on the person's biological sex?

A. Yes.

Q. On page 577, the next page, the first full paragraph at the top of the first column it says, "In trans women, feminizing hormonal therapy will lead to hypospermatogenesis and eventually azoospermia. The azoospermia will become irreversible after some time."

Azoospermia means the person has no sperm; is that right?

A. Mm-hmm. Yes, that's correct.

Q. And do you agree with this sentence that feminizing hormone therapy will lead to irreversible azoospermia after some time?

A. Sorry, which one are you asking if I agree with?

Q. Basically the second sentence, the azoospermia from the feminizing hormonal therapy, you know, do you

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agree that feminizing hormonal therapy will lead to azoospermia after some time?

A. I think that that is an over generalized -- over generalized statement. That I'm not aware of any research to suggest that all trans women will develop azoospermia after -- after being on estrogen for a certain period of time.

Q. So do you think these authors are incorrect?

A. I don't agree with that -- that sentence. I'm not seeing their citation for that -- that sentence, but if they're -- if it's 30 or 31, I would have to review that later in the paragraph, but I'm not aware of data suggest that all trans women are -- will become azoospermic after a period of time.

Q. Do you agree that some women will -- do you agree that some transgender women on feminizing hormonal therapy will become azoospermic after some time?

A. Yes.

Q. How long do you think this would take to occur?

A. I think it's extremely variable. I've had patients that have participated in a pregnancy unintentionally while treating with estrogen, and other patients that have had questions about their fertility and had -- and I've advised them that a course being estrogen should not be considered contraception because you may

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have the ability to make sperm on treatment.

So I think everyone's fertility potential is different at baseline, and then however long you're treated with hormonal interventions and how those interventions impact each person is different.

So I think for some people there would be no difference in fertility, and for other people there would be significant decrease in fertility if treated with estrogen for a prolonged period of time.

Q. Do you tell patients that they may suffer irreversible azoospermia?

A. I don't use that word because I don't think they know what it means, but I talk to patients about their risk of infertility when starting estrogen.

Q. And are you -- are you aware of any -- sorry, give me one second.

Are you aware of any biological male who started puberty blockers for gender dysphoria at Tanner stage 2 and then progressed to estrogen hormonal therapy and while continuing to use estrogen therapy was able to contribute sperm to a successful pregnancy?

A. The way you phrased that implies to me that the person was attempting to achieve a pregnancy while treated with estrogen, and I don't think that -- that that's

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the right way to think about it because a person wanting to achieve pregnancy would come off of their hormone treatment and wouldn't expect to be successful at achieving a pregnancy while on those interventions.

So the short answer to your question is no, but the scenario is impractical. The patient that is, say, has been treated with those interventions and would like to achieve pregnancy using their own gametes would discontinue treatment before attempting.

Q. And are you aware of any biological male treated for gender dysphoria with puberty blockers starting at Tanner stage 2 who then progressed to estrogen for at least five years who was able to successfully reproduce?

A. So again, I would say that I have -- I haven't -- I don't have awareness of a person that was treated at Tanner stage 2 and then started estrogen and has participated in producing a pregnancy, but I also haven't heard of anyone attempting to achieve fertility while being treated with those interventions, and so I think that's why I'm struggling to answer your question.

Q. So my question is really a biological male being treated for gender dysphoria with puberty blockers at Tanner stage 2 then five years of estrogen and then

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halts the treatment.
Are you aware of any such individual who was able to successfully reproduce after stopping the estrogen?
A. I'm neither aware of any such individual, nor am I aware of such individuals who have tried and failed.
Q. What about puberty blockers for a biological male at Tanner stage 2 followed by two years of estrogen; are you aware of any biological male who then stopped the estrogen and was able to successfully reproduce?
A. I'm not personally aware, but would find that to be quite plausible.
Q. But you don't know of any?
A. No.
Q. I'm going to show you as Exhibit 14 an article entitled "Consensus statement on the use of" -- we'll just shorten it to "GnRH hormone analogs in children."
MARKED FOR IDENTIFICATION:
EXHIBIT 14
12:00 p.m.
BY MR. MILLS:
Q. This is a consensus statement published it looks like in the AAP Journal of Pediatrics.
Are you familiar with this article?
A. Yes.

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Q. If we could go to page E758, the first column under "Conclusions."
"Despite a" -- sorry, this is the second sentence in the conclusions.
MS. WILLIAMS: Just a second.
All right, go ahead.
BY MR. MILLS:
Q. "Despite a considerable body of literature on the use of GnRHas, few rigorously conducted and controlled prospective studies are available from which to derive evidence-based recommendations."
Do you agree that that's true as to the use of GnRHa agonists in children?
A. So I agree that there's -- so I do believe that there is adequate literature to support the use of GnRH analogs for the treatment of gender dysphoria. These are -- they're not randomized controlled trials as maybe implied here in the conclusion, and so in that way I would agree.
Q. So the statement doesn't say randomized controlled trials. It says, "...few rigorously conducted and controlled prospective studies are available."
You would agree that that is correct, that there are few rigorously conducted and controlled prospective studies available?

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A. I would agree that there's not controlled prospective studies, but there are prospective studies, so in that way I would agree.
Q. The bottom of that paragraph says, "Use of GnRHas for conditions other than CPP requires additional investigation and cannot be routinely suggested."
CPP is central precocious puberty; is that right?
A. That's right.
Q. So the consensus in 2009 was that puberty blockers should not be routinely used for conditions other than central precocious puberty?
A. Can you point me to the sentence that you just read again? I'm sorry.
Q. Yeah, it's the last sentence in the conclusion section.
A. Yeah, so I guess it depends on what they're calling routinely suggested. If they're saying that professionals who are competent in assessing gender dysphoria should not use GnRH agonists to treat gender dysphoria, then I would disagree. If they're -- but if that's -- if they're saying that, then I would disagree. If they're saying that -- that using GnRH agonists routinely without that caveat, then I would agree.

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Q. Which do you read this as saying?
A. I think that they're implying that GnRH agonists should not be used in the way that I'm using them in treatment of gender dysphoria and so, therefore, I would disagree.
Q. Flipping back to page E756, the bottom of the first column, "Outcomes Reproductive Function," the very last line basically in the first column on E756 "Conclusions" --
A. Okay, hold on.
Q. Yep. Yeah, the very last line on E756.
A. Okay.
Q. "Conclusions: The available data suggests that gonadal function is not impaired in girls treated with GnRHas. Nevertheless, available data are limited. Long-term data on fecundity and ovarian reserve of treated patients of CPP are needed."
So in 2009, the effects of puberty blockers for central precocious puberty on fertility were not fully known; is that correct?
A. Well, I'll tell you that there is research related to this question, and I believe I cited it in my report outlining that a group of women treated for central precocious puberty and followed for fertility outcomes appeared to have no diminishment in their fertility.

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There's not a pathophysiologic reason that I would expect GnRH agonists to impair future fertility.

As a pediatric endocrinologist, when I'm prescribing GnRH agonists for central precocious puberty, I do not, and I don't think other pediatric endocrinologists, do warn of a risk of infertility.

So with all that said, there's certainly more research that could be done on every topic including this one, but I don't have an expectation that GnRH agonists impair someone's fertility who don't have another reason for impaired fertility.

Q. But would you agree with the consensus statement that long-term data on fecundity and ovarian reserve of treated patients with CPP are needed?

A. I'm not sure that I would agree based on the fact that -- that this isn't something that I -- I don't -- I don't know that the -- I don't think that the question about GnRH agonists causing infertility independently is one that is commonly debated amongst pediatric endocrinologists.

I think that if the -- if the group here that wrote this is saying that they're -- we would benefit from more data to prove this assertion, then I can support that, but I'm not accustomed to weighing the risk of infertility as a potential risk when

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deciding about treating central precocious puberty with patients with that condition.

Q. I'd like to show you a follow-up statement to this one, which I'm marking as Exhibit 15.

MARKED FOR IDENTIFICATION:
EXHIBIT 15
12:08 p.m.

BY MR. MILLS:

Q. Entitled "Use of gonadotropin-releasing hormone analogs in children update by International Consortium."

Are you familiar with this article?

A. I'm not sure if I've read this article completely or not.

Q. Sure. You would agree it's titled "Guidelines" at the top?

A. I see the word guidelines there, yes.

Q. Yeah. So on this first page in the middle of the abstract toward the end of the abstract paragraph it says, "Although there have been many significant changes in GnRHa usage, there is a definite paucity of evidence-based publications to support them."

Do you agree with that statement?

MS. WILLIAMS: Counsel, if he hasn't read this, I don't know. Do you feel comfortable?

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A. I'd like to at least read the entire abstract --

BY MR. MILLS:

Q. Sure.

A. -- before answering.

Q. Sure.

MS. WILLIAMS: Okay.

A. Okay, what was your question?

BY MR. MILLS:

Q. So the sentence says, "Although there have been many significant changes in GnRHa usage, there is a definite paucity of evidence-based publications to support them."

Do you agree with that description of GnRHa usage?

A. There have been significant changes in GnRH usage.

Q. Sorry. Do you agree that there is a definite paucity of evidence-based publications to support how GnRHas are currently used?

A. No, I wouldn't use the word paucity. I presented research related to the use of GnRH agonists for the treatment of gender dysphoria, so I would -- I would disagree.

But in reading this abstract, it seems like the authors here are -- are intentionally trying to avoid the type of discussion we're having today about

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the -- the decision to use GnRH agonists for treatment of gender dysphoria, but rather outlining its use. So I wouldn't -- I wouldn't say that the authors here are -- have been tasked to answer the question about the recommended treatment of gender dysphoria.

Q. You would agree that they are trying to point out what they call the deficiencies in the literature, though, correct?

A. I'm not sure what their intention is.

Q. So on page 365, the start of the second column under -- this is in section "Use of GnRHa and the management of transgender adults" that were in albeit in the second column, the first full sentence.

"The impact on BMD is concerning since lumbar spines e-scores at age 22 years were found to be lower than those observed prior to treatment suggesting a possible permanent decrement in BMD. Thus, it is unclear how long GnRHa can safely be administered."

Do you agree with that statement?

MS. WILLIAMS: Again, do you want to read it? I mean, it's up to you, but I just want to give you the opportunity if you don't recall reading this article.

A. I'll just read Section 7 real quickly and then I can

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respond. Is that okay?
Yeah, so -- so you've read a sentence that's related to bone mineral density questions and the use of GnRH agonists, and this is a pretty big topic that we can certainly talk about. You know, I think that agreeing or not agreeing with this one sentence, you know, is hard for me to do.
I think that bone mineral density is an important topic. It's one that I counsel patients on and talk to them about when we're making use of GnRH agonists and how long to use them, when to assess for bone mineral density, how would we measure this. So it's an important topic.
It would be concerning to me if someone had low bone mineral density at baseline and was planning to using GnRH agonists for an exceedingly long period of time because I would be concerned about their bone density and would want to follow that, but in other clinical scenarios it would be less concerning.
So I think that, you know, there's lots to say about this topic. I agree that it's an important topic and happy to talk more about it.
Q. Sure. My basic question is, do you agree with just the way they put it which is that, "It is unclear how long GnRHa can safely be administered in the context

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of a gender dysphoria intervention"?
A. I think that sentence by itself is hard to -- it's hard to agree with out of context, right?
Q. Sure.
A. So if you're saying that how long GnRH agonists can be safely administered without measurable difference in bone mineral density, sure. Is that difference clinically significant? Does it result in fracture? Does the risk of low bone mineral density outweigh the benefit of the intervention?
So I don't know if -- if asking me if I agree with this, it is unclear how long GnRH agonists can be safely administered without explaining that larger context can make any sense.
Q. Do you think it is clear how long GnRHa can safely be administered?
A. I think in certain scenarios, absolutely. So if I had a patient that has no risk factors for low bone mineral density, has clear gender dysphoria, and has a plan to use GnRH agonists for -- for two or three years, has normal bone mineral density at baseline, I do not have any concern about using GnRH agonists for that patient in terms of their bone mineral density. In other clinical scenarios, I would have more concern.

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Q. What about for six years for that patient?
A. Again, it depends on the clinical scenario. I have some patients that have been treated with GnRH agonists for six years, but if they don't need GnRH agonists that long, then I would prefer not to extend it for that amount of time.
Q. Because of in part of risk to bone mineral density?
A. Yes.
Q. All right. So the next sentence here is, "The effects of GnRHa on adolescent brain maturation are unclear."
Do you agree with that sentence?
A. I think that the question about GnRH agonists on brain maturation is odd for me because I don't -- I don't know that I understand why GhRH agonists would have an effect on brain maturation themselves.
So while I -- I may agree that I haven't seen studies specifically answering that question, I'm also not aware of studies that are outlining a concern related to this question specifically.
Q. So you are aware of no studies showing that there is no effect of GnRHa on adolescent brain maturation?
A. I'm aware that individuals with delayed puberty, for example, don't score different -- differently in cognitive testing, and that delaying puberty in and of itself with GnRH agonists I haven't -- I haven't heard

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of a plausible pathophysiologic reason why that would interfere with brain maturation in the way that's described, but, no, I haven't seen a study outlining exactly what you're asking.
Q. All right. The next sentence says, "GnRHa therapy prevents maturation of primary oocytes and spermatogonia and may preclude gamete maturation, and currently there are no current methods to preserve fertility in early pubertal transgender adolescents."
Just the first part of that sentence, "GnRHa therapy prevents maturation of primary oocytes and spermatogonia..."
A. Spermatogonia.
Q. Thank you. "...and may preclude gamete maturation," do you agree with that?
A. Yes.
Q. And currently there are no proven methods to preserve fertility in early pubertal transgender adolescents; do you agree that that's true?
A. Yes.
Q. If we could go back to Exhibit 2, which was the question and answers you gave on the Michigan website --
A. Do you feel like you're coming up to a good pause break in a little bit?

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Q. Yeah, that sound good. Are you good for just a few more minutes?
A. Sure, yep. Where are we at?
Q. The question and answer document should be marked as Exhibit 2. It's on the second page here, and we are under the heading "What are the risks or benefits of delaying puberty," the third paragraph under that heading.
You say, "We're also really cautious about using medical interventions to treat dysphoria because it delays growth spurts and bone density accrual."
Do you still agree with that statement of your practice?
A. Yeah, I think that the key here is delays because we do expect growth and bone density accrual to occur with future exposure to sex hormones.
Q. If we could go back to Exhibit 8, which was your book chapter and go to page 177. We're under "Special considerations for youth," then under "Bone Density" the second sentence, "When puberty is suppressed at Tanner stage 2, there is a concern for relative decrease in bone mineral density compared to untreated peers." And then skipping the next sentence, "However, another study demonstrated a decline in bone mineral density z-score during GnRH agonist treatment

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without full catchup by age 22."
I think this was the same study that the last source we used discussed.
So you would agree that puberty blockers at a minimum delay growth spurts, right?
A. I just want to go back because you skipped the one sentence that I felt like --
Q. Sure.
A. I'm not sure why you skipped one of the three sentences, but just to read the whole thing might be helpful. But maybe I'm not answering.
You asked a question that was different from I think what you read, so --
Q. Sure.
A. -- what do you want me to address right now?
Q. So puberty blockers at a minimum delay growth spurts; is that right?
A. Yes.
Q. And they delay bone density accrual?
A. Yes.
Q. And there is at least some evidence that bone density may not ever fully catch up; is that right?
MS. WILLIAMS: Objection.
A. So there's -- there's this one study that I'm referencing here that showed catchup, catchup towards

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normal at age 22 in -- I don't remember if it was the -- I think it was the trans girls, maybe it hadn't caught up to the z-score that they were at before.
What I would say is that I haven't heard any -- when we're talking about benefit density and z-scores, what are we really asking? We're really asking about the fracture risk in our elderly years. So what I haven't heard or seen any evidence of is an increased risk for osteoporosis in middle-aged people that were treated with GnRH agonists.
So we can say that GnRH agonists delay bone density accrual, that there's catchup with sex hormone exposure, complete catchup, almost complete catchup. Is 22 measuring too soon? Who knows. I think if we waited longer, we might see complete catchup, but ultimately what we really care about is fracture risk.
So at the -- even with the change in z-score outlined in Citation 34 here, I don't believe that to be enough of a change to result in meeting the clinically significant osteoporosis.
Q. And to go back to what we said earlier, none of your patients that you treated for gender dysphoria are beyond the age of 27; is that right?
A. Correct.
Q. If you could go to page -- the same page 177 the next

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paragraph the start. "There are a few little data regarding the final impact of prepubertal suppression and gender-affirming hormone therapy on stature."
Do you still agree with that statement?
A. Yes.
Q. So you don't know whether the effect of puberty blockers on stature is reversible?
A. Well, I know a lot about how pubertal suppression affects stature and talk about it with every single patient that I see.
Q. But you don't know whether the effect of puberty blockers on stature is reversible?
MS. WILLIAMS: Objection.
A. Well, I -- so just to be clear, stature means final height. So if you are -- so I would expect that the use of GnRH agonists in combination with gender-affirming hormones does have an effect on stature. That, for example, a trans boy who has delayed fusion of growth plates and then a more robust growth using testosterone may achieve a slightly taller stature than otherwise, which is typically very exciting for a trans masculine person who might be at risk for short stature. And for trans feminine folks the use of GnRH agonists plus estrogen may result in a slightly shorter final stature, lots of evidence to

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support that notion I think I haven't seen, but just as a pediatric endocrinologist understanding how these hormones work and how kids grow, I think that GnRH agonists do have an impact on stature, usually an impact that is desired.

BY MR. MILLS:

Q. Just a couple more if you're okay. Getting close.

I'm going to show you an article that you coauthored, marking as Exhibit 16, in the Journal, looks like, of Clinical Endocrinology.

MARKED FOR IDENTIFICATION:
EXHIBIT 16
12:27 p.m.

BY MR. MILLS:

Q. And are you familiar with this article?

A. Yes.

Q. And you were a coauthor on it?

A. Yes.

Q. If we could look at page 1565, the second paragraph. So it begins, "The literature on the impact of GAHT," which is I believe is gender-affirming hormone therapy, "in transgender youth is limited."

Would you agree with that sentence?

A. Well, I believe this is talking about bone density, correct?

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Q. That's right.

A. So I think it -- in this paper we are outlining the literature, so I guess it's up to the reader to say how limited it is.

I would say that it's -- it's limited to the extent that these are the main articles that we have to reference. So there is -- there is data to -- to review to answer questions about bone density, but certainly more -- more study on this topic is welcomed.

Q. The second to last sentence of that paragraph, "In one of the largest studies of bone mass development, trans girls had low BMD z-scores at the initiation of the study and after three years of estrogen therapy." I believe this was the same study we were just talking about.

Do you still agree that this is one of the largest studies of bone mass development?

A. Yeah, so if we -- if we explore that sentence a little bit more, the interesting thing here is that trans girls start with low bone mineral density before treatment and then continue to have low bone mineral density at the end of treatment. So it's interesting that there is this difference in baseline bone density in trans girls which, you know, there's -- there's

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potential reasons why that -- that may be, but this isn't saying that bone density in those girls worsened from its baseline z-score.

Q. Your next sentence says, "These findings raise concerns about prolonged GnRHa therapy with and in some" -- sorry -- "without and in some groups with sex hormone therapy on bone health in transgender youth and adults."

Do you agree that the findings raised concerns about prolonged GnRH therapy without and sometimes with sex hormone therapy on bone health?

A. Bone health is certainly a factor that we're using when we're making decisions with patients and families about GnRH agonists length of time on them. I think that GnRH agonists serve a purpose for patients with gender dysphoria, but shouldn't be used in the absence of other -- of -- of an indication for use for gender dysphoria.

Q. So are you saying you no longer have concerns about prolonged GnRH therapy --

A. I would have concern -- sorry. I would have concern about using GnRH agonists longer than required unnecessarily because that would potentially be -- there would be potential risk to bone density without subsequent benefit.

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Q. And you don't have data about how long GnRHa can safely be administered?

A. I think I answered that question.

Q. Page 1567, the bottom of the first column. This is about four sentences up from the bottom. The sentence is connected to Citation 506.

"Further research is also needed to determine optimal timing and duration of gonadotropin hormone agonist therapy in transgender youth as it relates to bone health and to determine the prevalence of osteoporosis, osteopenia, and fractures among transgender youth and adults."

Do you still agree with that sentence?

A. I think more research in this area would be great.

Q. On page 1569 in the second column, the first full paragraph the second sentence, "Prospective studies are needed to determine the timing and duration of gonadotropin hormone agonist therapy in transgender youth that optimizes peak bone mass"; do you still agree with that sentence?

A. I think a specific study to help address that question would be wonderful, but the fact that a study doesn't exist doesn't preclude me from safely using GnRH agonists.

Q. But you wrote last year that, "Prospective studies are

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needed to determine the timing and duration of GnRH therapy," correct?
A. Sorry, I wrote what?
Q. You wrote last year that, "Prospective studies are needed to determine the timing and duration of GnRHa therapy in transgender youth that optimizes peak bone mass," correct?
A. I'm not sure I wrote that sentence, but it's in this article that I'm authored on.
I agree that more studies on prospective studies on this topic would be needed to help answer that question more definitively, but still doesn't preclude me from using GnRH agonists.
Q. Do you recall giving a talk at the University of Michigan around October 21st, 2027 [sic] with a co-presenter Dr. Ellen Selkie entitled "Doctrine care for transgender children and adolescents?
MS. WILLIAMS: Objection. I think 2027.
MR. MILLS: 2017.
BY MR. MILLS:
Q. Yeah, a talk at University of Michigan October of 2017 with Dr. Selkie, do you recall that talk?
A. I'm not sure that I have a strong memory of it, but I certainly know Dr. Selkie and believe you that I gave this talk.

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Q. Sure. You've coauthored papers with Dr. Selkie, right?
A. Yes.
Q. So you agree she is knowledgeable in this field?
A. Yes.
Q. Okay. I just have a short video clip I wanted to show you which I don't know how we marked it, but it would be Exhibit 17, I believe.
MARKED FOR IDENTIFICATION:
EXHIBIT 17
12:34 p.m.
COURT REPORTER: And I will not be taking it down stenographically.
MR. MILLS: Okay.
A. Sorry, what year is this?
BY MR. MILLS:
Q. 2017.
A. So that's what I used to look like?
(Video playing.)
BY MR. MILLS:
Q. You were talking about puberty blockers here --
A. Yes.
Q. -- is that right?
So blocking puberty would prevent pubertal development during the same time as one's peers, correct?
A. Yes, the average peer in that age group would be going through pubertal changes.
Q. And that effect would be irreversible, right?
A. What effect exactly?
Q. In other words, you could not go back in time and go through puberty as the same time as one's peers did?
A. That's correct.
Q. And could that disconnect negatively effect a person's psychological well-being?
A. I think that -- I hear from patients that -- that as they're seeing their peers start puberty, oftentimes they're hoping that they will soon be able to go through puberty as well so, yes, that can be socially difficult.
Q. And it sounds like it can cause -- can cause social distress?
A. In patients that were -- that are feeling social distress related to a delay in their puberty, that social distress would be less than the distress associated with the -- going through endogenous puberty or else the GnRH agonist wouldn't be indicated.
Q. But blocking of puberty could cause social distress, correct?

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MS. WILLIAMS: Objection.
A. Social distress in the way that we've been discussing a desire to be progressing through puberty with -- at the same age as other peers, yes, but typically that would be in a pubertal direction aligned with their gender identity.
BY MR. MILLS:
Q. Puberty is also connected to emotional development; is that right?
A. So I think that emotional development does occur in adolescent years. How much of that is related to chronologic age progression versus pubertal progression I think is open to discussion, but I would -- I would posit that simply chronologic age progression also is important for emotional development.
Q. But by blocking puberty, you are at least delaying some aspect of emotional development, correct?
A. To whatever extent pubertal progression is related to emotionally development, yes, but again I would argue that chronologic age progression is I would think more important for emotional development.
I can't point to a citation to -- to make that point. I would say as a pediatric endocrinologist seeing patients with delayed puberty,

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I don't consider those patients to be emotionally stunted due to their delayed puberty, so in that way I would -- I would downplay the point that emotional development is somehow stunted by using GnRH agonists.
Q. But you'd agree that a person whose puberty has been blocked would not have the same emotional development pathway as their peers who are going through puberty?
A. I think that's hard for me to say. I don't -- I don't know that I have a specific expertise in emotional development, but I would say that -- that I don't see clinically patients with emotional immaturity compared to peers simply because they're on GnRH agonist treatment.
MR. MILLS: I think that's a good stopping point, if that works for everybody.
(Recess taken at 12:39 p.m.)
(On the record at 1:42 p.m.)
BY MR. MILLS:
Q. I'm handing you what I'm going to mark as Exhibit 18.
MARKED FOR IDENTIFICATION:
EXHIBIT 18
1:42 p.m.
BY MR. MILLS:
Q. This is an article you coauthored, "Gender affirming multidisciplinary care for transgender and nonbinary

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children and adolescents."
Do you recognize this article?
A. Yes.
Q. If we could flip to page 108. At the very bottom it says, "Longitudinal studies from Amsterdam Clinic patients document that only 1.9 percent of adolescents stop puberty suppression and did not go on to start GAHT gender-affirming hormone therapy."
Is this consistent with your experience?
A. I would say that the majority of patients that are prescribed pubertal suppression do go on to start gender-affirming hormone therapy. In my experience, the number is higher than 1.9 percent.
Q. About what percent would you say it is in your experience?
A. I think only about 5 percent.
Q. So that would mean that somewhere between, if you use this study, in your experience 95 to 98 percent of patients who start puberty blockers will go on to cross-sex hormones; is that right?
A. Yes, which makes sense given that the progression into Tanner stage 2 is that sort of predictive time where we're better able to understand the persistence into adulthood of one's gender identity, but still the pubertal suppression is used to take that extra time,

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so I think that lower number is a testament to the ability to accurately diagnose gender dysphoria and prescribe pubertal suppression to the correct candidates.
Q. So a provider should assume that a patient prescribed puberty blockers is almost certain to progress to hormonal therapy?
A. That is definitely not how I think about it. I would say that when I'm prescribing pubertal suppression I am myself keeping a very open mind and encouraging the patient and the family to keep an open mind to allow continued exploration of gender identity during that time of pubertal suppression and make no assumptions.
Q. But as a matter of fact, you know that 95 percent -- 95-plus percent of those patients will go on to hormonal therapy?
A. That's right. So I need to be cognizant of the fact that for the ones that don't, I need to, you know, help -- help to recognize when discontinuation of pubertal suppression is appropriate with patients that no longer require it.
Q. So would you consider hormonal therapy part of the standard course of treatment for gender dysphoria that starts with puberty blockers?
A. It's -- the treatment with gender-affirming hormones

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is part of the recommended -- is a recommended option for therapy to treat gender dysphoria as outlined by WPATH and the Endocrine Society, yes.
Q. I guess what I'm asking is, if it's 95 to 98 percent who go on to hormonal therapy, would you consider that to be the standard course of treatment?
A. I don't consider therapy to be a standard course of treatment. I consider every patient to be an individual person with individual needs and decisionmaking.
Q. Do you tell patients that 95 to 98 percent of those who start puberty blockers will go on to cross-sex hormones?
A. I'm not sure if I've used those exact percentages, but I -- I talk in great detail about the potential for transition to gender-affirming hormones when starting pubertal suppression.
Most patients and families assume that they will progress to hormones because they feel stable in their gender identity, and yet it's my job to continue to think critically about each patient and help them to think critically about themselves.
Q. And so do you tell families the risks of cross-sex hormones before you start puberty blockers?
A. I do talk about the implications of pubertal

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suppression followed by gender-affirming hormones when starting pubertal suppression, yes.
Q. And do you think that is the best practice to use before prescribing puberty blockers?
A. Yes.
Q. If we could go back to Exhibit 1, which is your Advances in Pediatrics article. We're on page 10, and this is the third full paragraph about five sentences in. It starts, "Although the effects." It's right after footnote 57, if that helps.
A. Yep, okay.
Q. So it says, "Although the effects of GnRH agonists are reversible, they are often started with the intent of initiating cross-sex hormones later on, and the combination of the two results in permanent and semipermanent effects."
So would you agree with just the first part of that sentence still that puberty blockers are often started with the intent of initiating cross-sex hormones later on?
A. I'm not sure I love the word intent. I think that the -- I'm oftentimes meeting with a patient that has very clear -- has been very clear in their gender identity from a very early age, and I may think to myself that it's very, very unlikely that that gender identity

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will change and that it would -- it is very, very likely that this person will be eligible for -- for gender-affirming hormones in the years to come, but I'm still using my diagnostic abilities and working with patients each time I see them to confirm that the trajectory of the plan is still correct.
Q. So do you disagree with what you wrote in 2017 which is that puberty blockers are often started with the intent of initiating cross-sex hormones later on?
A. I think I'm talking about the semantics of the word intent, so I don't disagree with the premise that when we're starting cross-sex -- when we're starting GnRH agonists, many of those patients will start gender-affirming hormones.
Q. Okay.
A. But I would just maybe point out that the intent can change as a patient's clinical course change -- changes.
Q. And then the second half of that sentence of what we just read, "The combination of the two results in permanent and semipermanent effects," do you still agree with that?
A. Yes.
Q. I would like to show you what I'm marking as Exhibit 19, which is a book chapter you wrote in

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"Pubertal suppression and transgender youth."
MARKED FOR IDENTIFICATION:
EXHIBIT 19
1:50 p.m.
BY MR. MILLS:
Q. That word continues to be a challenge.
Anyways, I believe after the front matter I've just excerpted your chapter from this publication. Do you recognize --
A. Yes.
Q. And you coauthored this chapter?
A. Yes.
Q. If we could turn to page 80 in the chapter. The first full paragraph toward -- the last sentence of the first full paragraph it starts with, "The intervention with a GnRH agonist." Do you see that?
A. Mm-hmm, yes.
Q. So I'll just read that. "The intervention with a GnRH agonist is "reversible" and allows time for a further gender identity exploration prior to committing to feminizing medications." And then you say, "Initiation of treatment with a GnRH agonist in a transgender girl at pubertal stage 2 requires discussion about several other considerations. The adolescent will continue to grow, but at a prepubertal

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speed while on GnRH agonist therapy.
"If estrogen is initialed later in adolescence, a growth spurt and subsequent growth arrest will occur likely resulting in a shorter final adult height than if no intervention were pursued."
Do you still agree with that section that I just read?
A. Yes.
Q. So skipping one sentence, but now we're talking about -- yeah, so skipping one sentence, "Spermatogenesis will not occur if puberty is suppressed. Therefore, a child treated with GnRH agonist medication followed by estrogen would not have the opportunity to preserve sperm using the standard methods."
Do you still agree with that what I just read?
MS. WILLIAMS: Objection.
A. Yeah, so I agree, but I would probably say if I were to, you know, rewrite the sentence, spermatogenesis will not occur while puberty is suppressed, because I think the sentence misses the element of the conversation we were having earlier about how one may still have the potential for fertility if they elect to go through puberty at a later time endogenously.
But the point remains that discussions

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around -- around the use of hormones and fertility matters are important to discuss when counseling patients and families on pubertal suppression.
Q. So is the reason you put reversible in quotation marks in this passage because, as the next paragraph explains, if you follow up puberty blockers with estrogen, then the consequences are not all reversible?
A. I'm not sure that that's the reason I put it in quotations. I think I put it in quotations because that's a word that's taken from the early Dutch protocol literature where they were using words like reversible, partially reversible, and irreversible to describe the GnRH agonist hormones and surgery.
Q. But you would agree that following puberty blockers with estrogen results in irreversible changes?
A. Yes. For example, breast development.
Q. In your clinic, do you use an informed consent form before starting puberty blockers?
A. We do not use an informed consent form in our -- in our clinic.
Q. And is that true also you don't use a form before starting cross-sex hormones?
A. Correct.
Q. Puberty blockers were historically used in the

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chemical castration of rapists; is that right?
A. I do believe that that's been attempted.
Q. And men taking GnRH agonists for prostate cancer experience a complete loss of sexual interest; is that right?
A. I don't know that that's always the case.
Q. Is it usually the case?
A. I don't know, I don't treat prostate cancer, but I know that men with low testosterone can have decreased libido, but I don't know if I would describe that as in the terms that you described.
Q. Sure. If we could go to page 83 of this same chapter. The last sentence before the estrogen heading at the bottom of this second column, the last sentence before estrogen, "Testosterone treatment likely increases the risk of polycythemia, sleep apnea, weight gain, and cystic acne, and possibly increases the risk of elevated liver enzymes, hyperlipidemia and hypertension"; you still agree with those -- that statement of risks?
A. Yes.
Q. On the next page right before conclusions, the sentence before conclusions, "Estrogen treatment likely increases the risk of thrombolic embolic disease, particularly synthetic ethanol, estradiol,

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hypertriglyceridemia, gallstones, elevated liver enzymes, and weight gain, and may increase the risk of hypertension and hyperprolactinemia."
Putting aside my butchering of scientific words, do you agree with that statement of the risks of estrogen still?
A. Yes. I also just point out that this would be the case if we were using estrogen to treat cisgender women with low estrogen, and the concerns about the potential risks of testosterone would be the case if we're treating cisgender men with low testosterone, and this is why we know how to prescribe these medications appropriately and monitor patients on these medications.
Q. And what is -- what is venous thromboembolism?
A. Blood clots.
Q. And is that life-threatening?
A. It can be.
Q. And long-term estrogen administration to a male increases the risk of those life-threatening blood clots?
MS. WILLIAMS: Objection.
A. I would -- I haven't had a patient that has had this condition, but I would say that women are at higher risk for venous thromboembolism than men, and treating

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a trans woman with estrogen puts her in a similar risk category as other women due to that fact that estrogen is a prothrombotic hormone.
BY MR. MILLS:
Q. So long-term estrogen to a biological male does increase the risk of thromboembolic events?
A. In the absolute sense, yes. I like to explain that when someone is being treated with gender-affirming hormones, you are adopting the health -- health risks of the affirmed sex and maybe eschewing the health risks of the sex assigned at birth.
A common example that I use with testosterone would be going bald. If you never started testosterone, you probably would never go bald. If you take testosterone, you've got the same chance of going bald as brothers in your family, and the same holds true with other medical problems that are sex specific if they're related to hormones.
MARKED FOR IDENTIFICATION:
EXHIBIT 20
1:59 p.m.
BY MR. MILLS:
Q. I'm showing you what I've marked as Exhibit 20, which is an article by Getahun and others entitled "Cross-Sex Hormones and Acute Cardiovascular Events in

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Transgender Person." I believe this is one of the articles you cited in your report.

If you would flip with me to page 11, on the second column the first full paragraph.

"A distinguishing feature of our study is that it represents one of the largest cohorts of transgender persons in the United States, and to our knowledge is the only study of this size that carefully validated trans feminine or transmasculine status in the participants."

And then going over to page 212, the bottom paragraph in the first column.

"In summary, the presence that he demonstrated that cross-sex estrogen is a risk factor for VTE and probably ischemic stroke among trans feminine persons."

And then going back to page 209. Again, the bottom paragraph of the first column.

A. Sorry.

Q. Yep, 209. So this is the last paragraph in the first column.

"The trans feminine cohort had an increase in post index date incidents of VTE compared with either referenced cohort, and the difference seem more pronounced with increased follow-up with two- and

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eight-year risk differences of 4.1 and 16.7 per 1,000 persons relative to cisgender men and 3.4 and 13.7 per 1,000 persons relative to cisgender women."

So the -- the authors of this study found that transgender females on estrogen were significantly more likely to have a VTE compared to cisgender males; is that right?

A. Yes.

Q. And they were also much more likely to have a VTE compared to cisgender females?

A. So let me just read these numbers again.

Q. Sure.

A. So I guess it's depending on your -- your -- how you'd like to use the term "much more likely." This is saying that, if I'm reading it correctly, that out of every thousand persons there was three more that had this event in the two-year follow-up, and 13 more out of a thousand in the eight-year follow-up, so that's more and statistically significant. Whether that is clinically significant or meaningful in a way that would prevent someone from deciding that the benefits of estrogen outweigh the risks is maybe a different question.

But I would also say that we have in pediatrics the risk for thromboembolism is extremely

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low, so this article is talking about adult patients, and so the -- when I'm seeing a patient with a clotting problem, I oftentimes consult with my hematology counterpart to discuss safety of estrogen treatment.

Transdermal estrogen is known to be less thrombogenic than oral estrogen, so we make that decision that someone has a higher thrombotic risk, but in general, young healthy adolescents are at very low risk for clotting regardless of whether they're treated with estrogen.

Q. And that's not true of adults, correct?

A. Adults have a higher risk for clotting compared to adolescents.

Q. And what proportion of the patients you start on hormonal therapy continue as adults, to your knowledge?

A. The majority continue as adults. So if I was an adult endocrinologist reading this article, I would be using that to make decisions on the administration route for estrogen based on the patient's thrombotic risk factors.

Q. But you don't consider these statistics when you're considering whether to decide -- whether to start an adolescent on hormonal therapy?

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A. Well, I just explained how I do consider it. I am assessing a transgender girl's thrombotic risk if she has thrombotic risk factors, then consulting with hematology and oftentimes changing the route of administration of the estrogen.

Q. But you're not considering the risk of that same girl once she becomes an adult?

A. I wouldn't say that that's true. I would say that that same girl would continue to see an adult provider who would continue to assess her thrombotic risk.

Q. Do you tell patients considering estrogen that they may be at significantly higher risk for a VTE compared to cisgender males or cisgender females?

A. I do talk about increased thrombotic risk and advise patients to not smoke cigarettes because that increases everyone's risk for clotting, which is a common thing to avoid when anyone is taking any form of estrogen.

Q. If we could go back to Exhibit 8, which was the first chapter we talked about from the transgender medicine book. If we could go to page 178 of your chapter, the start of the second paragraph under, "Fertility."

"Development of mature sperm and oocytes occurs during puberty, therefore, progressing through natural puberty is a requirement for fertility."

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Do you still agree with that statement that progressing through natural puberty is a requirement for fertility?
A. Yes.
Q. And by natural puberty you mean puberty of the person's biological sex?
A. I mean endogenous puberty, puberty created by the body itself.
So if you have a person that has hypogonadism and is cisgender, you'd be giving them hormones, but that person would not be able to reproduce either. Does that make sense?
Q. But I guess I'm asking a slightly different question which is that progressing through puberty of the person's biological sex is a requirement for fertility?
A. You have to go through puberty aligning with your biologic sex using your own body's hormones, yes.
Q. If we skip -- skip a sentence and then right after the number 36 you say, "Patients considering GnRH agonist therapy for gender dysphoria may not decide to allow their natal puberty to progress in later adolescence choosing instead to bridge to gender-affirming hormone therapy. If that decision is made, there will never be maturation of sperm or eggs and no opportunity for

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gamete preservation."
Do you still agree with what I just said?
A. Yes. Someone that was on GnRH agonists followed by hormones and continues on hormones will not have maturation of their germ cells.
Q. So they would be infertile?
A. At the present time, yes. If that person desired fertility, then again I would advise them to discontinue their hormones.
Q. So skipping the short paragraph right after the number 21, "Patients presenting after puberty should be advised that future fertility could be compromised by prolonged use of gender-affirming hormones."
Do you still agree that future fertility could be compromised by prolonged use of gender-affirming hormones?
A. Yes.
Q. If we go back to Exhibit 1, which was the Advances in Pediatrics, and we go to page 10, and this is about midway through the big paragraph closer to the bottom, the sentence starts with, "A child who starts on GnRH agonist therapy." Just let me know if you see it.
A. I got it.
Q. Okay. "A child who starts on GnRH agonist therapy at a similar stage 2 and continues on the" -- I think it

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should be "medication as cross-sex hormones are introduced later in adolescence will never have spermatogenesis or menarche and will not have the opportunity to bank gametes using cryopreservation."
Do you still agree with that statement?
A. This is almost the exact same statement that we just read, so I have the same answers.
Q. So that's a yes?
A. Well, I think that that person would not have -- they would not be fertile while taking these interventions, and if they desired fertility, my advice would be to discontinue treatment.
Q. Unless putting aside the possibility of discontinuing treatment, this child would never be able to reproduce naturally or artificially?
A. Well, that's a weird way to say it. If you discount this option, then -- then you never could do it? That's not how I typically would talk.
Q. Well, that is my question.
A. Okay, can you say it again?
Q. Yeah. So putting aside the possibility of discontinuing treatment, this child could never reproduce naturally or artificially, correct?
A. So I think that that's not 100 percent accurate for -- in terms of some protocols, and at -- at some centers

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transgender men could be stimulated to ovulate despite not having gone through puberty, and this -- this is a -- and germ cells can be harvested from testicular tissue.
None of this is standard of care or outside of what I would say experimental, but to say never, I'm not sure that I can agree with that completely given the experimental progress of genetic -- of fertility science.
Q. And are you aware of children being born using those experimental methods?
A. No.
Q. So if we take a biological male who starts puberty blockers at Tanner stage 2 and then goes on to estrogen, let's say he continues those interventions until age 45 then decides to align with his biological sex and holds treatment, would he go through natural male puberty at age 45?
A. I don't know the answer to that question, but I think that it's probable that he would.
Q. You're aware of no evidence showing that he would?
A. I'm not aware of anyone that has done that to prove whether it would be possible.
Q. How likely is it do you think that he would be able to successfully reproduce?

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A. I don't know how likely it would be. I think that his fertility could be compromised.
Q. Do you think there's a greater than 50 percent chance that his fertility would not develop?
A. Yes.
Q. Same question for a biological female. If she goes through puberty blockers at Tanner stage 2 and then testosterone and then discontinues interventions at age 38, can she go through female puberty and become -- and have a child?
A. There's a couple of different variables here, of course, because the female potential for fertility is marginal even in cisgender women at 38 sometimes, so I would say it's possible, but I think that it would be more likely at a younger age.
Q. Do you think the chance in the scenario I outlined would be less than 50 percent that she would be able to reproduce?
A. I'm less certain that it would be less than 50 percent in this scenario than in the biologic male scenario.
Q. And why are you more certain in the biological male scenario?
A. It seems to take less time for the -- the ovary to produce oocytes after suppression compared to spermatogenesis.

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Q. So if these -- if these individuals, and just talking generally about adolescents who started at puberty blockers at Tanner stage 2 and then went on to cross-sex hormones, if they were to halt that treatment and start going through their biological sex puberty, would that also mean that they would develop secondary sex characteristics associated with their biological sex?
A. Yes.
Q. So if they wished to remain living with their transgender identity, this would likely heighten their distress?
A. That's possible, yes.
Q. So a male who -- a biological male who wishes to be able to reproduce would then suffer a permanently lower voice?
A. In order to progress far enough into male puberty to have spermatogenesis, I would expect the voice to deepen.
Q. And a female who wishes to reproduce would suffer breast enlargement that would only be reversible via surgery?
A. Yes.
Q. And so when you say that they can choose to become fertile later, that would come at the cost of the irreversible effects that you're avoiding to begin with puberty blockers; is that right?
A. Yes.
Q. And do you tell patients that?
A. Yes.
Q. And are you aware of any literature discussing that issue?
MS. WILLIAMS: Object to form.
A. Yes. That's -- we talked about a lot of issues, but there's certainly literature that I highlighted in my rebuttal report outlining how -- how patients and families think through fertility conversations when considering gender-affirming care.
BY MR. MILLS:
Q. But you aren't aware of any long-term outcome studies examining patients who started puberty blockers at Tanner stage 2 then progressed to hormonal therapy and then wanted to become fertile, correct?
A. Correct, and so that is something that needs to be discussed when considering treatment.
Q. And you're not aware of any literature studying that specific issue; is that right?
A. Is that different than the question you just asked?
Q. Yeah. So my first question is about long-term outcome studies.

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A. Okay.
Q. But is there any literature about that specific issue? Again, thinking about the cohort of patients who started blockers at Tanner 2 and then went on to cross-sex hormones and then wanted to become fertile, are you aware of any literature that tries to examine what happens with those patients?
A. No literature talking about what happens to those patients. The topic is obviously discussed in the literature we've been reviewing together.
Q. If we could go back to Exhibit 19, which is, I believe, the other book chapter. This is page 79, the first column in the middle. It's about three sentences -- sorry, two sentences before footnote 7.
A. Okay.
Q. It starts, "Fertility for transgender men on sex steroid treatment testosterone has not been well studied."
Do you agree with that sentence still?
A. I think since that publication there's been a bit more literature on the subject, but I -- I would still agree with that statement.
Q. Has there ever been a live birth using sperm from a male who was administered puberty blockers at Tanner stage 2 followed by cross-sex estrogen?

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A. I don't know.
Q. But you're not aware of one?
A. No.
Q. Have you studied the literature regarding mental health problems in adolescents -- sorry -- in adults -- I'll start over.
Have you studied the literature regarding mental health problems in adults resulting from sterility?
A. No.
Q. And are you aware of any literature exploring mental health problems in adults resulting from sterility caused by puberty blockers, cross-sex hormones, or potential transition surgeries?
A. Not that I'm aware of.
Q. I'd like to show you what we'll mark as Exhibit 21, which is a short research presentation that you're listed as a coauthor on.
MARKED FOR IDENTIFICATION:
EXHIBIT 21
2:21 p.m.
BY MR. MILLS:
Q. Was this a study done through your clinic?
A. Yes.
Q. So on page 209, this table in the first block on the

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right under quote, it says, "A 17-year-old trans woman gave the quote, "I have lost 100 percent of my sex drive, all of it.""
Was this one of your patients?
A. I don't know who it was because it's a deidentified study.
Q. But all of these adolescents were recruited from your gender clinic?
A. There's seven physicians in our clinic so I don't know if I took care of this patient or not.
Q. But this was a patient in your clinic?
A. Yes.
Q. Did you have any follow-up indicating that this changed?
A. Again, this is a deidentified study so I don't know who this is.
Q. Have you seen this in other patients, trans female patients?
A. Diminishment in sex drive? Yes.
Q. Would you say that's common?
A. I would say it's not uncommon. Sometimes patients report, for example, diminishment in erections as a very positive finding, positive effect of hormone treatment.
It's something that I ask about when I'm

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treating trans feminine individuals, and if it is a problem, then it's something that we would discuss and potentially address.
Q. Are you familiar with Marci Bowers?
A. Yes.
Q. She is president of WPATH; is that right?
A. Yes.
Q. And she's one of the foremost surgeons in the field of gender transition, right?
A. She's a well-respected surgeon, I would agree with that.
Q. You said in your report that, "Uniformly, providers in this field are motivated by a desire to promote health and well-being in adolescents."
Would you say that about Dr. Bowers?
A. I don't know Dr. Bowers other than as the president of WPATH and a surgeon that I've heard of that is well-respected in the field, so beyond that I can't say.
Q. Well, your report says, "Uniformly, providers in this field are motivated by a desire to promote health," so I'm just wondering if that applies to Dr. Bowers.
A. I would think so, although Dr. Bowers isn't a pediatric endocrinologist. She doesn't do the type of care that we're discussing today.

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Q. Would you say that -- would you say that Dr. Laura Edwards-Leeper is motivated by a desire to promote health and well-being in adolescents?
A. I'd hope that anyone that's a licensed professional in any field is motivated to do good. To speak specifically about individuals, makes me uncomfortable.
Q. Would you say that about Dr. Paul Hruz?
A. I think that Dr. Hruz also has the best interests of children in mind and wouldn't disparage any person individually for any reason.
Q. And would you also agree that legislators in Alabama who voted this law are motivated by a desire to promote well-being in adolescents?
A. I would hope so, although my hope is that by listening to experts in the field that they would decide that their -- that their output in that regard falls short.
Q. You're not aware of any evidence, though, that legislators in Alabama who voted for this law were motivated by transgender animus?
A. No.
Q. I'm going to show you what I'm marking as Exhibit 22, which is an article in the Carolina Journal at Duke.
MARKED FOR IDENTIFICATION:
EXHIBIT 22

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2:26 p.m.
BY MR. MILLS:
Q. If we could go to page 3 of the article it says, "Bowers" -- the second paragraph, "Bowers seemed to acknowledge these challenges saying that, "Really about zero biological males who fought puberty at the typical Tanner 2 stage of puberty around 11 years old will ever go on to achieve an orgasm.""
Did I read that correctly?
MS. WILLIAMS: Have you had a chance to read this article?
A. (Witness shakes head in the negative.)
MR. MILLS: I'm not going to be asking about other parts of this article.
A. Yes, you read that correctly.
BY MR. MILLS:
Q. Is that consistent with your clinical experience?
A. No.
Q. What percentage of your biological male patients would you say who block puberty at the typical Tanner stage 2 go on to achieve an orgasm?
A. I don't -- I don't have a number for you, but just to explain why I said no, even prepubertal children can have that -- the rhythmic orgasm of the muscles of the phallus when exposed to stimulation, so I think that

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that -- I'm not sure if -- I'm not sure what the context of the conversation is, but I think that one thing that I do talk a lot about with patients is that the process of going through masculinizing puberty is important. It is -- in male adolescents the process of going through male puberty at a time where they explore their bodies in a different way than a trans girl would on pubertal suppression, and so the way that that person may choose to be intimate would be affected by pubertal suppression, and so those sort of -- those sort of topics are again something that I do spend time on talking about with patients and families considering pubertal suppression.
Q. Would you agree that most biological males who block puberty at Tanner stage 2 then progress to estrogen will never achieve an orgasm assuming they continued the estrogen?
A. I don't know.
Q. Do you tell biological males considering puberty blockers that you don't know the answer to that question?
A. I talk to them about the topic that I just discussed with you in a similar way to what I -- how I discussed it, but don't -- I don't -- I don't talk about orgasms specifically.

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Q. So you think Dr. Bowers is wrong?
A. I don't know the answer to that question other than to state that I believe that even prepubertal boys can achieve orgasm, and so I -- I don't -- I don't know what to say more than that.
Q. How often do prepubertal boys have orgasms? What percent of boys do you think experience that?
A. It would be a very low percentage. Remember that prepubertal boys don't have sex or interact with their genitals in a sexual way, the same way that an adult trans woman may learn to do.
Q. So if we set aside the very low percentage of boys who had prepubertal orgasms, would you then agree that Dr. Bowers is correct that the biological male who blocks puberty at Tanner stage 2 then progression to estrogen and continues estrogen will never achieve an orgasm?
MS. WILLIAMS: Objection.
A. I don't know the answer to that question.
BY MR. MILLS:
Q. I'm going to be showing you something which is marked as Exhibit 23, which is an article from the Free Press entitled, "Top 10 doctors blow the whistle on sloppy care."
MARKED FOR IDENTIFICATION:

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EXHIBIT 23
2:30 p.m.
BY MR. MILLS:
Q. I think we can go to page 5 of this article at the very bottom of the page of page 5.
A. Which part of page 5?
Q. Yeah, the very last part of page 5.
A. Okay.
Q. So I'll read it. "Bowers told me she now finds early puberty blockade inadvisable. I'm not a fan of blockade at Tanner 2, I really am not. She told me using the clinical name Deniliquin the first visible signs of puberty manifest, the idea all sounded good in the very beginning. She said, "Believe me we're doing some magnificent surgeries on these kids and they're so determined and I'm so proud of so many of them and their parents. They've been great, but honestly I can't sit here and tell you that they have better or even as good results. They're not as functional. I worry about their reproductive rights later. I worry about their sexual health later and ability to find intimacy.""
Do you disagree with Dr. Bowers?
A. I don't disagree. I think she's talking about sort of this process of what is Tanner 2. You know, if you

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say that the very first -- very first sign that a testicle has grown slightly larger as Tanner 2, that's not really allowing a child -- a young trans girl to have tangible evidence of secondary sex characteristics, so I wouldn't -- I would similarly not advise using blockers at the very first whiff of puberty, but that you really do need to experience some pubertal development in order to help that diagnostic pathway.

And what Dr. Bowers is saying is that the longer someone goes into puberty, she's feeling like there's better surgical outcomes, so that -- this is a topic that comes up when we're talking about the timing of starting GnRH agonists.

Q. So she says, "I'm not a fan of blockade at Tanner 2 anymore," but in the chart we looked at in your publication earlier, Tanner 2 is when you listed starting puberty blockers. So I guess I'm not seeing where she's redefining what Tanner 2 is.

Are you saying she's talking about a different stage than you're talking about?

A. Nope. I'm saying that these topics are something that we would talk about with patients when we're deciding when to intervene with GnRH agonists. So for some patients the progression past Tanner 2 would be so

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disruptive from a mental health standpoint that any of the advantages that Dr. Bowers is talking about would not outweigh the risk of waiting longer to intervene.

So just like all of the different topics that we've been talking about, the potential risks and benefits of GnRH agonist therapy, these are really important things to have conversations with patients and families about.

Q. So would you say that you are not a fan of blockade at Tanner 2?

A. I'm a fan of blockade at Tanner 2 if it's clinically indicated.

Q. And do you disagree with Dr. Bowers that patients who are blocked at Tanner 2 are not as functional?

A. I don't know what she means by that.

Q. I assume she means sexually functional; do you agree with her?

MS. WILLIAMS: Objection.

A. I do think that there could be benefit from a sexual function perspective to wait longer to block -- to use GnRH agonists, and from a gender dysphoria standpoint advantages to intervening sooner.

BY MR. MILLS:

Q. If we could go back to Exhibit 1, which was your article from Advances in Pediatrics. This is on page

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13 of Exhibit 1, and this is the last full paragraph on page 13, a sentence that starts with "While."

You say, "After a while," you say, "long-term health data is sparse with regards to adolescents."

Do you still agree that long-term health data is sparse with regards to adolescents on medical gender transition?

A. No. I think that since 2016 there's been quite a bit of literature outlining that type of data.

Q. So in the eight years since 2016, you think there is now long-term health data that is not sparse?

A. I think that there's -- there's long-term health data that I would not -- not classify as sparse.

Q. And which studies would those be?

A. I think the -- the -- the retrospective studies by Turban are an example of -- of longer-term data suggesting benefits of gender-affirming care for adolescents.

We have more longitudinal studies such as the Chen study outlining outcomes on gender-affirming hormones. Those -- those are examples.

Q. Do you agree that the Chen study goes up to two years after treatment initiation?

A. Yes.

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Q. Would you characterize two years after treatment initiation as long-term health data?

A. I don't think so.

Q. So Chen would not provide long-term health data?

A. I'll grant that.

Q. Psychotherapy poses no risk to fertility; is that right?

A. Correct.

Q. It poses no risk to ability to attain an orgasm?

A. I wouldn't think so.

Q. Psychotherapy poses no risk to breastfeeding capability?

A. No.

Q. It poses no risk to stature development?

A. No.

Q. It poses no risk to bone density?

A. No.

Q. It poses no risk to heart disease?

A. No.

Q. It poses no risk of blood clots?

A. No.

Q. It poses no risk of stroke?

A. No.

Q. It poses no risk of underdeveloped penile tissue?

A. No.

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Q. Are you aware of any studies showing that psychotherapy without medical interventions does not eliminate gender dysphoria?
A. Sorry, can you say that again?
Q. Sure. Are you aware of any study showing that psychotherapy without medical interventions does not alleviate gender dysphoria?
A. I think -- I'm not sure I can cite a study that's specifically answering that question, but the fact that patients have gender dysphoria despite psychotherapy would presume that conclusion.
Q. So in response to my question, you are not aware of any study showing that psychotherapy without medical interventions does not alleviate gender dysphoria?
A. I'm not aware of a study that takes a group of people with gender dysphoria, exposed them to psychotherapy alone, and then cures all their gender dysphoria, no.
Q. That wasn't my question. My question was, are you aware of any studies showing psychotherapy without medical interventions does not alleviate gender dysphoria?
A. No.
Q. When you started prescribing medical gender transition interventions in your current clinic, was that around 2017?

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A. 2015.
Q. 2015, okay. Sorry, just catching up.
So if we could go back to Exhibit 6, this was one of your articles entitled "Transgender and gender nonconforming adolescent care."
A. 6?
Q. That's right. This is page 2, the second paragraph under "Gender Identity," the second paragraph under "Gender identity."
The second to last sentence says, "Estimates for the likelihood of gender dysphoria persisting from childhood into adulthood range from 2 to 27 percent depending on the study."
You still agree with that statement?
A. I think this is a tricky one. I don't know that I agree with that statement because we're talking about using the term gender dysphoria to describe old studies that were using other definitions of children captured in their studies. So I -- I would agree that that range sounds accurate if you're asking me the percentage of children that express a difference in gender identity during childhood, how many of them are transgender adults, I think that range sounds accurate.
If you're saying how many people -- what

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percentage of people that currently meet the diagnostic criteria for gender dysphoria, I would posit that the percentage is higher.
Q. And by old studies using other definitions, do you mean like the DSM-IV or what are you referring to?
A. So some studies, some of this literature is using DSM-IV, gender identity disorder in childhood criteria. Some of the studies are using referred patients to mental health clinician for gender concerns. So the -- so the denominator is important when you're trying to understand the phenomenon of persisting gender identity. Fortunately, we don't have to make decisions about treatment in prepubertal youth so we can allow puberty to begin and help clarify things for us.
Q. But you agree that using the DSM-IV definition may alter the expected results from what you're seeing today under the DSM-5?
A. Well, I -- I don't know, but I think if we're using the term gender dysphoria to describe people that were diagnosed in a time that that term didn't exist, then we have to be careful.
Q. You're not aware of any updated studies along these lines analyzing persistence from childhood into adulthood using DSM-5 criteria of gender dysphoria?

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A. No.
Q. And you're not aware of any studies examining persistence from adolescents into adulthood using the DSM-5 definition of gender dysphoria, are you?
A. Well, we do have -- have studies examining the percentage of people that discontinue treatment, so I'm not sure if that answers your question.
You would assume that if someone is continuing on treatment they have persistence of their gender dysphoria or their gender identity and the high rate of continuation of treatment suggests a high rate of persistence.
Q. But you don't have any evidence outside of continuing medications in terms of showing persistence from adolescence into adulthood, correct?
A. I can't think of a study specifically asking that question.
Q. And in terms of the literature considering continuing interventions, you're not aware of any of that literature that controls for the use of medical gender transition and establishes the likelihood that adolescent gender dysphoria will persist into adulthood, are you?
MS. WILLIAMS: Objection.
A. I'm sorry, could you repeat that question?

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BY MR. MILLS:
Q. Sure. So you talked about the studies that examined continuation of using the interventions, and my -- my question is, are you aware of any literature that controls for using medical gender transition and establishes the likelihood that adolescent gender dysphoria will persist into adulthood?
A. No.
Q. In your clinic you don't track patients once they hit 18, do you?
A. Many of my patients are older than 18, so I tend to see patients until they're 21 or 22.
Q. You don't track people once they hit 22, then?
A. Patients that graduate from clinic and see adult providers, no.
Q. So you wouldn't know if any of those patients' gender dysphoria persisted past age 22?
A. I wouldn't know the percentage of patients, no.
Q. And most of your patients are on medical transition interventions; is that right?
A. Yes.
Q. And so you wouldn't know how many adolescent patients not on medical interventions would see their gender dysphoria resolve, do you?
A. Not from my own clinical experience. But I would say

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that I have seen many patients with gender dysphoria that for one reason or another were not able to access gender-affirming care and in follow-up those patients tended to have persistence of their gender dysphoria.
Q. Other providers in the United States didn't start this course of treatment for medical gender transition until around -- until after 2006; is that right?
A. I think that most pediatric gender clinics were not in place before that year, that's correct.
Q. You don't know if adolescents with gender dysphoria who do not receive medical interventions are likely to be transgender as adults, do you?
A. Say that one more time, please? Sorry.
Q. Yeah. You don't know if adolescents with gender dysphoria who do not receive medical interventions are likely to be transgender as adults, do you?
A. I do expect that transgender adolescents who do not receive medical interventions will continue to be transgender as adults.
Q. But you have no long-term data supporting that view?
A. Right. I can't point to a specific study taking a group of transgender adolescents that are not being offered treatment tracking them into adulthood, but we do have retrospective data from, for example, the US Transgender Survey exploring, you know, patients that

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currently identify as transgender and their experiences earlier in their life.
Q. So I'm going to show you what I've marked as Exhibit 24, which is an article entitled "Continuation of gender-affirming hormones among transgender adolescents and adults" by Roberts and others.
MARKED FOR IDENTIFICATION:
EXHIBIT 24
2:49 p.m.
BY MR. MILLS:
Q. This was published in the Journal of Clinical Endocrinology and Metabolism, right?
A. Yes.
Q. Are you familiar with this article?
A. I have seen it.
Q. So on page 2 in the second column, the first paragraph just before "methods" the second to last sentence, "In the current study, we assess the rate of treatment discontinuation after starting gender-affirming hormones among TGD adolescents." And then go over to page -- the next page. In the second column in the middle, the third sentence of the first full paragraph, "The four-year" -- oh, sorry, that's not the right sentence.
So there's a link to Figure 3 and then it

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says, "Patients who are younger than 18 years of age." Do you see that on the second column --
A. Yes.
Q. -- on that page?
Okay. And then the next sentence is, "The four-year continuation rate among people who started treatment under 18 years of age was 74.4 percent, and the rate among people who were greater than or equal to 18 years was 64.4 percent."
So this study found that over 25 percent of minor patients had discontinued hormonal therapy after only four years, correct?
A. First I'd just like to point out the sentence that you started to read and then stopped was just explaining that patients who were younger than 18 years of age when starting hormones were less likely to discontinue than patients who were 18 years or older, and I don't dispute the findings of this article.
I think that -- I think the way that the question is framed would suggest that all of the patients that stopped treatment stopped because they had a change in their gender identity, where I don't think that that is accurate that patients stopped treatment for a whole host of potential reasons.
Q. But you would agree that over 25 percent of patients

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in the study under 18 years old stopped --
A. Yes.
Q. -- treatment within four years?
And this study doesn't say what percentage of people may have stopped interventions later, does it, to your knowledge?
A. Later than what?
Q. Beyond four years.
A. No.
Q. Sorry, if you'll just give me one moment.
I'm going to show you an exhibit that I'm marking as Exhibit 25. It's an article that you cite in your report by van der Loos and others, "Continuation of gender-affirming hormones."
MARKED FOR IDENTIFICATION:
EXHIBIT 25
2:53 p.m.
BY MR. MILLS:
Q. Do you recognize this article?
A. Yes.
Q. So if we go to page 872, the E of the first paragraph under "Results" it says, "Overall 282, 59 percent of all 480 eligible, i.e., minimum age of 18 years and at least one year of gender-affirming hormone treatment participants, had gonadectomy."

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So 59 percent of the participants in this study had their sexual organs removed, correct?
A. Yes.
Q. And after that removal, are individuals supposed to continue hormonal therapy?
A. Yes. After gonadectomy, some sex hormone is important for the body's health.
Q. So for 59 percent of these patients, 59 percent of these study participants, they were medically required to continue hormonal therapy, correct?
A. Well, I don't -- I think I'd have to reread the article about how old these people were. I think there's some controversy about how long to continue sex hormones in older people.
This is also in Europe where the rates of gonadectomy are lower in the United States, but, yes, people that generally have gonadectomy benefit from continuing to have sex-hormone exposure in their body usually in the form of testosterone and estrogen replacement therapy.
Q. All right. If we could go back to Exhibit 19, which was part of your book chapters on the duration of pubertal suppression. This is page 76, and I'm under "Endocrine clinical practice guidelines" the second column, and I'm at the bottom of the second paragraph

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under that section.
You say, "There has been limited literature published on treating patients prior to 13.5/14 years of age."
Do you still agree with that statement?
A. Yes. This is referring to gender-affirming hormone treatment.
Q. The next sentence, "Rigorous" -- actually -- oh, so you're talking these the Endocrine Society guidelines. You say, "These guidelines also note that rigorous study and evaluation is needed to determine the effects of prolonged pubertal delay on bones, gonads, and brain development."
Do you agree with the guideline's note on those issues?
A. Yeah, so, I mean, I think we're like quoting me quoting the guidelines, so I guess if you want me to agree to something specific in the guidelines, I'd like to see the guidelines. I agree with this sentence as I wrote it.
Q. So I guess I would say, do you agree that rigorous study and evaluation is needed to determine the effects of prolonged pubertal delay on bones, gonads, and brain development?
A. I think that I would certainly welcome more study on

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long-term outcomes in these areas on long-term pubertal suppression, but given that we do have -- we do have evidence to inform us on how GnRH agonists do interplay with these things and use that to make informed decisions with patients on GnRH agonists use today.
Q. Would you say that evidence is rigorous?
A. Well, I would, for example, say we talked about bone density studies in some detail today, I would call those studies rigorous.
Q. Including the one that found no full catchup by age 22?
A. Right. So that's data that we can now use to discuss with patients the potential risks and benefits of GnRH agonists and determine length of treatment.
Q. So if you go back a page to page 75 here, this is near the bottom of the second column where we're talking about WPATH guidelines, it's right after you say number 1 starting puberty suppression, and two starting sex therapy.
The next sentence is, "Puberty suppressing hormone eligibility may begin as soon as adolescents have the onset of puberty to Tanner stage 2 which they note may occur as early as nine years of age, although it is stated that the evaluation of this approach has

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only been studied for adolescents who are at least 12 years old."

Would you agree that the evaluation of this approach has only been studied for adolescents who are at least 12 years old?

A. No. The -- the original Dutch protocol involved pubertal suppressions at 12 or Tanner stage 2, so that's where that sentence comes from, and I -- I'd have to look at the articles, but I do believe more contemporary research related to GnRH agonists includes folks younger than 12, but I'd have to -- I'd have to look to make sure.

Q. You're not aware of any literature that specifically considers patients who started puberty blockers before age 12?

A. So again, I'd like to look at individual studies to be sure. Like if we're -- if we're -- if we're thinking about, like, the Chen study, for example, the study involved gender-affirming hormones, but many of those children were treated with GnHR agonists prior to starting hormones, and I believe that many of them were younger than age 12. So I don't have -- I don't have a citation off the cuff, but I no longer think that this is accurate, but don't have -- don't have something more definitive to say.

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Q. You mentioned the Dutch studies. Are you saying that some of those children were under the age of 12 when they started puberty blockers in the Dutch protocol?

A. No. I think I was saying that the original Dutch protocol I think as it was worded was using age cutoffs instead of pubertal staging as their primary decision point.

Q. Yeah, got it.

So if you flip over to page 77, the bottom of the first column about three sentences up, "However, the published guidelines offer less nuance and guidance around topics commonly encountered when treating transgender youth. For example, if GnRH agonists are started in early puberty, when should they be discontinued, especially if gonadectomy is not practical or desired."

Do you agree that the current guidelines are still lacking on that question?

A. I think that gender medicine is very nuanced because everyone is an individual with individual goals and needs, so to protocol-ise gender-affirming care is really challenging.

So I agree that, you know, a protocol doesn't contain the nuance of -- of the character of the types of conversations and decisions that

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clinicians face every day, but you use the tenets of the standards of care and clinical practice guidelines in practicing medicine with -- with actual real live people every day, using those tools in your toolkit to understand what is potentially the best next step for each person.

Q. So these -- the guidelines for medical gender transition differ from the guidelines that you would use for something like precocious puberty, correct?

A. I think there's nuance there too because, you know, I think when I'm seeing a patient with precocious puberty, the decision to start treatment is not straightforward. You're balancing things like the importance of height, what the height prediction is, what the parent's heights are, what the social -- social or emotional challenges a young person might face going through precocious puberty, and so, no, a simple protocol to practice medicine doesn't work. That's why doctors are people and not robots.

Q. So the next sentence here is, "What about the large percentage of adolescents seeking medical care well after the onset of puberty or GnRH agonists helpful for these patients?"

You agree that the published guidelines still do not offer much guidance on that question?

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A. I think that's one of the reasons that I wrote this chapter, right, because the -- the -- you know, the Endocrine Society guidelines and WPATH Standards of Care again provide that framework, but then in the real world a patient comes in, you know, after Tanner stage 2 and we have the same conversations like we -- like we had before about what would GnRH agonists do, what wouldn't they do, what are your goals, what's the source of distress, and so, no, I don't think that the guidelines speak to that to the degree that clinicians see it in practice.

Q. And then the next sentence, "If so, should GnRH agonists be considered for adult transgender patients presenting for care?"

And then you say, "While peer-reviewed studies attempting to tackle these questions are sparse, we've attempted to guide the reader through the various situations."

You agree today that peer-reviewed studies on those questions are sparse?

A. Yeah, those specific scenarios I would agree.

Q. And then the last sentence in that paragraph is, "In writing this section, we have relied on personal clinical experience, input from other experts in the field, published clinical guidance, and the limited

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available data on medical treatment and outcomes for transgender individuals."

You still agree that there is limited available data on medical treatment and outcomes for transgender individuals?

A. As I -- as I outlined in my reports, there is literature outlining safety and efficacy and I would not currently categorize that as limited.

Q. So you disagree with what you previously wrote?

A. I would say that today the -- I would not describe the available literature as limited.

Q. So you think in the four years since 2019 the available data has gone from limited to sufficient?

MS. WILLIAMS: Objection.

A. Well, I think -- I think that when I wrote this article and used the word limited, I felt that the literature was sufficient to use these interventions at that time, so I think that the -- the body of literature was sufficient then and now and, no, I would not use the word limited today.

BY MR. MILLS:

Q. Even though you cannot point to any long-term outcome studies that examine any period longer past the age of 22?

A. Since the publication of this article, correct.

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Q. So what is your basis for changing your position?

A. I think it -- I think it has to do with whether -- how we're using the word limited. You know, I think I'm using the word limited in this paper in the -- in the framework of like most authors do in writing a paper calling for more literature on a subject, but not in a way that means limited as in not enough to proceed with care.

Q. The Standards of Care 8 say, "The long-term effects of gender-affirming treatments initiated in adolescence are not fully known."

Do you agree with that statement?

A. Sorry, this is from WPATH Standards of Care 8?

Q. Mm-hmm.

A. Could you read it again?

Q. "The long-term effects of gender-affirming treatments initiated in adolescents are not fully known."

MS. WILLIAMS: I'm sorry. Are you going to be asking him about things from the SOC8?

MR. MILLS: Just about this statement.

A. Okay, so you want me to answer whether I agree with that statement?

BY MR. MILLS:

Q. Mm-hmm.

A. Not fully known, I think that I can support that.

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Q. Okay. Well, I guess we'll look at Standards of Care 8 for a minute.

You're familiar with Standards of Care 8?

A. Yes.

Q. And do you regularly consult it in your practice?

A. I read it enough now that I don't reconsult it, but yes.

Q. I will have that marked as Exhibit 26.

MARKED FOR IDENTIFICATION:
EXHIBIT 26
3:10 p.m.

BY MR. MILLS:

Q. WPATH Standards of Care 8, and this is largely just the adolescent chapter.

If you could flip to page S46, and the first column, the end of that initial paragraph, on the third sentence up from the end of that first paragraph, "Despite the slowly growing body of evidence supporting the effectiveness of early medical intervention, the number of studies is still low and there are few outcome studies that follow youth into adulthood." WPATH wrote this in 2022.

Do you disagree that the number of outcome studies is still low?

A. I think that -- that given the fact that the treatment

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pathway that we've been talking about has only existed since the 1990s naturally up comes data into older adulthood is low.

Q. It also says, "The number of studies is still low."

Do you see that?

A. Yes.

Q. And do you agree with that statement?

A. I think that compared to other areas of medicine, the number of studies is low yet sufficient to endorse the practice -- practice care that -- the care outlined in WPATH's standards.

Q. Earlier you said that between 2019 and 2023 the evidence became no longer limited.

Do you disagree with WPATH that there's a slowly growing body of evidence?

A. No.

Q. The next sentence is, "Therefore, a systematic review regarding outcomes of treatments in adolescence is not possible."

Do you agree with WPATH on that point?

A. I don't know if I would have agreed that a systematic review is not possible at the time of this writing. I -- but I don't have a reason to disagree. I didn't attempt to conduct a systematic review at that time.

Q. So do you believe that a systematic review regarding

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outcomes of treatments in adolescents is possible now?
A. I don't know.
Q. Are you aware of any systematic reviews regarding outcomes of treatments in adolescents?
A. I know that there have been attempts at systematic reviews around various topics in -- in this field, some about pubertal suppression, some about the care in general, yes.
Q. So if we go down a little bit in that column, the second to last sentence it's referring to the de Vries study in 2014.
"The 2014 long-term follow-up study is the only study that followed youth from early adolescence pretreatment mean age of 13.6 through young adulthood posttreatment mean age of 20.7."
Are you aware of any -- first, do you agree that when this was published in 2022 that 2014 study was the only study that had a long-term follow-up?
A. Yes.
Q. And are you aware of any new studies since SOC8 was published that had long-term follow-up?
A. I'm not. I think that the -- the evidence supporting gender-affirming care comes from long-term studies like the ones that we're talking about now, also retrospective data and cohort-type data.

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Q. All right. The WPATH Standards of Care 8 deviates from the Dutch approach used in the de Vries 2014 study because it doesn't prescribe age cutoffs; is that right?
A. Yes.
Q. So the Dutch protocol used age cutoffs at age 16 for cross-sex hormones; is that right?
A. Yes.
Q. And you typically give cross-sex hormones closer to age 14?
A. Who me?
Q. Mm-hmm.
A. Not necessarily. I think that I do have patients that are 14 that have been good candidates for hormones and others that it made more sense to wait until an older age.
Q. So if the Dutch study provides the only long-term outcomes study, there is no long-term study about the use of gender -- medical gender transition that WPATH guidelines prescribe, is there?
A. I'm not sure that there's long-term studies of patients following the -- what is this, 2008?
Q. '22.
A. No, sorry, 2022 model of care, no.
MR. MILLS: All right. This is probably a

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good time for a ten-minute break, if that works for everybody. We can go off.
(Recess taken at 3:16 p.m.)
(On the record at 3:26 p.m.)
BY MR. MILLS:
Q. So, Dr. Shumer, I'm going to show you another clip of Dr. Selkie speaking with you in the presentation we talked about earlier.
(Video played.)
BY MR. MILLS:
Q. Do you agree with Dr. Selkie that there is not as much evidence for medical gender transition as there is for other treatments for children?
A. First I just want to point out that that was like a four-second clip of a -- I don't know what. She said "but" and then it trailed off, so I would be interested to know what she said afterwards. But I would also add that, yes, there are certainly treatments that we use in pediatrics that have been around for decades, and naturally if a modality of treatment has only been around for a couple decades there's going to be less long-term outcomes data on that particular intervention, so clearly that's true.
I'd just like to point out, though, that this is the case with all advances in medicine. When

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a new -- when a new treatment for significant medical condition emerges and there's significantly improved -- significant improvement in whatever condition it is you're treating, then you -- you note that there's not going to be, you know, decades-long outcomes data and use that information when understanding whether this new treatment modality might be beneficial.
Q. So there's less evidence supporting medical gender transition of adolescents than there would be, for example, about protruding precocious puberty?
A. I think those are really difficult to compare because people have been treated for precocious puberty for longer using GnRH agonists. The outcomes that you're measuring for precocious puberty are perhaps simpler to -- to measure; you know, final height, for example, or onset of the first period.
The outcomes that you're attempting to measure when assessing treatments for gender dysphoria are more challenging to measure, quality of life measures, and -- and so I'm not sure if I would agree that there's more articles published about the treatment of precocious puberty.
There's certainly a lot of articles published about transgender medicine, but patients have been treated for longer for that condition for

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sure.
Q. So would you say that the evidence base supporting medical gender transition of adolescents is greater or less than the evidence base supporting treatments of precocious puberty?
A. I don't know.
Q. I'd like to show you one more clip, if I could, from the same presentation.
(Video played.)
BY MR. MILLS:
Q. Do you agree with Dr. Selkie that we don't have good evidence about the long-term risks for young healthy people who start medical gender transition in adolescence?
A. I don't think that's the way I would describe the current state of the literature. I think that we have a lot of knowledge about the long-term effects of having a normal male hormone profile, for example, or normal female hormone profile, for example. We don't have decades-long studies demonstrating that the -- the long-term outcomes for certain health problems are identical to those that are seen in other people with those same hormone profiles, but we also have shorter term research to help demonstrate that we would expect those long-term outcomes data to be reassuring.

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Q. So I guess I'm not clear. Do you agree with her or not that we really don't have good -- good evidence about the long-term risks for young healthy people who start medical gender transition in adolescence?
MS. WILLIAMS: Objection.
A. We certainly don't have longitudinal follow-up studies of patients that had these treatments that are now living in their sixties and seventies. That would be -- that's the type of research that we're developing now, but we do have sufficient literature on the effects of how these medications work and their side effect profile to have meaningful conversations about risks and benefits and prescribe them when appropriate.
BY MR. MILLS:
Q. And you know from studies like the VTE one that we talked about earlier today that the risk profile could vary based on use in transgender individuals, correct?
A. Yes, so we reviewed that I agree, yeah.
Q. So back to Exhibit 19, which is the book chapter we were talking about I think just before SOC8, the duration of pubertal suppression.
A. 19 you said?
Q. That's right. And I'm on page 83, and this is the second column the end of the first full paragraph just

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before the heading that says "Testosterone."
You wrote, "However, prior to the accrual of long-term data, providers should be cautious when starting gender-affirming hormones in early adolescence."
Do you still agree with that statement?
A. Yes. I'm cautious when prescribing hormones in all situations, but especially in early adolescence.
Q. I'd like to show you an exhibit -- let's see where are we -- Exhibit 29, which is an article you wrote, you coauthored, entitled "The role of ascent in the treatment of transgender adolescents."
MARKED FOR IDENTIFICATION:
EXHIBIT 29
3:34 p.m.
BY MR. MILLS:
Q. And I'm on page 5 first full paragraph.
So you say, "There may be clinical situations where patients with carefully diagnosed gender dysphoria who otherwise meet eligibility and readiness criteria are not able to provide meaningful consent due to cognitive or verbal disability. In other medical conditions such as cancer or diabetes, medical interventions would never be withheld from these patients provided parents or guardians are

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available to make proxy medical decisions. This comparison requires acknowledgment that treatment of gender dysphoria with pubertal suppression in cross-sex hormones continues to remain controversial is the subject of continued research and requires careful individualized assessment, whereas the decision to treat of cancer of diabetes with medical interventions is typically not controversial."
You wrote this or you coauthored this article, correct?
A. Yeah. In 2015, yes.
Q. And do you still agree with the passage that I just read?
A. I generally agree, although I would also say that, you know, because gender identity is something expressed by the patient and that diabetes and cancer are more easily measured without the patient's cognitive participation, those are -- that's another difference making decisionmaking around gender dysphoria more complicated than diabetes or cancer.
Q. Sure. But do you also agree that medical gender transition is different from treatment for cancer because of what you say here, it is the subject of continued research?
A. I think both are the subject of continued research.

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Q. So do you no longer agree that medical gender transition is different from treating conditions like cancer or diabetes?
A. I just outlined one reason, one way that it's different. I don't think that they're the same, but being the subject of continued research is not a difference.
Q. Do you think the evidence base for diabetes treatment is greater or less than the evidence base for medical gender transition in adolescents?
A. It depends on what aspect of diabetes treatment.
Q. So you no longer think that the difference in research distinguishes medical interventions for gender dysphoria from cancer or diabetes?
A. I don't think that's what I said.
Q. Well, you said is the subject of continued research makes it different from cancer then. Now you're saying it's no longer different?
A. I'm not saying -- I'm not saying that. So if we read the whole paragraph again, you know, I'm saying that there's -- the point here is that -- that ascent is important in the treatment of transgender youth. Whereas, when youth aren't able to provide ascent in cancer and diabetes, you would still proceed anyway. That wouldn't be advisable in -- in -- in someone with

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gender dysphoria.
Do I think that this area of medicine is controversial? Clearly, because we're meeting here today to talk about it. Do I think that gender medicine is the subject of continued research? Absolutely. There are certain tenets of diabetes care that are better researched than elements of gender dysphoria. You know, new medicines to treat type 2 diabetes in children like Ozempic and Victoza, you know, are just now getting studied.
So we're always learning in medicine and we're always trying to advance care to make patients healthier, but the crux of this paragraph is really just that meaningful ascent is really important in -- when working with gender diverse youth.
Q. You would say there is no difference between the evidence base of your day-to-day treatment of diabetes for patients in your clinic as there is of treatment for your gender dysphoria patients?
MS. WILLIAMS: Objection.
A. I wouldn't say that.
BY MR. MILLS:
Q. Which one would you say is supported by greater evidence?
A. You know, I think -- I think that the question doesn't

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make sense without context. So if you're asking me is there greater evidence that insulin will keep you alive when you have type 1 diabetes or --
Q. Sure.
A. -- should we use GnRH agonists, then, yes, there's more evidence that insulin will keep you alive if you have type 1 diabetes.
Q. And that medicine was used before 2006, correct?
A. Yes.
Q. So you would say the medical gender transition of adolescents is a newer field of medicine than using insulin to treat type 1 diabetes?
A. Yes.
Q. If a patient with type 1 diabetes is unable to provide consent and doesn't want insulin, should the patient still get it?
A. Yes.
Q. Why is that?
A. Because there is a clear cause and effect between getting the insulin and living and -- and -- and so we would figure out a way for that child to get treatment with insulin.
Q. If a patient with gender dysphoria does not want medical interventions, that patient would not receive it, correct?

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A. Correct.
Q. And why is it different?
A. In -- in lots of different ways. There isn't a clear -- in the same way that no insulin equals dying, yes, insulin equals living. The conversation around the potential risks and benefits using treatments for gender dysphoria is much more nuanced and involves consideration of personal values and attitudes on gender, your gender identity, how it's affecting you on a day-to-day, so it's -- it's a more complicated decision that requires patient involvement and input to determine what the best course of treatment is.
Q. And if a patient with gender dysphoria wants medical interventions, that patient would ordinarily receive them?
A. There's certainly situations where a patient may want an intervention, but doesn't meet criteria to receive it, so wanting it by itself is not sufficient.
Q. I'm going to show you what I'm marking as Exhibit 30, which is labeled, "Metaanalysis hormone therapy, mental health, and quality of life among transgender people, a systematic review."
MARKED FOR IDENTIFICATION:
EXHIBIT 30
3:42 p.m.

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BY MR. MILLS:
Q. And this was a systematic review conducted prior to SOC8 funded by WPATH.
Are you familiar with this document?
A. I have seen it, yes.
Q. Okay. So page 1 of the abstract says, "We sought to systematically review the effect of gender-affirming hormone therapy on psychological outcomes among transgender people."
Page 2 under "Search Strategy" it says, "This review is one of a series of systematic reviews conducted for WPATH to inform the 8th revision of the standards of care." If you want to see on page 13, it says funded by WPATH, but it's not important to my questions.
Page 12 the end of the first full paragraph under the "Discussion" it says, "It was impossible to draw conclusions" --
MS. WILLIAMS: I'm sorry, where are you?
MR. MILLS: Page 12 the end of the first full paragraph under "Discussion."
MS. WILLIAMS: After Table 6?
MR. MILLS: That's right.
BY MR. MILLS:
Q. "It was impossible to draw conclusions about the

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effects of hormone therapy on death by suicide."
Do you agree that it's impossible to draw conclusions about the effects of hormone therapy on death by suicide?
A. I don't dispute that the totality of literature isn't adequate in addressing that question. I'd also point out the other finding that wasn't read which demonstrates improvements in quality of life and decrease in depression and anxiety symptoms among transgender people.
So while I think that it is seemingly hard to draw conclusions about death by suicide, the -- the improvements in other areas of mental health are notable and I would -- I would hypothesize that people with improved quality of life, decreased depression and anxiety symptoms are less likely to die by suicide. However, I agree that the literature can't currently answer that question.
Q. So on that other literature the next paragraph begins, "Uncontrolled confounding was a major limitation in this literature. Many studies simultaneously assess different types of gender-affirming care and did not control for gender-affirming surgery status making it difficult to isolate the effects of hormone therapy."
Do you agree that uncontrolled confounding

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is a major limitation in the medical gender transition of minors literature?
A. I think it's a limitation and I think it's important to understand that gender identity care for people, for adolescents specifically, is a challenging thing to measure without any confounding. That, you know, what is confounding? If you have -- if you have a new penicillin and you're comparing it to the old penicillin, you can put a bacteria in a culture dish and put another one in a different culture dish and everything else is the same and just introduce the two penicillins and see which bacteria resolves faster, and there's not a lot of confounding because everything else in that experiment was exactly the same.
But when you're talking about comparing adolescents receiving gender-affirming care in Boston, in LA, in Chicago, and San Francisco, seeing different providers, having different sociopolitical environments, those things can confound results, and this is certainly not unique to gender-affirming care, but a problem with measuring all sorts of different complex care modalities.
Q. So the next paragraph, the third paragraph under "Discussion" says, "Another source of potential bias

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was recruitment of participants from specialized clinics that imposed strict diagnostic criteria as a prerequisite for gender-affirming care. The dual role of clinicians and researchers as both gatekeepers and investigators may force transgender study participants to over- or understate aspects of their mental health in order to access gender-affirming care."
Do you agree that that's another source of potential bias?
A. Potentially. If I was reading any article outlining outcomes of gender-affirming care, I would be interested to know how patients were recruited, what the modality of care was at that institution in order to better understand if the patients in that study were similar to the patients that I treat.
Q. You mentioned a minute ago evidence regarding quality of life, depression and anxiety. If you look at Table 6 on page 13 it lists outcome, quality of life, depression, anxiety, death by suicide as the four outcomes. Under strength of evidence it lists low for qualify for the quality of life, low for depression, low for anxiety, and insufficient for death by suicide. And the footnote E connected to low says, "Evidence downgraded due to study limitations included uncontrolled confounding and imprecision because of

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small sample sizes."

Do you agree that the strength of evidence for quality of life, depression, and anxiety outcomes are all low?

A. So according to the definition as presented, I would. I would also just warn that when you hear something like the strength of evidence is low, that doesn't mean that the evidence is bad or poor or incorrect.

And also just to point out that when you're talking about quality of life, another alternative would be worse quality of life as an outcome. So the fact is that in a systematic review there was findings of improved quality of life for patients that are receiving gender-affirming care categorized as low strength based on the criteria as presented, and I don't disagree with that.

Q. And you would agree low strength of evidence means that -- relative to high strength of evidence, low strength of evidence means that it's more likely that the actual effect is different from what the study found, right?

A. I agree based on the things that we've been talking about. The petri dish example, the only logical conclusion of the difference in clearing the bacteria is that the antibiotic worked better or worse than

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penicillin.

When there's potential confounding in a complex medical problem, the ability to be certain about whether the intervention is the cause of the change is more limited, similarly to the strength of evidence supporting many complex health -- health treatment modalities.

Q. So on page 13 the bottom of the page, the new paragraph that begins at the bottom of the first column of the page, actually specifically the very last sentence in the first column, "Studies assessing the relationship between gender-affirming hormone therapy and mental health outcomes in transgender populations should be prospective or use strong quasiexperimental designs, consistently report type, dose of hormone therapy, adjust for possible confounding by gender-affirming surgery status, control for other variables that may independently influence psychological outcomes, and report results separately by gender identity."

This isn't necessarily describing a randomized controlled trial, correct?

A. Correct.

Q. But it is explaining a higher strength of evidence study design than currently exists, correct?

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A. I think that it's preventing guidance on the type of studies that would be required to strengthen the statements made in this report.

Perhaps I -- perhaps some studies that currently exist meet some of these criteria, but it's, you know, similarly to the end of most scientific articles prescribing next steps to better understand the problem at hand.

Q. But you would agree that at least according to these authors there are study designs short of randomized controlled trials that would be higher quality than the ones they've examined?

A. Yes. For example, the Chen study is a prospective study that was published after this systematic review.

Q. And do you think the Chen study is a high quality study design?

A. I find it to be very helpful to me in my practice because the type of care that's described in the Chen study is similar to the type of care that I practice, and so I would.

Q. And you agreed earlier that the Chen study doesn't have data or conclusions beyond two years from starting cross-sex hormones, right?

A. Correct.

Q. So we can look at the Chen study for a minute. So I'm

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marking the Chen study as Exhibit 31, and you're obviously familiar with it; it's what we've been discussing.

MARKED FOR IDENTIFICATION:
EXHIBIT 31
3:54 p.m.

BY MR. MILLS:

Q. So on page 241, the second page of the article in the middle of the first column at the end of that second paragraph it says, "Evidence has been lacking from longitudinal studies that explore potential mechanisms by which gender-affirming medical care affects gender dysphoria and subsequent well-being."

This -- this study was published in 2023; is that right?

A. Yes.

Q. So would you agree with the authors that in 2023 evidence has been lacking from longitudinal studies that explore potential mechanisms by which gender-affirming medical care affects gender dysphoria and subsequent well-being?

A. There was limited longitudinal studies on this topic prior. I think that was mentioned in the metaanalysis that we just read, and so this is an attempt to expand that literature.

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Q. So on page 242 under the results, this is the second column, it lists that there were 6,114 observations from 315 participants, and it says there were five study visits and 162 participants completed all five study visits.

So about 50 percent completed each of the five study visit questionnaires; is that right?

A. That seems to be what they're saying.

Q. On page 243 in the middle of the second column, three sentences up from the "Appearance Congruence" heading it says, "Two participants died by suicide during the study, one after six months of follow-up and the other after 12 months of follow-up."

So those two individuals could not complete a study visit at 18 or 24 months, right?

A. That's correct.

Q. And two suicides out of 315 participants implies a .6 percent suicide rate; is that right?

A. I don't know. I can do the math with you again. Can you give me those numbers?

Q. It's 2 out of 315, so roughly .6 percent --

A. Okay.

Q. -- does that sound right?

A. Yes.

Q. And that's substantially higher than the adolescent

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suicide rate in the United States generally; is that right?

A. I -- I would be cautious about implying that -- that the -- this represents an actual rate of suicide when you're -- you know, when you're -- if you're using the statistics to say what would be the expected suicide rate if the study were replicated, the -- the range of possible based on the sample size would be quite broad, so I don't think this study is able to say that suicide is more likely as a result of gender-affirming care, but I do agree that .6 percent is higher than the suicide rate in the United States.

Q. So over on page 244 on the table there, Table 1, do you see near the bottom of Table 1 it says, past use of GnRH agonists no was 92.1 percent of participants? So 92.1 percent of the participants had not received puberty blockers; is that right?

A. Yes.

Q. And is that a higher percentage of patients than would not have received puberty blockers in your clinic?

A. As I said, the majority of patients are presenting older than -- than Tanner stage 2. I -- so I think that the percentage of patients that are treated with GnRH agonists is likely higher than the study, but not substantially higher let's say.

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Q. But if they were not treated using the puberty blocker, then is it safe to say that most of these participants went through puberty aligned with their biological sex?

A. Well, we can see exactly how many did based on these numbers.

92 percent of people went through at least some puberty aligned with their biologic sex.

Q. Page 241 the top of the first column, the very first full sentence, "Depression and anxiety symptoms decreased significantly and life satisfaction increased significantly among youth designated female at birth, but not among those designated male at birth."

So biological males saw no improvement in depression, anxiety, or life satisfaction; is that right?

MS. WILLIAMS: Objection.

A. I'm just going to back up for a second to read the beginning of the paragraph.

BY MR. MILLS:

Q. Sure.

A. Okay, I'm with you.

So, yes, during the -- during the course of this study, statistically significant differences in

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depression and anxiety and life satisfaction variables specifically were statistically significantly better in those designated female at birth compared to male at birth, and then the authors continue on to discuss that in more detail.

Q. So in this study, biological males did not see statistically significant improvement in depression, anxiety, or life satisfaction, correct?

A. Yes.

Q. Over on page 247, sorry, 249, the first full sentence on 249 it says, "Finally, our study lacked a comparison group which limits our ability to establish causality."

Do you agree with that statement?

A. Yes.

MARKED FOR IDENTIFICATION:
EXHIBIT 32
4:02 p.m.

BY MR. MILLS:

Q. I'm going to show you what I've marked as Exhibit 32, which I believe you cite in your rebuttal report a commentary by de Vries and others on the Chen paper. This is called "Growing evidence and remaining questions in adolescent transgender care."

On page 276, which is the second page, the

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first column in the middle, and I'm three sentences down from -- let's see. This long paragraph in the middle I'm on the one, two, three, fourth sentence, starts with, "However, other possible determinants of outcomes were not reported, particularly the extent of mental healthcare provided throughout GAH treatment."

So you agree that the Chen study did not control for psychological therapy, correct?

A. Correct.

Q. And it did not control for use of other psychiatric medications?

A. I don't believe so.

Q. So the study cannot exclude the possibilities that psychological therapy or other psychiatric medications could account for any positive change?

A. That's correct.

Q. And the study also does not -- the Chen study also does not control for the fact that testosterone may have mood elevating effects?

A. Right. The reader for this prospective study, just like any prospective study, has to think critically about what the intervention was, what the outcomes are, think about these potential confounders, and then draw conclusions.

Q. So the next sentence, "To date, international

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guidelines for transgender adolescent care recommend a psychosocial assessment and involvement of mental health professionals in a multidisciplinary care model. Whether participating centers in the current study followed that approach is, unfortunately, unclear. Future studies that compare outcomes with different care models are needed preferably using similar results."

Do you agree with that statement?

MS. WILLIAMS: I think it said "similar measures."

MR. MILLS: Oh, I'm sorry, "similar measures," yep.

A. I don't -- I don't know that I agree completely because I'm -- I know the centers that conducted the study, and they are centers that have a psychological assessment and involve mental health professionals in a multidisciplinary care model, so whether it was unclear in the article, it's clear to me that those -- that the clinics that did this, that performed this study meet those criteria.

BY MR. MILLS:

Q. Okay. But you would agree that future studies that compare outcomes with different care models are needed?

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A. Yes.

MARKED FOR IDENTIFICATION:
EXHIBIT 33
4:05 p.m.

BY MR. MILLS:

Q. I'm going to show you what I'm marking as Exhibit 33, which is the protocol submitted for the Chen study.

Are you familiar generally with these types of prestudy protocols?

A. I suppose I am.

Q. Yeah?

A. Yes.

Q. Okay. So page 34, and the pagination skips ahead so it's only on like page 5 or so. The one, two, third sentence says, "The MANOVA analyses will investigate the changes over time in gender dysphoria, depression, anxiety, trauma symptoms, self-injury, suicidality, body esteem, and quality of life."

So the protocol proposes these eight measures to study; is that right?

MS. WILLIAMS: Objection. Do you need to read this or do you need more time to answer?

A. I can answer --

MS. WILLIAMS: Okay.

A. -- that question.

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MS. WILLIAMS: Go ahead.

A. It does.

BY MR. MILLS:

Q. Okay. And flipping to page 43, the table there explains the measure that will be used for each of those -- or the surveys that will be used for each of those measures; is that right?

A. Yes.

Q. So if we go back to the Chen study on page 242, and this is Exhibit 31, and you look at the second paragraph under "measures," at the end of that paragraph it says, "Higher scores on these measures reflect greater appearance congruence, depression, anxiety, positive effect, and life satisfaction respectively."

So this study didn't report on the effects on gender dysphoria, did it?

A. I'm not sure. I'd have to go through and see if that is mentioned or not, but I don't see it in that statement right there.

I think that I -- I think that the -- you know, the implication is that there's -- you know, when a study is trying to measure lots of things, that they may only be publishing the most, you know, positive sounding material.

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You know, in talking to some of the investigators that wrote this paper, I know there was constraints on word limits and such that they certainly would have been happy to present every piece of information, and that information is available, but that the goal of the journal article in the New England Journal of Medicine was to present the most, you know, important or groundbreaking material.

So the fact that every measure isn't documented in this journal article may be true, but also not something that the authors are hiding from.

Q. Did the authors explain to you why they've refused to release the data for these other variables?

A. I don't know anything about releasing or not releasing the data.

Q. That didn't come up in conversation with them?

A. No.

Q. Would you consider it relevant to your treatments whether gender-affirming care helps alleviate gender dysphoria?

A. Yes.

Q. But this study didn't provide any evidence on that measure, did it?

A. Not that I can see right now. It provides evidence based on the outcome measures that we've reviewed.

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Q. They also omitted results going back to those original eight categories on trauma symptoms, self-injury, suicidality, body esteem, and quality of life, correct?

A. Can you point to me where you're at so I can --

Q. Sure. This was in the protocol, those eight --

A. Okay.

Q. -- measures on page 34. Page 34 the middle of the first paragraph, "The analysis will investigate the changes over time for depression, anxiety, trauma symptoms, self-injury, suicidality, body esteem, and quality of life."

A. Okay, yep.

Q. So they omitted results on six of those eight proposed measures, correct?

A. I don't know that to be correct. I would need time to cross-tabulate and -- but certainly not everything that is offered up in this protocol is reproduced in the manuscript.

Q. So the manuscript lists depression, anxiety, appearance and congruence, positive effect, and life satisfaction, so the only -- of the eight on the protocol, only depression and anxiety are among the ones that are actually reported, correct?

A. Unless there's other mentions in other places that we're omitting, that would be correct.

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Q. And to your knowledge, the authors haven't provided this data regarding those variables for public analyzes, have they?

A. I don't have information about that.

Q. You haven't seen the data?

A. No.

Q. The authors would have no reason to hide positive results, would they?

MS. WILLIAMS: Objection.

A. No.

BY MR. MILLS:

Q. It's more likely that they didn't report those measures because they showed negative effects, isn't it?

MS. WILLIAMS: Objection.

A. So by negative effects I think you're implying that perhaps there was a deep diminishment in one of these variables, and I have no -- no reason to believe that there was a diminishment in one of these, and if there was a statistically significant negative outcome, I would expect that that would be published.

BY MR. MILLS:

Q. You expect that would be published in the New England Journal of Medicine?

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A. Yes.

Q. Would you publish it if you found that?

A. What do you mean?

Q. If you conducted this study and found statistically significant negative effects, would you publish that study?

A. Yes.

Q. And you think the New England Journal of Medicine would accept it?

MS. WILLIAMS: Objection.

A. I don't know if it would be accepted.

BY MR. MILLS:

Q. These researchers are all advocates for medical gender transition; is that right?

A. They're providers of gender-affirming care.

Q. And they advocate in their own interests, correct?

MS. WILLIAMS: Objection.

A. I don't know that I would agree with that statement. I don't know all of these individuals, but the ones I do know are doctors that are motivated by the health and wellness of their patients.

BY MR. MILLS:

Q. Would you say they should release the full data of the other measures that they omitted?

A. I don't know that they haven't. I don't have any --

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Q. If they haven't, should they release it?
A. I don't -- I don't have any reason to suggest that they -- that they shouldn't.
Q. So you would agree they should release the data?
A. I think all -- all research conducted is -- that all published research data is typically open access and should be publicly available.
Q. And you think the same of the data that they gathered in this article, the Chen article?
MS. WILLIAMS: Objection.
A. I don't -- I don't know if there's a particular reason that someone would or would not, but yes.
BY MR. MILLS:
Q. I'm going to show you what I'm marking as Exhibit 34, which is an article you cited in your report by Turban and others, "Access to gender-affirming hormones."
MARKED FOR IDENTIFICATION:
EXHIBIT 34
4:15 p.m.
BY MR. MILLS:
Q. You're familiar with this report?
A. Yes.
Q. And it used the 2015 US Transgender Survey as the source of data, correct?
A. Yes.

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Q. And this was an online survey, correct?
A. Yes.
Q. And the participants were drawn from the websites of transgender advocacy organizations, correct?
A. I'm not sure if that's how the websites are described, but the recruitment is pretty well outlined in the US Transgender Survey itself if we wanted to reference it.
Q. So if I were to say it said that the outreach involved developing lists of active transgender LGBTQ and allied organizations who served transgender people, does that sound correct?
A. Yes.
Q. So page 3 of the Turban study under population -- study population, this is near the end of the paragraph, "So this was assessed by choosing hormone therapy in response to the question, "Have you ever wanted any of the healthcare listed for your gender identity or gender transition? Mark all that apply." Options included counseling, therapy, hormone treatment, HRT, puberty blocking hormones, and none of the above."
So this particular study focused on wanting hormones, specifically hormone therapy, right?
A. So this study focuses on desire for and access to

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hormones historically and current mental health, yes.
Q. Okay. The 2015 US Transgender Survey participants are not representative of the actual transgender population in the United States, right?
A. Sorry, say that again.
Q. Yeah. The 2015 US Transgender Survey participants are not representative of the actual transgender population in the United States, correct?
MS. WILLIAMS: Objection.
A. I'm not sure that I would agree with that statement.
BY MR. MILLS:
Q. Okay. I'm going to show you what's marked as Exhibit 35, which is the report of the 2015 US transgender study.
MARKED FOR IDENTIFICATION:
EXHIBIT 35
4:18 p.m.
BY MR. MILLS:
Q. And if we could go to page 26. It jumps around a bit; it's very long.
So 26 just before outreach, the last two sentences, "It is important to note that respondents in this study were not randomly sampled and the actual population characteristics of transgender people in the US are not known. Therefore, it is not

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appropriate to generalize the findings in this study to all transgender people."
Do you agree with that statement?
A. Yes.
Q. And it would necessarily exclude those people who no longer identified as transgender, correct?
A. It would because they wouldn't be responding to the survey as they're not transgender.
Q. And this survey was anonymous, right?
A. Yes.
Q. So researchers would have no way of verifying the self-reported survey responses, correct?
A. That's correct, just like many similar surveys that are used in research.
Q. And individuals who died including by suicide cannot fill out the survey?
A. Individuals who died prior to the survey being available? That's correct.
Q. So they would be excluded?
A. As a transgender person alive during this study period, yes.
Q. If you could flip to page 126 of this transgender survey footnote 12, the second sentence, "While puberty blocking medications are usually used to delay physical changes associated with puberty in youth ages

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4 to 16 prior to beginning hormonal replacement therapy" --
A. Sorry, where are we?
Q. Footnote 12 the second sentence.
MS. WILLIAMS: And I believe it's 9 to 16. You said 4 to 16.
MR. MILLS: I'm losing my eyesight.
BY MR. MILLS:
Q. "While puberty blocking medications are usually used to delay physical changes associated with puberty in youth ages 9 to 16, prior to beginning hormone replacement therapy, a large majority, 73 percent, of respondents who reported having taken puberty blockers in question, 12.9 reported doing so after age 18, in question 12.11."
After age 18 is not when puberty blockers are typically prescribed; is that right?
A. I think it depends on what you mean by puberty blockers. We've been using this word kind of loosely.
So, you know, if the word puberty blockers is the word that's used in the survey question, you know, I think it's worth pointing out that GnRH agonists are the name of the medication that we're talking about when -- when talking about treatment at Tanner stage 2, but other folks may consider other

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medications such as antiandrogens to be puberty blockers, so that's a little bit hard to answer.
Q. GnRH agonists are not typically prescribed after age 18, correct?
A. Not as typically. I think that some trans women are now being prescribed GnRH agonists if they're having trouble with testosterone suppression on estrogen, but more commonly it's used in early adolescence.
Q. So you said you treat people through age 21, 22 and you're familiar with other clinics.
In that age range above age 18, what percentage of your patients would you say are on puberty blockers using either definition of puberty blockers, so including both GnRH agonists and the androgen intercepters?
A. So for trans women older than 18, probably for including both of those, 85 percent, because the majority of patients are on spirolactone and estrogen as an antiandrogen. For trans masculine individuals, a much lower percentage, maybe 20 percent, as testosterone itself is typically sufficient.
Q. So are you surprised that this survey found 73 percent of respondents report having taken puberty blockers after age 18?
A. Again, I think it -- it's all about what patients are

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-- are referring to when they're saying puberty blockers. So do I think that all of the patients that answered a question about puberty blockers actually received GnRH agonists? No, I think that's a lower percentage.
Q. So using this survey to answer questions about GnRH agonists poses a significant risk of bias because of this misunderstanding about puberty blockers?
MR. MILLS: Objection.
A. I think that when you're -- when you're interpreting any study, you know, you have to understand what the survey is asking and what the question being asked is. So when the -- when there's -- when the US Transgender Survey is answering questions about access to gender-affirming care in early adolescence, that in comparing people that didn't have access to that care and showing a difference that's helpful information to understand what access to that care may do for someone's future health.
BY MR. MILLS:
Q. To your knowledge, the survey did not ask whether the participant had gender dysphoria, correct?
A. Not to my knowledge.
Q. So nothing in this survey tracks whether the kids who wanted puberty blockers or cross-sex hormones had

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gender dysphoria, right?
A. There's not any -- there's a retrospective study, so there's no tracking of anything. It's a survey answered at one moment in time.
Q. But you would only prescribe puberty blockers or hormones for gender transition to someone with gender dysphoria, correct?
A. Yes.
Q. So going back to the Turban article on page 12, and again this is Exhibit 34, under "Strengths and Limitations" on page 12, the third sentence says, "Limitations include its non-probability cross-sectional design which produces generalizability and limits determination of causality."
So this study cannot determine causality, right?
A. That's correct.
Q. The next sentence is, "It is possible that people with better mental health status at baseline are more likely to be able to access GAH, thus confounding associations between GAH access and adult mental health outcomes measured."
You agree with that statement?
A. Sorry, I'm trying to find the sentence just to read it along with you.

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Q. Yeah, it's --
A. "It is possible"?
Q. Yeah, "it is possible."
A. I agree that it is possible.
Q. Okay. And then the next sentence says, "Nonetheless, this measure isn't perfect for investigating mental health changes following GAH, and future longitudinal studies are needed."
Do you agree with that statement?
A. I agree that it's imperfect. I think just to point out that between all of the sentences I read were -- were the strengths in this strengths and limitations section that addressed some of those things that we -- that we've discussed.
Q. Toward the bottom, the second to last sentence says, "The 2015 US TS sample is younger with fewer racial minorities, fewer heterosexual participants, and higher educational attainment when compared with probability samples of TGD people in the United States."
Do you agree with that statement?
A. Yes.
Q. And this bias would affect all studies that use this survey; is that right?
A. This -- that's right. When examining data from the US

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Transgender Survey, it's important to understand what the population is surveying, how that population -- who is in that population, and then ask yourself is that population a relevant population to the clinical question that you have.
Q. If we could go back to Exhibit 4, which is your article "Serving Transgender Youth." This is on page 8 of that article, and I'm in the second full paragraph on page 8 the second sentence.
It says, "In general, adolescence is marked by a search for identity and personal transformation and at times impetuous decisionmaking."
Do you still agree with that statement?
A. Yes.
Q. Flipping back to page 6, toward the very last sentence on page 5 over to page 6 -- sorry. On the very bottom of page 5, "In our view, it is often unrealistic to expect an adolescent to sort through the myriad of issues related to gender variance without the help of a professional."
Do you still agree with that statement?
A. Yes.
Q. And you would agree that as a child gets older, the child is more likely to have a better understanding of complex topics like gender identity?

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A. Yes.
Q. And is that one reason why you delay cross-sex hormones?
A. Yes.
Q. I'm going to show you a short clip from your presentation with Dr. Selkie.
MARKED FOR IDENTIFICATION:
EXHIBIT 36
4:31 p.m.
(Video plays.)
BY MR. MILLS:
Q. So do you agree with the Dutch researchers that 10 to 11 is not the ideal age to be making decisions about medical transition?
A. Did the Dutch say that in something you're reading?
Q. Well, that's just how you characterized them in the video.
A. Oh.
Q. But I guess I should just say, do you think that 10 to 11 is the ideal age to be making decisions about medical transition?
A. Not permanent transition which is why we think the -- the leadup to -- that was the leadup to me explaining why we use GnRH agonists instead of using gender-affirming hormones at the start of puberty.

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Q. In fact, the Dutch protocol didn't allow even the use of puberty blockers until the age of 12; is that right?
A. In their first cohort of patients that's what they did, yes.
Q. Do you think 10- to 11-year-olds can weigh the long-term fertility risks associated with medical gender transition?
A. I think that it's possible to talk about fertility in an age appropriate way with a 10-year-old, but there's not -- but there's certainly the -- the ability to -- to discuss complex topics like fertility changes and evolves over time as a child gets older and progresses through adolescence.
Q. So you would agree that a 19-year-old would have a better capability to understand or discuss fertility issues than 10- to 11-year-old?
A. On average, a 19-year-old would certainly be able to discuss fertility in a more complex way than a 10-year-old would.
Q. To go back to -- sorry. That video we can note is Exhibit 35 just so we don't get out of order here.
To go back to Exhibit 1, which was your article --
MS. WILLIAMS: I think that was 36.

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MR. MILLS: Was it? Okay, sorry.
A. Yeah, 35 is the US --
BY MR. MILLS:
Q. Oh, 35 is the US Transgender Survey, got it.
A. So going back to 1?
Q. Yes, No. 1. This is on page 14. This is the first full paragraph sentence number 3 on page 14.
MS. WILLIAMS: Is that the third, "in our experience"?
MR. MILLS: That's right, "in our experience."
MS. WILLIAMS: Are you there?
A. Okay.
BY MR. MILLS:
Q. "In our experience, many adolescent patients, even those who are not transgender, are often reticent to discuss their future fertility. A conversation can be more complex in transgender adolescents who may have some desire to accomplish biologic" -- sorry -- "some desire to have biologic children, but who bristle at the idea of using their own anatomy to accomplish this."
Does that still describe your experience?
A. Yes.
Q. If we could go back to Exhibit 8, which was one of

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your book chapters, the one in Transgender Medicine, and look at page 178. And this is the second sentence on page 178 at the top.
"Transgender youth, especially those presenting prior to or around the onset of puberty, are seldom concerned about the impact of medical interventions on fertility and often even less interested in discussing this topic. This ambivalence is likely age appropriate shared by their cisgender peers and may not predict their future feelings."
Do you still agree with that statement?
A. I do. I think -- I think that the topic of fertility is a tricky one and requires a lot of careful discussion, so I think in all of these passages that we're reading, at least the ones that I wrote, my intention is to express that complexity.
Q. Should medical gender transitions ever be prescribed when a parent or guardian does not consent?
A. Sorry, could you say that one more time?
Q. Sure. Should medical gender transition interventions ever be prescribed when a parent or guardian does not consent?
A. I do not believe so.
Q. You've never prescribed puberty blockers or cross-sex hormones absent a parental consent?

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A. Correct.
Q. And you think others should not as well?
A. I -- I'm a pretty strong advocate for, you know, parental involvement in healthcare decisionmaking when it comes to gender-affirming care, especially in light of the fact that I think oftentimes a child that is engaging in transition without consent of their parents may be unsafe, and if they're financially or emotionally supported by that parent, that, you know, as we've been talking about generalizability this whole time, as you've mentioned the Dutch study and other similar studies involved patients that have psychosocial support, so the literature would support that notion that these interventions are helpful in that context, so I do believe that parental consent is important and would suggest it be obtained when considering initiating gender-affirming care.
Q. If a parent did not consent to insulin for their type 1 diabetic children -- child, would you prescribe it anyway?
A. Yes.
Q. And why -- why the difference?
A. Well, I feel like I answered this question before, but it is a little -- maybe it's a little bit of a different question.

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I think again, you know, insulin is pretty clear. If you have type 1 diabetes, your body doesn't make insulin and you need insulin to live, so there's a clear no insulin and you die, insulin and you live. Whereas, the -- the decision around gender-affirming care there's a lot more nuanced and involves the details related to the patient's experience with their gender, patient and family values, discussion of risks and benefits, decisionmaking that is shared amongst the clinician and the patient and the parent, and so they're very different conditions with very different treatments, and so my answer is different for those two -- for those two different conditions.
Q. I'd like to show you the de Vries 2014 study that we've talked about a couple of times. You're familiar with that study?
A. I guess I have to see it first to know which one you're talking about.
Q. Sure. There are several. This is the 2014 study that earlier we talked about because study path described it as the only long-term follow-up study --
A. Okay.
Q. -- through young adulthood. So this is that study, correct?
A. Yes.

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Q. And we talked about this earlier. The mean age of adult follow-up was 20.7 years old; is that right?
A. Yes.
Q. To your knowledge, is the brain still developing at age 27 years old?
A. Yes.
Q. Would you be interested to know what follow-up looks like past age 20.7?
A. Yes.
Q. Could that affect your treatment decisions?
A. Certainly if all of these patients are doing very poorly now compared to the general population, that would be surprising, and I would like to -- it would be interesting to know that. It's not what I would expect, but to answer your question, yes.
Q. All right. I'd like to show you another paper you wrote that talked about this study. This is Exhibit 38.

MARKED FOR IDENTIFICATION:
EXHIBIT 38
4:41 p.m.

BY MR. MILLS:
Q. This was an article you coauthored with Dr. Spack entitled "Transgender medicine long-term outcomes from the Dutch model."

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On page 2 discussing this study, the second full paragraph on page 2 it starts by saying, "It should be noted that the patients described were well supported, brought to care in early adolescence, and cared for as part of a carefully structured multidisciplinary care team in a small supportive country. Generalizing the Dutch clinics success to clinics in other settings might be problematic."

Do you still agree with that statement?

A. Well, I think we have to remember that when this was written, Dr. Spack had developed the clinic at Boston Children's Hospital modeled after the Dutch clinic and so, therefore, was trying to replicate as closely as possible the -- the Dutch clinic because of this point that we're making in this article, and -- and since 2015, similar clinics around the country are similarly modeled. So, yes, it's something that should be considered, but also the reason why the care is provided the way it is.
Q. So you would still say that generalizing the Dutch clinic's success to clinics that may use other models might be problematic?
A. Like, is there another model that you're -- that you're thinking of? I think most care in the US is performed in this model, so if you're not using a multidisciplinary model of care like the one -- like the care that we've been talking about, then it seems like you wouldn't be following standard of care, perhaps, and may not be generalizable, but also wouldn't be recommended.

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Q. Even this sentence, though, "Brought to care in early adolescence," I think you testified earlier that most of your patients do not present in early adolescence; is that right?
A. That's right. The patients that present to care in our clinic are more -- are better represented in studies like the Chen study.
Q. So the Dutch patient population you would say is different from your patient population?
A. In that way, yes.
Q. This Dutch study, and we can look at the method section on page 697, "Participants include 55 young adults." So you would agree the sample size is 55?
A. Yes.
Q. And there was no controlled group here who did not receive medical interventions; is that right?
A. Well, they are comparing the mental health and quality of life outcomes, I believe, to the general population, so it's a pseudo control group in that way.

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Q. But the general population would not be those adolescents with some gender incongruence?
A. That's correct. There's not a control group of patients with gender incongruence that are not receiving treatment.
Q. Okay. And then it says a little ways down in this third column, "The young adults were invited between 2008 and 2012 when they were at least one year past their GRS," which I believe is the gender reassignment surgery; is that your understanding?
A. Yes.
Q. So the whole sample size of 55 had also received surgeries, correct?
A. At the end time point, yes.
Q. And then it lists further down a couple sentences later, "Nonparticipation was attributed to," and then several things, the last one of which, "One trans female died after her vaginoplasty owing to a postsurgical necrotizing" --
A. Fascitis.
Q. -- "fascitis."

So that would be over 1 percent of the 55 participants died due to gender-affirming interventions?
A. Yes.

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Q. And then it says, "Nonparticipation was N equals 15 out of the 55 who did," and there were 55 who did participate, so over 20 percent of the participants dropped out during the study; is that right?
A. Well, it says here 15 were not one year postsurgical so they didn't meet that criteria.
Q. Mm-hmm.
A. So there's six -- okay, so, sorry, let me try to answer your question again. What was your question?
Q. So of these --
A. So they break it down --
Q. Right. There were 70 people, but 15 of the 70 did not participate because of these various factors; is that right?
A. They weren't included in the --
Q. Analysis.
A. -- analysis, yes.
Q. This -- this death from the -- after the vaginoplasty, are you aware that the death was of consequence of puberty suppression?
A. I don't -- I don't have information to confirm or deny that.
Q. So you don't know if that death was because the patient's penis was too small for the regular vaginoplasty and so surgery had to be attempted with a

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portion of the intestine?
MS. WILLIAMS: Objection.
A. I don't know. I do know that patients that I take care of that are adults that receive surgery at the institution that I work in do not require intestinal tissue for successful surgery. So if this is -- if that was the case, that isn't a complication that I see today.
BY MR. MILLS:
Q. Those patients you're talking about, did they start puberty blockers at Tanner stage 2?
A. Yes.
Q. And you follow every gender-affirming surgery that happens at your hospital?
A. I talk to the surgeon in my institution about patients that are treated at Tanner stage 2, and he has guided me to that he's able to accomplish vaginoplasty successfully despite blockade at Tanner stage 2.
Q. So do you consider him a more adept surgeon than Dr. Bowers?
A. I don't know.
Q. This study didn't control for psychotherapy, did it?
A. No.
Q. And all the subjects were getting psychological counseling during the same time as these medical

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interventions?
A. Yes.
Q. And the bottom of page 697 here, "Participants" -- this is the final paragraph, "Participants were assessed three times posttreatment, during treatment at initiation of cross-sex hormones, and posttreatment one year after gender reassignment surgery."
So this study provides no evidence about the long-term outcomes of puberty blockers and cross-sex hormones without surgeries, correct?
A. Correct. The patients in this study that are included in the final analysis all had surgery.
Q. So flipping over to page 699, the top, that first line in Table 2 UGDS, that's a gender dysphoria scale; is that right?
A. Yes.
Q. And from T0 which was at intake to T1 which was while on puberty supervision, gender dysphoria increased from 53.51 to 54.39; is that right?
A. The mean number is higher. I don't think that they're reporting that to be a statistically significant difference.
Q. They don't report that to be a statistically insignificant difference, though, do they?
A. I do believe they do because the standard deviation

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overlaps, so that is a -- is not a -- is not statistically different.
Q. And is that what a p-test measures?
A. Yes. The p-test is telling us that from T0 to T2 there is a statistically significant difference. There's not a p-value reported for T0 to T1, that's right.
Q. So you don't know whether that p-value would be statistically significant?
A. Well, it's true that I don't know what the p-value is, but if you just look at the numbers, the mean of 53 with a standard deviation of 8, and the mean of 54 with a standard deviation of 7, so that means that those bell-shaped curves would overlap almost completely, and so I am quite confident that those are not statistically significant.
There's not a statistical significant decrease in -- or statistically significant increase in the score from T0 to T1 without pulling out a calculator.
Q. And without a p-value or a calculator, you wouldn't know whether that would be statistically significant?
A. I just explained why it's -- why it isn't.
Q. But putting that aside, the mean for gender dysphoria worsened from T0 to T1; is that right?

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MS. WILLIAMS: Objection.
A. Well, when you're saying worsened, you're implying that there's a meaningful difference in the numbers, and if it's not statistically significant, then -- then I don't -- then it wouldn't be an accurate statement.
So, yes, I don't have a p-value to share with you on those means in standard deviations. Yes, I believe that they are -- that the T1 is not statistically significantly higher than T0. So, no, I wouldn't make an assertion about the difference between those numbers 53.51 and 54.39.
Q. The Dutch protocol excluded those with significant psychological comorbidities, correct?
A. It sounds right. If we wanted to find the place in the methods section where they talk about their inclusion criteria, I can confirm the wording on that.
Q. That's okay. Page 702 the bottom of the first column of text, the last sentence in the first column of 702 says, "These individuals of whom" -- sorry, I'll wait until you get there.
A. 702.
Q. Yeah. "These individuals of whom an even higher percentage than the general population were pursuing higher education seemed different from the transgender

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youth in community samples with high rates of mental health disorders, suicidality and self-harming behavior, and poor access to health services."
Do you agree that -- that the latter community would describe your typical patient population -- sorry, I'll phrase it a different way.
Does your patient population look more like the individuals in the Dutch protocol or what the authors describe as the transgender youth in community samples?
A. Probably somewhere in between because I still think there's a bias towards people with better access to healthcare that are going to receive care at pediatric gender clinics, and the most -- most high risk patients with the least access to mental healthcare, patients living in poverty, or without any parental support, are not being included in the patient population that I see.
Q. So page 700 in the middle it says, "The participants were, other than more likely to be pursuing higher education, families were supportive 80 to 90 percent." The next paragraph. "Many participants reported having never or seldom been called names or harassed. The majority had experienced sexual transitioning as easy."

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Do you think that sample is representative of the patients that are presenting to your clinic?
A. Certainly a percentage of patients that I see are well described by those -- those descriptions, and then others are struggling more than the -- the patients described in this -- in this study.
Q. So I guess I'm asking, you know, are you experiencing patients with these -- who are coming in with these same high levels of positive objective well-being?
A. So I think I'm answering your question when I say that, yes, some patients are very similar to this group of patients and then others are not.
Q. So percentages are you experiencing those type -- the types of patients with high objective well-being to the same high percentages as the Dutch protocol participants were?
A. Perhaps slightly lower percentages, although, again, there is still a bias in terms of who is presenting to gender care because the -- there needs to be some degree of support from family to bring patients to clinic.
Q. I'm going to show you the 2020 article de Vries wrote, which I'll mark as Exhibit 39.
MARKED FOR IDENTIFICATION:
EXHIBIT 39

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4:57 p.m.
BY MR. MILLS:
Q. This is "Challenges in timing puberty suppression for gender nonconforming adolescents."
Are you familiar with this article? I believe it's cited in your report.
A. Yes.
Q. All right. So in the middle of the second column, the second to last sentence in that first paragraph, "This older adolescent group did not only have more mental health difficulties, but also at a later age of onset of gender incongruents."
A. I'm sorry, I didn't pick up where you started. This is the second column --
Q. Second column right under -- right past footnote 4.
A. Okay, I'm there. Thank you.
Q. So she's -- describe --
A. Could you just read it again and ask me the question again? I'm sorry.
Q. Yep, yep, sure. She's describing another study that was written in Pediatrics by Sorbara, et al --
A. Okay.
Q. -- and she's comparing them to the Dutch study. So she says, "Interestingly, this older adolescent group did not only have more mental health difficulties, but

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also a later age of onset of gender incongruents."
A. Okay.
Q. And then skipping to just past footnote 5 on the same column she says, "Authors of case histories and apparent report study warned that gender identity development is diverse and a new developmental pathway is proposed involving youth with postpuberty adolescent onset transgender histories.
"These youths did not yet participate in the early evaluation studies. This raises the question whether the positive outcomes of early medical intervention also applied to adolescents who more recently present in overwhelming large numbers for transgender care."
You would agree that the author of this is the same as one of the authors of the 2014 study we were just talking about?
A. Yes.
Q. And she identifies what she calls "new developmental pathway."
Are most of your patients aligned with this new developmental pathway involving youth with postpuberty adolescent onset transgender histories?
A. So I think that there's a lot of variability in the types of patients that we're seeing. There are

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patients that have seemingly later onset of gender dysphoria than are described in the Dutch paper. There's other patients that had earlier onset of gender dysphoria, but are presenting to care in later adolescence, and then, of course, some patients that are very similar to the patients described in the Dutch article. So on the whole, the average age of presentation is older than the age described in the original Dutch article.
Q. And would you agree with her that this raises the question whether the positive outcome seen in the 2014 study also applied to adolescents who were recently present in overwhelming large numbers for care?
A. I think that that study by itself, you know, would be -- would be best at answering questions related to the younger presenting cohort, and then, you know, other studies examining older adolescents and even adults are -- can be impactful in understanding how later presenting patients may or may not benefit from treatment.
Q. But you aren't aware of a similar long-term outcome study like the 2014 focused on what she calls is the new developmental pathway?
A. Correct.
Q. So the bottom of page 2 the first column of the same

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2020 note we're reading says, "Systematic studies on the rate of adolescents who discontinued their transitions after they have started gender-affirming hormones or surgeries with lasting effects are lacking at present."
Do you agree that there's a lack of systematic evidence about how many adolescent presenting patients de-transition?
A. I -- I think that there is a -- I don't have the citation, but there is a recent article outlining long-term continuation or non-continuation of hormones in -- in adolescents who've started gender -- gender care, but I don't disagree that more systematic follow-up is an important question to continue to study.
MR. MILLS: All right. I think we're almost through. Can we just take a five-minute break? Would that work for everyone?
(Recess taken at 5:03 p.m.)
(On the record at 5:09 p.m.)
BY MR. MILLS:
Q. So I'd like to flip back to the Standards of Care 8, if we could, which was Exhibit 26, and I'm looking at page S51. Yep, you're good.
A. Okay. S51?

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Q. That's right, S51.
A. Okay.
Q. So the first full paragraph, the last two sentences of that first full paragraph in the first column on S51.
A. Okay.
Q. See it here?
A. Yep.
Q. It starts, "There are no studies -- There are no studies of the long-term outcomes of gender-related medical treatments for youth who have not undergone a comprehensive assessment. Treatment of this context, e.g. with limited or no assessment, has no empirical support and, therefore, carries the risk that the decision to start gender-affirming medical interventions may not be in the long term best interests of the young person at that time."
Do you agree with that statement?
A. Yes.
Q. So a provider who prescribes medical gender transition interventions for an adolescent who's never had any mental health evaluation for gender dysphoria, would not be following the WPATH guidelines, correct?
A. So it says a comprehensive assessment, so I just want to be careful that that doesn't necessarily mean that it has to be a certain type of health professional.

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A comprehensive assessment must be performed, in our clinic it is a mental health professional. In most pediatric gender clinics it is, but it needs to be someone that's competent in doing a psychosocial assessment and diagnosing gender dysphoria.

Q. So you think someone can receive medical gender transition interventions consistently with WPATH who's never had a mental health evaluation for gender dysphoria?

MS. WILLIAMS: Objection.

A. So I -- I think in my mind comprehensive assessment is a mental health assessment, so -- but I just wanted to be clear on the words in WPATH, that they use the word comprehensive assessment. I agree that a mental health assessment is important.

BY MR. MILLS:

Q. Okay. I'd like to show you what I'm marking as Exhibit 40, which is an article in the Los Angeles Times entitled, "This abortion doctor is not ready to leave Alabama."

MARKED FOR IDENTIFICATION:
EXHIBIT 40
5:13 p.m.

BY MR. MILLS:

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Q. Have you read this article?

A. No.

Q. So on page 1 it's dated April 2023. You can see in the first two paragraphs of the article it discusses a Dr. Leah Torres, a 43-year-old OB-GYN.

To your knowledge, Dr. Torres is not an endocrinologist, correct?

A. Correct, not to my knowledge.

Q. Or a pediatrician, to your knowledge?

A. No. I don't know her. I had nothing -- no information other than what's here in the article.

Q. Sure. And so you don't know if she has any mental health training?

A. I don't know.

Q. So page 10 of the article in the middle -- actually toward the bottom, the third to last paragraph on page 10, "When meeting trans patients, Torres is upfront that she has been practicing such care for only a year. Full disclosure she tells them this area of medicine is pretty new to me. She also points out that this is a relatively experimental area of medicine without a lot of data."

Just from that description, does that -- does that sound like someone you would consider to be qualified to practice pediatric gender medicine?

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A. I don't think I can take two sentences from a quote and make that determination.

Q. All right. So two paragraphs above what we just read, "Torres does not believe adolescents seeking hormones require mental health evaluations. "No, I don't need a psychologist or psychiatrist to evaluate someone who's telling me this is how I felt for years," she said. "I know that how they felt for years is not pathological.""

In your view, is Dr. Torres providing care in accord with WPATH Standards of Care 8?

MS. WILLIAMS: Objection.

A. So I want to just pick apart these two sentences before I answer.

So a psychologist or psychiatrist is not necessarily required to be the person that does the mental health evaluation, and that her comment that how someone's feeling, their gender identity is not pathological, I would agree with.

Q. Even though it's a DSM-5 diagnosis?

A. Gender dysphoria is -- is a DSM diagnosis, but a difference in gender identity is not. So the author wrote Torres does not believe adolescents seeking hormones require mental health evaluation, but that's not her quote. And so I don't know what evaluation

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Dr. Torres would perform in determining whether someone has unmet mental health needs, but I wouldn't be able to assess that just from these lines in this article.

Q. It doesn't sound like Dr. Torres is performing gender medicine in the context of a multidisciplinary clinic, does it?

MS. WILLIAMS: Objection.

A. I would have a hard time answering that question without more context.

BY MR. MILLS:

Q. Assuming she's the only provider that talks to a patient, is she performing in the context of multidisciplinary care?

A. No.

Q. So she's not performing in accord with WPATH Standards of Care 8, correct?

MS. WILLIAMS: Objection.

A. Well, I don't know how she's actually performing. I -- that second question is unrelated to the previous one.

BY MR. MILLS:

Q. So the article goes on to say that Dr. Torres prescribes youth cross-sex hormones on their first visit, including a visit via telehealth, regardless of

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whether they had a mental health evaluation.
Would you do that in your clinic?
A. Can you direct me to where that's stated?
Q. If you could just answer the question. We don't need to look at the article again.
A. Well, I guess it would be important to know is she talking about an adolescent or an adult. I certainly have prescribed hormone interventions to patients on a first visit, and prescribing on a first visit with the doctor or performing telehealth visits would not be outside of the standard of care, so.
Q. So the next paragraph, the last paragraph on page 10, "One transgender patient Torres recently started seeing through telehealth was referred to her because the teen's pediatrician and staff at a psychiatric hospital did not respect his gender identity and used his own name. He told Torres he had known he was a boy for years. Torres," the next page, "told him straight up that she would prescribe a low dose of testosterone."
Do you believe that Dr. Torres is providing care in accord with WPATH Standards of Care 8?
MS. WILLIAMS: Objection. Counsel, if you're going to ask about the article, he should be able to read the article.

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BY MR. MILLS:
Q. The sections I've described outline how she has cared for this child, and I'm asking the care for this child was that in accord with WPATH Standards of Care 8.
A. I think it's hard for me to comment on what her care actually is like, but, you know, I think that I would suggest that mental health evaluation is important for adolescents with gender dysphoria prior to proceeding with hormone, and that's why I practice the way I do.
Q. So she may be treating a condition that has never been properly diagnosed, correct?
A. I think it's hard to say based on the author's report of her conversation with her, but --
Q. The passages I've read you have no concerns with how Dr. Torres is practicing gender medicine for adolescents?
A. I'd like to reserve concern until I knew more about how she actually structures her visits and sees patients.
Q. The next page, page 11, the second to last paragraph, "I will do whatever I can within legal parameters," Dr. Torres said later."
You would agree that WPATH itself cannot do anything about Dr. Torres's practice of gender medicine?

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A. What? Say that again.
Q. Can WPATH do anything to stop Dr. Torres from using her current approach to gender medicine?
A. I don't know what her current approach is exactly, but WPATH can't tell anyone what to do.
Q. And neither can the Endocrine Society?
A. No.
Q. Should adolescents be able to receive gender-affirming surgeries?
A. I think that there's some adolescents that benefit from masculinizing chest surgery, but I don't advise genital surgeries in patients under 18.
Q. Are you aware that Standards of Care 8 now permit surgeries under age 18, including the bottom surgeries you just mentioned?
A. I think that the WPATH doesn't actually discuss particular age cutoffs and more talks around patient readiness and individual factors.
Q. In fact, is it right to say that WPATH removed the age considerations that were in the initially published version of Standards of Care 8?
A. I believe that to be true.
Q. Do you know why they removed those age restrictions?
A. I do not.
Q. [redacted]

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[redacted]
MS. WILLIAMS: Objection.
A. I do not know.
BY MR. MILLS:
Q. Are you aware that the United States in this case is not challenging the law's ban on surgeries?
A. I was aware.
Q. Should they be?
A. That's not for me to say.
Q. You think it will harm children, though, if they can't access gender-affirming surgeries before the age of 19?
A. Before the age of 19.
Q. That's the age in Alabama.
A. I think it's -- it's possible that it can be harmful, but as a nonsurgeon, I have more experience with the -- the treatment of gender dysphoria with hormonal interventions.
Q. Have you told the United States that they should challenge the surgery component of the Alabama law?
MS. WILLIAMS: Objection.
A. I have not.
BY MR. MILLS:
Q. Why do you think they aren't challenging the surgery component of the law?

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MS. WILLIAMS: Objection.
A. I don't know.
BY MR. MILLS:
Q. I'm going to show you a clip. Do you recall doing a Facebook live streaming video with a group called "Stand with Trans" entitled "Ask the Expert" in February 2021?
A. I do believe so.
Q. Okay. I'm just going to just show a clip from that video if we can get it queued up here.
(Video playing.)
BY MR. MILLS:
Q. And I'll mark that as Exhibit 42 [sic].
MARKED FOR IDENTIFICATION:
EXHIBIT 41
5:24 p.m.
BY MR. MILLS:
Q. So in this -- in this video, you're talking about -- sorry. What types of surgeries are you specifically referring to in this video?
A. I was -- I was talking about OB-GYNs so I was talking about hysterectomy.
Q. Okay. And do you -- so in the video you said it should be an adult decision to completely reverse fertility potential.

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Do you still agree that it should be an adult decision to completely reverse fertility potential?
A. I do. I think that the decision around removal of gonads and therefore having no possibility of fertility is different than the hormonal interventions that we've been discussing so far which do not reduce fertility to zero, and my opinion is that -- that that decision is best made in -- in most people after 18.
Q. And that's -- you have that view despite the potential availability of artificial means of reproduction?
A. As in? What artificial means of reproduction are you referring to, like, sorry, just to understand your question a little better?
Q. Sure. A 17-year-old considering these surgeries could conceivably freeze her eggs, for instance, but despite that available option, you still don't think a person, a child, should be able to decide to have that surgery?
A. I think there could be a compelling case where a person has really significant gender dysphoria related to the uterus, and I'd be open to the idea that the benefits would outweigh the risks, but as a general matter, I -- I -- I encourage people to delay the decision on gonadectomy surgeries.

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Q. And you're a pediatric endocrinologist, correct?
A. Yes.
Q. You don't treat adults past the age of 22, thereabouts?
A. Sometimes I have patients that I have a hard time graduating because they don't want to say good-bye, so some patients are 23 or 24, but generally that's the oldest patients, group of patients that I would see.
Q. And why is pediatric endocrinology its own specialty?
A. I think that there's a wide range of endocrine problems that affect children that don't affect adults and so having a specialty devoted to pediatrics is important.
Q. So treatments may vary between adult and child practice it sounds like?
A. Generally in endocrinology or gender-affirming care?
Q. Generally in endocrinology.
A. Yes.
Q. And research on treatments for adults again generally in endocrinology may not be applicable to treatments for adolescents; is that right?
A. Yes.
Q. All right. I'd like to show you another study that you cited in your report, and this has to do with the -- one of the twin studies that we started talking

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about earlier.
So I will mark this as Exhibit 42.
MARKED FOR IDENTIFICATION:
EXHIBIT 42
5:28 p.m.
BY MR. MILLS:
Q. So again this -- you're familiar with this study? You cited it in your report; is that right?
A. Yes.
Q. If we could go under "Methods" on page 452.
MS. WILLIAMS: You mean 752?
MR. MILLS: Yep, I do. Yes, I do.
BY MR. MILLS:
Q. So it says, "For the review of case studies on twins, we searched several databases using the following keywords. For unpublished data sets we contacted the authors directly. We also included three twin pairs who attended the gender clinic of Ghent University." And then later on it says, "There were some case reports examined at our clinic," and then it says, "A total of 25 twin pairs were also available for analysis from a Toronto gender identity service."
So this isn't a randomized sample, correct?
A. Correct.
Q. And it would not be representative of the overall

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population of twins?
A. Well, that's, I think, open to the readers and open for the readers' determination. So having enough identical twins in one gender clinic there wouldn't be enough power to answer any question about -- about a genetic link so you need to widen the circle, so to speak. So they outlined how they recruited these twin pairs, and then it's for the reader to then assess how well does this recruitment strategy represent twins generally.
Q. And these -- these patients, or some of them, had been diagnosed with gender identity disorder. That is the old diagnosis under the DSM-IV; is that right?
A. Yes.
Q. And that's not the same is gender identity under the DSM-5?
A. There's -- the criteria are not identical.
Q. So this study does not examine twins in the context of the current diagnostic criteria for gender dysphoria under the DSM-5?
A. That's right. It's not -- it's specifically talking about gender identity disorder, which is similar to, but not the same as gender dysphoria.
And I think I also used this article to express biologic origin for gender identity more

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generally, so we're using gender identity disorder as a surrogate for gender identity.
Q. But not all persons with divergent gender identity have or had under the old diagnosis gender identity disorder; is that true?
A. That's true.
Q. So on page 755 under "Statistical Analysis" it says, "If we combine the same sex MZ and DZ twin pairs across sex, there were a total of nine 39.1 percent MZ twin pairs concorded for GID, and fourteen 60.9 percent discorded for GID. Of the 21 DZ twin pairs all were discorded for GID."
So that means, if I can try to translate that, that means that 39.1 percent of identical twins examined were found to both have gender identity disorder; is that a fair --
A. Yeah, I think the way that I would explain it is they're finding twin pairs where at least one of the twins has gender identity disorder, and then they're saying what percentage of the other also has gender identity disorder.
So in the monozygotic or you could say identical twins, 39 percent of the other twin also had gender dysphoria, and the fraternal, so to speak, dizygotic twin pairs none of the other twins had

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gender identity disorder.
Q. Right. You said gender dysphoria, but it's really just gender identity disorder?
A. Right, that's what I tried to say, yep.
Q. Okay. So 755 on the second column right at the top of the page, the higher concordance for GID and MZ than in DZ twins is consistent with a genetic influence on its genesis, although shared and nonshared environmental factors cannot be ruled out."
Do you agree with that statement?
A. Yes.
Q. Then the next sentence, "Indeed, from these case reports, very little is known about the "equal environment assumption." That is the assumption that MZ twins are not treated more similarly than DZ twins in ways that might affect their gender identity."
You agree with that statement?
A. I think I understand what they're saying, and in -- I would agree that the -- the point they're making is, you know, the assumption in twin studies is that the environment is the same when you are an identical twin or a fraternal twin because you're living in the same house, but could there be subtle differences in the environment if you are identical twins, are you treated differently in some way that isn't the case

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with fraternal twins, could this be explaining the reason that 39 percent of monozygotic twins are concorded where zero percent of dizygotic twins are concordant, that's the question that they're posing, so it's up to the reader then to think that through and make a determination.
Q. And so under "Statistical Analysis" on 755 the first column the second sentence, the one right after the one we already read was, "The difference in concordance between the MZ and DZ pairs was significant chi squared equals 8.18, so" --
MS. WILLIAMS: It says 8.08.
MR. MILLS: Sorry.
MS. WILLIAMS: That's okay.
MR. MILLS: I'm dying, 8.08.
BY MR. MILLS:
Q. So this chi squared test just asks whether there's an observed difference between two variables; is that right?
A. Yes.
Q. And it doesn't control for any other variables, correct?
A. Right. But again, that's sort of the point of a twin study is that you're doing almost everything you can to control the variables.

Q. But the -- in terms of formal statistical analysis, it doesn't control for any other variables?
A. Correct.
Q. And so it doesn't control for sexual orientation, right?
A. Correct.
Q. So if there were an overlap between sexual orientation and GID, that could account for some or all of any of this difference observed?
A. I don't really know that I understand what you mean. I think that -- could you explain the question a little bit more?
Q. Sure. So we can look at 755 at the bottom of the page, the very last full sentence. "In all the cases reported to be concorded for GID, there was also concordance for sexual orientation."

So if there's a relation between GID and sexual orientation, any differences between the identical and fraternal twin groups could be due to the sexual orientation concordance rather than gender identity disorder concordance, right?

MS. WILLIAMS: Counsel, we're at 7 according to Coty's clock, but if you want to answer that question.

A. Yeah, so I think what you're saying is -- is that all



of the twin pairs that are concordant also shared the same sexual orientation, so could the sexual orientation be somehow impacting the diagnosis of gender identity disorder.

I think that -- that -- that doesn't seem plausible to me, but I'm not sure I completely understand the question, but I -- I -- I think that regardless of someone's sexual orientation, whether they have a difference in gender identity or have gender identity disorder is -- is relevant, so I guess that would be my answer, but I'm still not sure I hit it out of the park because I'm not sure I understood the question completely.

MR. MILLS: That's all right. Great. Well, thanks so much for your time.

COURT REPORTER: Please put your order on the record for transcript. Do you want to order the transcript?

MR. MILLS: Yes, I would like to order the transcript.

MS. WILLIAMS: United States would as well.

(Deposition concluded at 5:39 p.m.

Signature of the witness was requested.)



Boe, Brianna, Et Al. v. Marshall, Steven T., Et Al.
Daniel Shumer, MD (#6502246)

ACKNOWLEDGEMENT OF DEPONENT

I, Daniel Shumer, MD , do hereby declare that I have read the foregoing transcript, I have made any corrections, additions, or changes I deemed necessary as noted above to be appended hereto, and that the same is a true, correct and complete transcript of the testimony given by me.

_____ _____
Daniel Shumer, MD Date
*If notary is required

SUBSCRIBED AND SWORN TO BEFORE ME THIS _____ DAY OF _____, 20___.

_____
NOTARY PUBLIC



Boe, Brianna, Et Al. v. Marshall, Steven T., Et Al.
Daniel Shumer, MD (#6502246)

E R R A T A S H E E T

PAGE_____ LINE_____ CHANGE_____
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_____ _____
Daniel Shumer, MD Date

CERTIFICATE OF NOTARY

STATE OF MICHIGAN )
) SS
COUNTY OF MONROE )

I, LEISA PASTOR, certify that this deposition was taken before me on the date hereinbefore set forth; that the foregoing questions and answers were recorded by me stenographically and reduced to computer transcription; that this is a true, full and correct transcript of my stenographic notes so taken; and that I am not related to, nor of counsel to, either party nor interested in the event of this cause.

LEISA PASTOR, CSR-3500, CRR,
Notary Public,
Monroe County, Michigan
My Commission expires: 9/7/27



Renee Williams
renee.williams3@usdoj.gov
April 22, 2024
RE: Boe, Brianna, Et Al. v. Marshall, Steven T., Et Al.
4/2/2024, Daniel Shumer, MD (#6502246)

The above-referenced transcript is available for review.

Within the applicable timeframe, the witness should read the testimony to verify its accuracy. If there are any changes, the witness should note those with the reason, on the attached Errata Sheet.

The witness should sign the Acknowledgment of Deponent and Errata and return to the deposing attorney. Copies should be sent to all counsel, and to Veritext at cs-southeast@veritext.com.

Return completed errata within 30 days from receipt of testimony.

If the witness fails to do so within the time allotted, the transcript may be used as if signed.

Yours,
Veritext Legal Solutions

Boe, Brianna, Et Al. v. Marshall, Steven T., Et Al.

Daniel Shumer, MD (#6502246)

ACKNOWLEDGEMENT OF DEPONENT

I, Daniel Shumer, MD , do hereby declare that I have read the foregoing transcript, I have made any corrections, additions, or changes I deemed necessary as noted above to be appended hereto, and that the same is a true, correct and complete transcript of the testimony given by me.

5/15/2024

Daniel Shumer, MD Date

*If notary is required

SUBSCRIBED AND SWORN TO BEFORE ME THIS 15th DAY OF May, 2024.

NOTARY PUBLIC

KENNARD LEE VANCAMP III
Notary Public - State of Michigan
County of Washtenaw
My Commission Expires Sep 19, 2030
Acting in the County of Washtenaw

KENNARD LEE VANCAMP III NOTARY PUBLIC MY COMMISSION EXPIRES SEP 19, 2030 County of Washtenaw STATE OF MICHIGAN

Boe, Brianna, Et Al. v. Marshall, Steven T., Et Al.

Daniel Shumer, MD (#6502246)

E R R A T A  S H E E T

PAGE_23_ LINE_13__ CHANGE_"gains" to "genes"_____

_____

REASON_wrong word_____

PAGE_35__ LINE_14__ CHANGE_"children in adolescence" to "children and adolescents"_____

REASON_wrong word_____

PAGE_36__ LINE_16__ CHANGE_"cross X hormones" to__ "cross sex hormones"_____

REASON_wrong word_____

PAGE_37__ LINE_21__ CHANGE_"diagnosis" to_____ "diagnose"_____

REASON_wrong word_____

PAGE_41__ LINE_23-24 CHANGE_"half progresses" to__ "has progressed"_____

REASON_wrong word_____

Please see page 273a for continuation of the Errata Sheet.

_____ 5/15/2024

Daniel Shumer, MD Date

Page 273a
Errata Sheet – Continued

PAGE: 45 LINE: 10-12
CHANGE: add open quotation mark before "appear" and add close quotation mark after "environment"
REASON: I am reading a passage, not using my own words

PAGE: 54 LINE: 3
CHANGE: "Casey" to "K.C."
REASON: Corrected name of legal case

PAGE: 61 LINE: 21
CHANGE: "higher" to "high"
REASON: wrong word

PAGE: 63 LINE: 7
CHANGE: "parents" to "patients"
REASON: wrong word

PAGE: 65 LINE: 1
CHANGE: "female body" to "female-bodied"
REASON: wrong word

PAGE: 70 LINE: 9
CHANGE: "persistent" to "persistence"
REASON: wrong word

PAGE: 83 LINE: 5
CHANGE: "involved" to "evolved"
REASON: wrong word

PAGE: 97 LINE: 2
CHANGE: "male body" to "male-bodied"
REASON: wrong word

PAGE: 98 LINE: 1-2
CHANGE: "female body" to "female-bodied"
REASON: wrong word

PAGE: 103 LINE: 3
CHANGE: "the" to "a"
REASON: wrong word

Daniel Shumer, MD

5/15/2024
Date

Page 273b
Errata Sheet – Continued

PAGE: 107 LINE: 24
CHANGE: "a course being estrogen" to "of course being on estrogen"
REASON: wrong words

PAGE: 117 LINE: 12
CHANGE: "adults" to "adolescents"
REASON: I went back to the source material to confirm that the wrong word was transcribed

PAGE: 117 LINE: 15
CHANGE: "e-scores" to "z-scores"
REASON: wrong scientific word

PAGE: 130 LINE: 16
CHANGE: "doctrine care" to "Doctoring: care"
REASON: wrong title

PAGE: 143 LINE: 25
CHANGE: "particularly synthetic ethanol, estradiol," to particularly synthetic ethinyl estradiol,"
REASON: wrong scientific word and position of punctuation

PAGE: 151 LINE: 25
CHANGE: "at a similar stage 2" to "at SMR stage 2"
REASON: I went back to the source material to confirm correct words, SMR is a medical abbreviation for Sexual Maturity Rating

PAGE: 162 LINE: 6
CHANGE: "fought puberty" to "block puberty"
REASON: I went back to the source material to confirm the correct word

PAGE: 164 LINE: 23
CHANGE: "Top ten" to "Top trans"
REASON: I went back to the article in question to confirm correct title

PAGE 165: LINE: 12
CHANGE: "the clinical name Deniliquin the first visible" to "the clinical name of the moment when the first visible"
REASON: wrong word, misssing words; I went back to the source material to find the correct language

Daniel Shumer, MD

5/15/2024
Date

Page 273c
Errata Sheet – Continued

PAGE: 168 LINE: 3
CHANGE: "You say 'After a while'" to "You say after, 'While,' you say,"
REASON: Wrong phrase, the quotation in question starts with the word "while"

PAGE: 182 LINE: 20
CHANGE: "GnHR" to "GnRH"
REASON: wrong scientific word

PAGE: 183 LINE: 21
CHANGE: "protocol-ise" to "protocolize"
REASON: protocolize is a word

PAGE: 189 LINE: 2
CHANGE: "up comes" to "outcomes"
REASON: wrong word

PAGE: 193 LINE: 10
CHANGE: "protruding" to "treating"
REASON: wrong word

PAGE 198: LINE: 21
CHANGE: "ascent" to "assent"
REASON: wrong word

PAGE: 198 LINE: 23
CHANGE: "ascent" to "assent"
REASON: wrong word

PAGE: 199 LINE: 14
CHANGE: "ascent" to "assent"
REASON: wrong word

PAGE: 201 LINE: 4-5
CHANGE: "no insulin equals dying, yes, insulin equals living" to "no-insulin equals dying; yes-insulin equals living."
REASON: more clear with edited punctuation

PAGE: 208 LINE: 1
CHANGE: "preventing" to "presenting"
REASON: wrong word

Daniel Shumer, MD

5/15/2024
Date

**Page 273d**
**Errata Sheet – Continued**

**PAGE: 212 LINE: 9**
**CHANGE: "page 241" to "page 247"**
**REASON: In review of the source material, the page number related to the discussion is wrong**

**PAGE: 220 LINE: 18**
**CHANGE: "deep diminishment" to "diminishment" (omit the word deep)**
**REASON: I don't believe I used the word "deep" because that doesn't make sense; perhaps the transcript caught a stutter, di- diminishment?**

**PAGE: 228 LINE: 9**
**CHANGE: "Mr. Mills" to "Ms. Williams"**
**REASON: The wrong lawyer is quoted, it should be Ms. Williams objecting to the question posed by Mr. Mills.**

**PAGE: 229 LINE: 13**
**CHANGE: "produces" to "reduces"**
**REASON: wrong word**

**PAGE: 238 LINE: 5**
**CHANGE: "27" to "20.7"**
**REASON: wrong number**

**PAGE: 269 LINE: 3**
**CHANGE: "concorded" to "concordant"**
**REASON: wrong word**

Daniel Shumer, MD

5/15/2024
Date

# EXHIBIT 40

Ex. 1



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# Advances in the Care of Transgender Children and Adolescents

**Daniel E Shumer, MD, MPH**[1], **Natalie J Nokoff, MD**[2], and **Norman P Spack, MD**[3]

[1]Assistant Professor, Department of Pediatrics and Communicable Diseases, Division of Pediatric Endocrinology, University of Michigan Health Systems, Ann Arbor, MI

[2]Fellow, Department of Pediatrics, Division of Pediatric Endocrinology, University of Colorado School of Medicine, Aurora, CO

[3]Senior Associate, Endocrine Division, Boston Children's Hospital, Boston, MA; Associate Clinical Professor of Pediatrics, Harvard Medical School, Boston, MA

## Abstract

Children and adolescents with gender dysphoria are presenting for medical attention at increasing rates. Standards of Care have been developed which outline appropriate mental health support and hormonal interventions for transgender youth. This article defines terminology related to gender identity, reviews the history of medical interventions for transgender persons, outlines what is known about gender identity development, and reviews mental health disparities faced by this patient population. We provide an overview of medical management options for transgender adolescents meeting diagnostic criteria for gender dysphoria including pubertal suppression, cross-sex hormones, longitudinal screening and anticipatory guidance. We describe current challenges in the field and provide information about how care is currently being provided in the US and Canada. We conclude with 5 brief case examples.

### Keywords

Gender dysphoria; transgender; gender identity; adolescent; child

## Introduction

The World Professional Association for Transgender Health (WPATH) first published Standards of Care for the heath of transsexual, transgender, and gender-nonconforming people in 1980, with the 7th Edition released in 2012.[1] In 2009, The Endocrine Society issued a clinical practice guideline for the treatment of transsexual persons, including support for pubertal suppression and cross-sex hormones in carefully screened and supported transgender adolescents.[2] In the 35 years since the publication of the first edition of the WPATH standards, transgender issues have emerged from the periphery of the general

Corresponding author: Daniel E Shumer, MD, MPH, Assistant Professor in Pediatrics, Division of Pediatric Endocrinology, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-5175, Phone: 734-764-5175, Fax: 734-615-3353.



Shumer
EXHIBIT NO. 1
FOR IDENTIFICATION
DATE: 4/26/24
Leisa Pastor, CSR, CRR

conscious to a center stage cultural, human rights, and medical topic in both lay media and scientific inquiry.[3,4] Gender management clinics have emerged to assess, support, and provide medical treatment for transgender adolescents across Europe and North America.[5–9] As transgender issues continue to emerge to the forefront of the public consciousness, the public is expecting knowledgeable, competent, and comprehensive mental health and medical care. Yet, only a minority of medical schools offer curriculum related to transgender-specific care.[10] This mismatch between provider education and patient expectation has left providers and health systems struggling to develop appropriate clinical care systems. This article will define critical terminology in the field, describe what is known about gender identity development, outline the current mental health disparities faced by transgender persons in general and youth specifically, address current guidelines regarding medical treatment of the pediatric transgender patient, highlight persisting challenges and barriers to care, and conclude with case examples.

## Definitions and Epidemiology

*Gender identity* describes one's internal feeling of gender, for example, boy or girl, man or woman, agender (identifying as having no gender), or a non-binary understanding of one's gender. This is in contrast to *biologic sex*, which is describes the chromosomal, hormonal, and anatomic determinants which result in characterizing people as male or female. A *transgender* person feels a discrepancy between their sex assigned at birth and their gender identity.[11] The term *cisgender* has subsequently been introduced to describe individuals who have a gender identity congruent with or the same as their sex assigned at birth. *Gender role* or *gender expression* describes how a person presents themselves as masculine or feminine in the context of societal expectations. *Gender attribution* describes the process whereby other observers view a person as masculine or feminine. For example, a transgender woman who appears masculine due to the development of male secondary sex characteristics may have a male *gender attribution* and struggle with "passing" as an affirmed woman. Finally, *sexual orientation* describes the persons one finds sexually desirable, for example, homosexual, heterosexual, bisexual, pansexual or asexual.[12]

*Gender dysphoria in childhood* and *gender dysphoria in adolescents and* adults are defined in the Diagnostic and Statistical Manual of Mental Health Disorders, Fifth Edition (previously referred to *gender identity disorder in previous editions*).[13,14] Both children and adolescents meet diagnostic criteria for gender dysphoria if they experience a marked difference between their experienced and assigned gender which persists for at least 6 months, and causes significant distress or impaired functioning.[14] A *transsexual* person, as defined by the WPATH Standards of Care, describes "individuals who seek to change or have changed their primary and/or secondary sex characteristics through feminizing or masculinizing medical interventions (hormones and/or surgery), typically accompanied by a permanent change in gender role."[1]

As evidenced by the American Psychiatric Association's decision to remove the stigmatizing word "disorder" from the lexicon, replacing *gender identity disorder* (GID) with *gender dysphoria*, there has been evolving depathologization for those whose gender identity differs from their sex assigned at birth. The idea that *gender identity* exists on a continuum and that

*gender diversity* should be celebrated has gained cultural traction and has resulted in greater acceptance of gender non-conforming people in certain communities. See Table 1 for a list of commonly used terms.

The prevalence of gender dysphoria has been difficult to estimate. A calculated prevalence from the Netherlands in 1996 suggested 1 per 11,900 natal Dutch males and 1 per 30,400 natal Dutch females were transsexual.[15] However, the frequency of new referrals to pediatric gender programs suggests that these numbers understate the current prevalence in the US. In addition, the proportion of natal male and natal female referrals appears to be closer to 1:1, conflicting with Dutch epidemiologic data.[5] In dramatic contrast to the Dutch data, a recent survey of 28,662 adults in Massachusetts found 0.5% self-identifying as transgender.[16] We suggest that as societal acceptance of gender diversity continues to advance and as barriers to care are removed, the transgender population will grow dramatically.

## Historical Perspectives

Prior to the isolation of sex hormones, their development into an injectable or oral compound to be administered, and development of surgical techniques, there were no options to change one's secondary sex characteristics. Charles-Édouard Brown-Séquard was among the first to conceptualize that hormones, or substances, may be secreted by a gland and enter the bloodstream to affect distant organs. He claimed to have injected himself with an extract derived from dog and guinea pig testes.[17]

Testosterone was discovered in 1935[18] and was synthesized from cholesterol soon after.[19] Estrone was isolated in 1929–1930 from the urine of pregnant women in the US[20] and Germany[21] with the discovery of estriol shortly afterwards.[22] Progesterone was discovered in 1934 by multiple groups.[23,24] The first orally active progestin was synthesized in 1938 and named "ethisterone" and was significantly androgenic.[25] The same group later synthesized estradiol, termed "ethynylestradiol,"[26] which was widely used for decades including in the care of transgender women.[27]

Magnus Hirschfeld was a Jewish German physician and sexologist who is known for advocating for the rights of homosexuals in turn-of-the century Germany. He coined the term "transvestite" and opened the Institute for Sexual Research in 1919.[28]

The first "modern" orchidectomy was performed in 1930 for a Danish natal male who sought a sex change. She then went a penectomy, implantation of ovarian tissue and vaginoplasty.[29] There are older examples from history, for example, the Hijiras, an Indian Caste of men who lived as women and underwent ritual castration or a surgery performed in Australian aboriginal men to create a rudimentary vagina.[30] There were additional published cases of penectomy for gender dysphoria in the 1940s–1950s in Germany.[30]

The first widely published case in of a transgender female in the United States was Christine Jorgensen, who appeared on the front page of the *New York Daily News* on December 1, 1952.[31] Christine, formerly George, served in World War II and after returning from war, started taking feminizing hormones. She underwent castration and sex reassignment surgery in Denmark, and later had a vaginoplasty in the United States.[32]

The earliest case reports in the medical literature of surgical treatment of a transgender individual were in Germany in 1940s[33] and in JAMA in 1953 by Danish physicians.[34] A 24 year old natal male presented with a desire to more fully live as a woman and was treated with estradiol monobenzoate injections and oral ethinyl estradiol. Per the patient's wishes, she underwent castration after permission was granted by the Danish Ministry of Justice.[34] Following the initial operation, the patient had a penectomy and plastic surgery of the scrotum to construct "labia-like formations." A vaginoplasty was not performed and not desired by the patient. The authors were ahead of their time, calling upon the "medical profession and authorities" to show a "more positive attitude toward the efforts at easing and facilitating the daily life of the victims of genuine transvestism" with an outline of suggestions to make this possible,[34] which resonate with current recommendations.

Harry Benjamin was a German-born sexologist and endocrinologist who knew Magnus Hirschfeld and became widely known for his 1966 book, The Transsexual Phenomenon.[35] He treated Christine Jorgensen. In 1979, the Harry Benjamin International Gender Dysphoria Association was formed, now re-named the World Professional Association for Transgender Health. The first "standards of care" were published in 1979, now in their 7th version.[1]

The first female-to-male (FTM) sex reassignment surgeries were performed at Johns Hopkins in 1966 after the Gender Identity Clinic was formed. The psychologist and sexologist John Money helped found the clinic and was later widely criticized for the John/Joan case.[36] Thousands of gender affirmation surgeries were later performed by Dr. Stanley Biber in Trinidad, Colorado, which was later coined, the "sex change capital of the world." He performed his first sex reversal surgery in 1969 at his patient's request and after learning from sketches of surgical procedures.

In the 1980s, GnRH agonists were first used for the treatment of central precocious puberty,[37] and would prove to be a key treatment option for younger transgender patients. Prior to the late 1990s, treatment of children or adolescents with gender dysphoria was not considered. In 1998, Drs. Cohen-Kettenis and van Goozen in the Netherlands published a report of a FTM transgender patient treated with triptorelin, a GnRH agonist, starting at age 13 years.[38] The Dutch practice methods of using pubertal suppression followed by cross-sex hormones for transgender adolescents subsequently became incorporated into the WPATH and The Endocrine Society Standards of Care.[1,2]

## The Development of Gender Identity

Expectant parents can now learn the chromosomal sex of their fetus with first trimester cell free fetal DNA and the anatomic sex on the second trimester ultrasound.[39] Many parents then spend the next few months preparing a nursery adorned in pink or blue, excited to welcome their new son or daughter into the world. The baby is born into a gendered world, where boys and girls dress differently and are often encouraged to pursue gender-specific games or styles of play. While these stereotypical gender roles vary by culture and change over time, the different expectations of boys and girls are thought to impact the development of gender identity. Children as young as 2 years learn to label themselves as a boy or a girl,

and by age 4–5, are able to understand that gender is a stable and lasting aspect of their identity.[40] Boys and girls have group differences in toy preference by as early as 12 months and can label other children as boys or girls by age 2.[41]

Development of gender identity development is complex, and likely a multifactorial process involving genetic, hormonal, and environmental factors. John Money and Anke Ehrhardt proposed the idea of brain sex,[42] which has drawn controversy.[43] Several brain structures appear to be sexually dimorphic,[44] which has led researchers to study whether transgender individuals have brain structures that more closely align with their affirmed gender. In one study, the volume of the bed nucleus of the stria terminalis in male-to-female (MTF) transgender persons was equivalent to the volume found in cisgender women.[45] However, others argue that such "dimorphisms" are better thought of as small differences with significant overlap.[43]

Studies of heritability of transgender identity have suggested that genetic factors may contribute to gender development. For example, in a recent review of twin studies, of 23 monozygotic male and female twin pairs where at least one twin met criteria for GID, 9 twin pairs (39.1%) were concordant for GID.[46] Studies have failed to firmly establish causative genes.[6]

The hormonal milieu of the developing fetal brain and its role on later gender identity has been another area of active research. Much of this research has been driven by the study of persons with disorders of sex development (DSD). Sex hormones, primarily androgens and estrogens, affect sex-specific changes in the developing fetus. During fetal life and infancy there are significant sex-specific differences in the normal concentrations of these hormones. It has been posited that these differences may contribute to group differences in behaviors observed between males and females later in life.[47,48] Populations of patients with various DSDs have served as natural experiments for this hypothesis. For example, infants with a 46,XX karyotype and congenital adrenal hyperplasia are most often raised as girls but have had fetal exposure to higher than normal concentrations of circulating androgens. In a meta-analysis, 5% of those assigned and raised female had gender dysphoria or a male gender identity, suggesting that prenatal androgen exposure may influence the development of a male-typical gender identity.[49] In another example, of 14 patients with 46,XY karyotype and cloacal extrophy raised female, 8 (57%) subsequently affirmed a male gender identity.[50] These and other studies (see Rosenthal[6] for a more complete review) suggest that the prenatal hormonal milieu, especially fetal androgen exposure, may play a role in gender identity development. Yet, the vast majority of transgender persons do not have an identified DSD or endocrinopathy.

Finally, individual environmental factors may influence the development of gender dysphoria. It has been suggested that the social relationship between the parent and infant[41] and cognitive learning about parental expectations and societal norms[51] contribute to gender development in all children. The observation that children with autism spectrum disorder (ASD) are disproportionately affected by gender dysphoria has contributed to the discussion of environmental factors and gender identity. Children with ASD may, as a result of social

cognitive impairment, feel less societal pressure to conform to their assigned sex at birth which may manifest as persistence of gender dysphoria.[52]

Children referred for assessment due to gender non-conformity may demonstrate gender non-conforming behaviors at a very young age, sometimes as early as 3 years.[53] Others persons may disclose a transgender identity later in adolescence or adulthood, without a history of gender non-conformity in early childhood.[6,54] Young children who are gender non-conforming or who identify as transgender may or may not continue to identify as transgender as adolescents and adults. In fact, there is evidence to suggest that for a majority of young children with cross-gender identity, this identity does not persist into adolescence; at the time of puberty their transgender identity may desist and perhaps evolve into a gay or lesbian sexual orientation.[55,56] However, those who have persistence of transgender identity and/or worsening of gender dysphoria in puberty are thought to be much less likely to identify as cisgender as adolescence continues. Clinicians can use worsening gender dysphoria at the onset of puberty as a diagnostic tool of persistent transgender identity and as a criterion for eligibility for medical intervention.[57,38]

There have been efforts to identify factors to differentiate prepubertal children who will persist in their transgender identity during adolescence and adulthood versus those who will desist. In a study of 53 adolescents in the Netherlands, those who persisted versus desisted in their gender identity had similar gender variant expression in childhood. Yet, those who experienced increased dysphoria in adolescence, starting between 10–13 years, were more likely to have a stable transgender identity. Important factors in early adolescence included: the social environment, feelings toward pubertal changes, and the emergence of sexual attraction.[58] Additional study of desistance versus persistence suggested that children who persist may have more severe symptoms of gender dysphoria in childhood and are more likely to undergo a social transition in childhood (live as the affirmed gender).[59] The uncertainty of future persistence, coupled with the idea that acceptance of a transgender identity in early childhood may be associated with persistence of transgender identity in adolescence and adulthood has led to controversy regarding the appropriate counseling and mental health treatment strategies for prepubertal children with gender dysphoria.

## Mental Health

Transgender persons continue to be disproportionately affected by bias, persecution, and harassment,[60] and have alarmingly high rates of depression, anxiety, self-harm behaviors and suicidality. A staggering 41% of transgender adults have attempted suicide. Rates of suicide attempt are higher among non-white transgender adults, those who are unemployed or underemployed, poor, less educated, and young.[61] Transgender youth who experience verbal and physical abuse are more likely to attempt suicide,[62] and transgender individuals are disproportionately victimized by physical abuse.[63] Transgender youth also have higher rates of alcohol, tobacco, cannabis, and other drug use,[64] and MTF persons, in particular, have higher rates of sex work and HIV.[65] In a recent study of mental health disparity, transgender youth had two- to threefold increased risk of depression, anxiety disorder, suicidal ideation, suicide attempt, self-harm behaviors, and utilization of both inpatient and outpatient mental health services compared to cisgender youth.[66]

The 2011 National School Climate Survey of LGBT youth surveyed over 8,500 students ages 13–20 years in the US, 700 of whom identified as transgender. Eighty percent of the transgender students reported feeling unsafe at school because of their gender expression and over half of gender nonconforming students had experienced verbal harassment. School policies that affect transgender students include school dress codes, gender segregated sports and physical education, gender segregated bathrooms and locker rooms, gendered pronouns, and binary-only options on school forms.[67]

It is therefore not surprising that youth presenting to gender management clinics are disproportionately affected by mental health comorbidities. At Boston Children's Hospital's Gender Management Services program, patients had a high prevalence of diagnosed psychiatric comorbidities (44%), history of self-mutilation (21%), history of psychiatric hospitalization (9%), and history of suicide attempt (9%).[5] Among 101 transgender youth ages 12–24 followed at the Center for Transyouth Health and Development at Children's Hospital Los Angeles, 15% had mild depression, 9% had moderate depression, and 11% had severe depression as rated on the Beck Depression Inventory. Half reported having thoughts about suicide, while 30% had attempted suicide.[64] As noted above, rates of ASD disorder may also be elevated among children and adolescents presenting with gender dysphoria, with a rate of 7.8% reported from the gender program in the Netherlands, a rate exceeding that in the general population.[68]

There is a lack of consensus among mental health providers regarding the goals of mental health treatment in pre-pubertal children.[12] Some argue that therapeutic goals should focus on reduction in dysphoria and acceptance of the biologic sex.[69] Affirmative approaches help families to support a child's transgender identity and assist children and families with the logistics of making a social transition.[70] There is less controversy about treatment goals for pubertal adolescents. Pubertal adolescents are less likely to desist, and supportive trans-affirmative mental health support is encouraged. The American Psychological Association recently published "Guidelines for Psychological Practice with Transgender and Gender Nonconforming People" containing 16 guidelines recommended for psychologists to assist with "culturally competent, developmentally appropriate, and trans-affirmative psychological practice."[71]

The WPATH Standards of Care and The Endocrine Society clinical practice guidelines describe comprehensive approaches aimed to mitigate mental health disparities and improve outcomes. Data from a pioneering Dutch group suggests that adolescents followed by a multidisciplinary gender team and treated with pubertal suppression followed by cross-sex hormones had improvement in psychological function with mental health outcomes in young adulthood similar to the general Dutch population.[72,73] The Endocrine Society guidelines recommend that children and adolescents with gender concerns be seen by a mental health professional with training in child and adolescent developmental psychology. The mental health professional should: 1) determine whether the individual fulfills DSM criteria for gender dysphoria; 2) inform the individual with respect to possibilities and limitations of sex reassignment and other treatments; and 3) assess for potential psychological comorbidities.[2] The WPATH Standards of Care requires adolescents meet eligibility and readiness criteria before proceeding with hormone treatments; medical interventions can be initiated only after

a referral from a qualified mental health professional.[1] Many multidisciplinary clinics require such documentation before hormones are prescribed. However, mental health providers with expertise in this area are limited, and many transgender youth may not have access to such providers given location, insurance coverage and cost.

## Sex Differentiation and Normal Puberty

Testosterone and estrogen are produced in the testes and ovaries beginning in early fetal life. Testosterone production in the fetus, and its subsequent conversion to dihydrotestosterone, leads to virilization of genital tissues and development of male genitalia. Absence of testosterone results in female external genitalia.[74]

After the "mini-puberty" of infancy, sex hormone production within the gonads enters a quiescent stage.[75] There is little difference in the hormonal milieu between prepubertal males and females, therefore, hormonal interventions are not indicated in prepubertal transgender children. The transgender prepubertal child can instead focus on better understanding their gender identity with the aid of a mental health professional and their family. When a prepubertal child makes a social transition, presenting themselves as their affirmed gender, their ability to "pass" as their affirmed gender is aided by the fact that they have not yet developed secondary sex characteristics.

Puberty, the life stage characterized by the development of secondary sex characteristics, begins with the activation of the gonadotropin releasing hormone (GnRH) pulse generator within the hypothalamus. Pulsatile GnRH leads, in turn, to pulsatile production luteinizing hormone (LH) and follicle stimulating hormone (FSH) within the anterior pituitary gland. LH inspires production of testosterone in testicular Leydig cells. It also leads to production of androgens in ovarian theca cells, which are then converted to estrogen. FSH causes germ cell maturation and testicular enlargement in males and the growth and recruitment of ovarian follicles in females.[76,77] Male puberty, driven by testosterone and dihydrotestosterone, is characterized by enlargement of the testes and phallus, development of facial and body hair, enlargement of the larynx and deepening of the voice, increase of lean muscle to fat ratio, and skeletal changes such as masculinization of the facial bones and jaw and widening of the shoulders. In female puberty, estrogen production results in development of glandular breast tissue and redistribution of fat to the buttock and hips. Ovarian and endometrial development leads to menarche.[78]

The onset of central puberty can be assessed clinically by the development of testicular enlargement and breast budding in biologic males and females respectively. The beginnings of testicular enlargement and thinning of the scrotal skin in biologic males, and the development of breast budding in biologic females, are hallmarks of sexual maturity rating or SMR (Tanner stage) 2. Pubic hair development and the development of apocrine body odor may develop prior to central puberty as a result of adrenal androgen production. These changes by themselves should not be considered evidence of central puberty.[79,80] The average age of onset of puberty is 10–11 years in females and age 11–12 years in males. Height velocity increases during puberty and peaks about 2.5 years after the start of the pubertal growth acceleration.[81] In biologic males, characteristics significantly affecting

gender attribution, such as facial hair development, completion of voice change, and masculinization of facial bones, occur later in puberty compared to genital development. The lateness of these changes within normal male puberty provides incentive for pubertal suppression in MTF individuals presenting in late puberty. In FTM individuals, breast development typically progresses from SMR 2 to 5 (fully developed) within 4–5 years and menses typically begin 2–2.5 years after breast budding.[78]

## Overview of Medical Management

The WPATH standards of care and The Endocrine Society clinical practice guidelines both recommend the diagnosis of gender dysphoria be made by a mental health professional with expertise in gender identity prior to considering a hormonal intervention.[1,2] Some multidisciplinary gender programs employ mental health professionals to perform assessments for referred patients; other programs rely on community-based mental health providers to make the diagnosis of gender dysphoria.[82] Primary goals of medical interventions include: (1) prevention of the development of unwanted secondary sex characteristics of the biologic sex, and (2) promotion of the development of desired secondary sex characteristics of the affirmed gender. Broader objectives include reduction in dysphoric feelings, reduction in co-morbid depression, anxiety and suicidality, and enhanced ability to "pass" as the affirmed gender with subsequent improvement in quality of life and general functioning.

## Prevention of the Development of Unwanted Secondary Sex Characteristics

Medical interventions that suppress sex hormone production, or that block sex hormone action, work to prevent the development of undesired secondary sex characteristics of the biologic sex (Table 2). These interventions include pubertal suppression using GnRH agonists, reduction in biologic hormone production using progestins, and use of androgen receptor antagonists such as spironolactone.[6]

Use of a GnRH agonist to completely suppress puberty starting at SMR 2 followed by introduction of cross-sex hormones in later adolescence was first described by a pioneering gender center in Amsterdam, The Netherlands.[57,38] The rationale for using GnRH agonist medications to suppress puberty include the following: (1) it allows a transgender adolescent protected time to explore their gender identity with their mental health professional and family without continued progression into their biologic puberty; (2) halting progression of puberty appears to improve behavioral and emotional problems, and reduces depressive symptoms;[72] (3) preventing the development of secondary sex characteristics of the biologic puberty can improve the ability to pass as the affirmed gender and obviate the need for procedures such as masculinizing chest surgery in biologic females, and electrolysis of facial and body hair, feminizing facial surgeries, and voice therapy in biologic males.

For example, a FTM patient who starts on GnRH agonist medication at SMR 2, and then starts on testosterone in later adolescence, may not require masculinizing chest surgery and will also forgo menstruation. If suppression occurs at SMR 3 or 4, prior to full breast

development, a less invasive chest surgery (for example, through an areolar incision rather than an inframammary incision) may be considered. A FTM patient presenting after full breast development has occurred would get less benefit from GnRH agonist treatment. While a GnRH agonist would suppress dysphoric menses, other more cost effective interventions, such as treatment with a progestin, may accomplish a similar result.

For MTF, use of GnRH agonist medication prior to the development of male secondary sex characteristics can dramatically improve gender attribution, the ability to pass as the affirmed female gender. For example, a MTF who starts on GnRH agonist medication at sexual maturity rating 2, who continues on it as estrogen therapy is initiated in later adolescence, and then proceeds with gonadectomy and vaginoplasty after age 18, will never develop masculine facial and body hair, will not have a deep voice, and will not have masculinization of the facial bones and skeletal frame.[12]

Both WPATH and The Endocrine Society Guidelines recommend consideration of GnRH agonist therapy only after the start of puberty (SMR 2).[1,2] Use of pubertal suppression to prevent puberty from starting, starting at SMR 1, is not recommended. This is because persistence of gender dysphoria during early puberty can be used as an important diagnostic tool, predicting continued transgender identity in older adolescence and adulthood. Additionally, starting at SMR 1 would add unnecessary treatment and cost for a prepubertal patient not requiring pubertal suppression.

GnRH agonist medications have been used extensively in the pediatric age group for treatment of precocious puberty for over 25 years. They are considered safe and reversible medications.[83] In the transgender population, theoretical risks include reduction in bone mineral density z-score while on treatment. However, new evidence suggests bone density accrual improves after starting treatment with cross-sex hormones.[57] Although the effects of GnRH agonists are reversible, they are often started with the intent of initiating cross-sex hormones later on, and the combination of the two results in permanent and semi-permanent effects. It is important that families receive counseling regarding the fertility effects of GnRH agonists and cross-sex hormones. A child who starts on GnRH agonist therapy at SMR stage 2 and continues on the mediation as cross-sex hormones are introduced later in adolescence will never have spermatogenesis or menarche, and will not have the opportunity to bank gametes using cryopreservation. Yet for many patients and families, after appropriate informed consent, the benefits of pubertal suppression still outweigh the risks.[1,2] GnRH agonists can be continued during treatment with cross-sex hormones. For example, a MTF individual may be concurrently treated with a GnRH agonist and estrogen until gonadectomy is performed, at which point GnRH agonist therapy would no longer be needed. A FTM individual may use a GnRH agonist and testosterone until masculinizing chest surgery, at which point monotherapy with testosterone should suffice to prevent continued menstruation.

GnRH agonists provide a constant level of stimulation to the GnRH receptor and, as a result, inhibit the pulsatile secretion of LH and FSH from the anterior pituitary. Common forms of administration include an intramuscular injection administered every 30 or 90 days (leuprolide acetate) or a subcutaneous implant, replaced annually (histrelin acetate). In our

experience, histrelin acetate implants in either the pediatric preparation (distributed in the US as Supprelin®, designed to deliver 65 mcg per day of active medication) or adult preparation (distributed in the US as Vantas®, designed to deliver 50 mcg per day of active medication) are both effective at suppressing puberty in transgender adolescents for longer than one year. GnRH agonists can also be given as intranasal preparations; however, there are no reports of use of this preparation in transgender individuals. Choice of GnRH agonist preparation in the US is often based on availability, insurance coverage, patient age and patient and family preference. We have often used Vantas® in situations where insurance coverage is denied because it is more affordable for out-of-pocket payment compared to other preparations. The use of any GnRH agonist preparation for pubertal suppression in transgender adolescents is considered "off-label" in the US. The Food and Drug Administration has not listed gender dysphoria as a clinical indication for their use, despite the fact that this is current standard of care.

In addition to GnRH agonists, other medications that reduce the production of sex hormones or inhibit their actions can be useful in the transgender adolescent. Even prior to the development of GnRH agonist medications, progestins, more specifically medroxyprogesterone acetate, had been used in the treatment of precocious puberty to suppress sex hormone production.[84] Progestins, including medroxyprogesterone acetate and norethindrone, reduce the pulsatile release of LH and also directly inhibit sex hormone production at the level of the gonad.[6] Medroxyprogesterone acetate can be given as an intramuscular injection every 3 months (Depo-Provera®) or as a daily oral medication (Provera®), and norethindrone as a daily oral medication (as Micronor® or Aygestin®). In our experience, treatment with progestins have been especially helpful in a few specific situations: (1) in a FTM individual who has already completed breast development and started menstruating, but who is either too young or still in the process of considering treatment with testosterone. In this situation, treatment with a progestin can aid in reducing dysphoria by suppressing menses; (2) in a MTF individual who has started on cross-sex hormone therapy with estradiol and who cannot receive GnRH agonist therapy due to lack of insurance coverage. In this situation, if the estrogen monotherapy is insufficient to bring testosterone down to a level which would support normal breast development, use of estrogen therapy with concurrent use of a progestin can help to promote normal breast development and minimize further masculinization from testicular production of testosterone. Note that, conversely, a FTM individual on monotherapy with testosterone will most often have adequate suppression of menses and should not require pubertal suppression with GnRH or treatment with a progestin.

Finally, spironolactone is an oral medication most commonly used as a weak diuretic, which also acts as a weak androgen receptor antagonist. This medication can be used by MTF individuals to reduce effects of testicular androgen production.[6] We most commonly use spironolactone when the patient is troubled by the development of facial and body hair. While spironolactone will not cause regression of the terminal hair follicles, patients on spironolactone therapy may require less frequent shaving or electrolysis treatments. Cyproterone acetate is another antiandrogen medication not approved for use in the US, but used in MTF patients in other countries.[6]

## Promotion of the Development of Desired Secondary Sex Characteristics

The use of hormonal interventions, often referred to as *cross-sex or gender affirming hormones*, to promote the development of desired secondary sex characteristics in transgender persons can be considered in carefully screened and counseled adolescents with gender dysphoria (Table 2). Specifically, the use of 17β-estradiol in MTF individuals, and testosterone in FTM individuals, are used to induce the development of the secondary sex characteristics of the affirmed gender. Broad goals of treatment are to improve psychological functioning and general well-being, and enhance the patient's ability to present as their affirmed gender in social life. The WPATH standards of care do not specify an age at which cross-sex hormones can be administered, but suggest that obtaining parental consent.[1] The Endocrine Society suggests that cross-sex hormones can be considered "around age 16."[2] In our practice, we have found that for many patients there is significant psychosocial risk in waiting until age 16 years to start cross-sex hormones if the patient is otherwise stable in their transgender identity. It is therefore our practice, and the practice of similar institutions, to consider cross-sex hormone treatment initiation as young as age 14 years.[5,6]

MTF individuals are treated with 17β-estradiol to induce female secondary sex characteristics. Treatment with 17β-estradiol will promote the development of breast tissue and development of a more feminine body habitus. These changes are more effective when testosterone production is reduced, either by using GnRH agonist medication or a progestin concurrently. Higher doses of 17β-estradiol would be required to produce feminizing changes if the testosterone concentration is in the normal male range.

17β-estradiol is available in oral, sublingual, transdermal, and intramuscular preparations.[6] We prefer to use oral or transdermal 17β-estradiol. In a patient who is concurrently being treated with GnRH agonist, we would use oral 17β-estradiol (Estrace®) 0.5 mg daily and increase gradually to 2 mg daily, with dose increases every 4–6 months, or transdermal 17β-estradiol (such as Climara® or Vivelle-Dot®) starting at 12.5 or 25 μg weekly. In our practice, adolescent patients on GnRH agonist therapy concurrent with 17β-estradiol are able to achieve normal breast development without need or desire for later breast modification surgery. Similar results may be possible using a combination of medroxyprogesterone and 17β-estradiol or norethindrone and 17β-estradiol. Without any concurrent suppression using GnRH agonist or progestin, patients require higher doses of estrogen to suppress testosterone production and overcome its androgenic effect on the breast tissue. Cosmetic results may be less favorable and higher dose estrogen therapy carries thrombogenic risk. Once a patient undergoes gonadectomy as part of gender confirmation surgery, monotherapy with 17β-estradiol is sufficient. Additionally, some centers use progesterone concurrently with estradiol to improve breast development, although the effects have not been adequately studied.

FTM individuals are prescribed testosterone to promote the development of male secondary sex characteristics. Testosterone is available via many different preparations including intramuscular, gels and creams, and patches. Testosterone for pubertal induction has classically been given as an intramuscular preparation (as testosterone cypionate or testosterone enanthate). Intramuscular testosterone, when used for male pubertal induction,

is often used starting at 25 mg every 2 weeks with gradual dose increases to 100–200 mg every 2 weeks. Many centers use testosterone cypionate or testosterone enanthate administered as a subcutaneous injection administered by the patient or his parent weekly. It can be started at 12.5–25 mg weekly increasing to 50–100 mg weekly.[6] Doses are adjusted to keep the testosterone concentration in the normal male range for age, and based on clinical response. The subcutaneous method allows for in-home administration after a brief in-office education on subcutaneous administration technique. Because testosterone for injection is suspended in oil, it does not draw readily through a standard insulin syringe. Instead, a thicker gauge needle, such as a 21-gauge-needed for drawing, and a 25-gauge needle for injecting, must be prescribed for administration.

## Longitudinal Screening and Anticipatory Guidance

Patients being treated with pubertal suppression, spironolactone, 17β-estradiol, and/or testosterone require continued support from a mental health professional, longitudinal follow-up to assess clinical response and development of untoward side effects of treatment, and laboratory monitoring. Rosenthal suggests that patients undergoing pubertal suppression using GnRH agonist medication should have a physical exam including monitoring of height, weight, and pubertal staging, as well as biochemical assessment of puberty using LH, FSH and estradiol or testosterone measurement every 3 months and a bone age evaluation annually. Additionally, due to the delay in bone density accrual in patients undergoing pubertal suppression, it is advised to follow bone health using measurement of calcium, phosphorus, alkaline phosphatase, and 25-hydroxyvitamin D annually, as well as consideration for dual-energy X-ray absorptiometry (DEXA) annually.[6] Spironolactone can cause hyperkalemia, therefore, we obtain a baseline electrolyte panel and repeat with each dose adjustment and when obtaining other laboratory evaluations. In patients prescribed 17β-estradiol or testosterone, Rosenthal suggests clinical follow-up every 3 months to assess height, weight, blood pressure, and pubertal progression. At these visits, LH, FSH, and estradiol and/or testosterone can be assessed. In addition, he suggests following calcium, phosphorus, alkaline phosphatase, 25-hydroxyvitmain D, complete blood count, renal function, liver function, fasting lipids, glucose, insulin and hemoglobin A1C, plus prolactin in male-to-female patients.[6] Patients who had puberty suppressed and who are subsequently being treated with cross-sex hormones can also be monitored for gains in bone density using DEXA.[6]

While long-term health data is sparse with regards to adolescents, some data exists in the adult transgender literature. In longitudinal studies of FTM adults, testosterone administration (250 mg IM every 2 weeks) is associated with lower high-density lipoprotein (HDL) and higher triglycerides after 6–12 months of treatment compared to baseline.[85–87] However, long-term testosterone administration does not appear to alter fasting insulin or glucose utilization in FTM adults compared to a pre-testosterone baseline assessment.[86] A Dutch study of FTM adults on testosterone did not suggest an increased incidence of cardiovascular events or increased mortality compared to the general population.[88]

Elevated blood pressure, fasting insulin and decreased insulin sensitivity have been reported in MTF adults treated with ethinyl estradiol.[86,87] Treatment of MTF adults with ethinyl

estradiol has also been associated with increased risk of cardiovascular death.[88] After 1990, 17β-estradiol was used in the Netherlands in favor of ethinyl estradiol due to its more favorable cardiovascular risk profile.

We advise discussing potential impairment to fertility, not only prior to starting cross-sex hormones, but also prior to starting pubertal suppression. Even though pubertal suppression using GnRH agonist medications by themselves do not impair future fertility, their use combined with cross-sex hormones does impair fertility. In our experience, many adolescent patients, even those who are not transgender, are often reticent to discuss their future fertility. The conversation can be more complex in transgender adolescents who may have some desire to have biologic children, but who bristle at the idea of using their own anatomy to accomplish this. If a patient has progressed far enough into natal puberty such that cryopreservation of sperm or oocytes is possible, this option should be discussed. Providers treating transgender adolescents should familiarize themselves with cryopreservation options in their community. The cost of preservation methods, especially the preservation of oocytes, is often a significant barrier.

## Gender Affirmative Surgery

Mental health and medical providers caring for transgender adolescents should become familiar with common surgical interventions used in the transgender patient population, and should be knowledgeable about what surgical resources are available in the community. Surgical interventions used in transgender persons for the purposes of transition are often referred to as *gender affirmation* surgeries. Procedures may include genital surgeries, chest surgeries, and a variety of other gender affirming procedures. The most common surgical procedures performed in MTF individuals include breast augmentation surgery, genital surgery including penectomy, orchiectomy and vaginoplasty, facial feminization surgeries, voice surgery, thyroid cartilage reduction, and hair reconstruction. Electrolysis or laser hair removal is also commonly performed. In FTM individuals, surgical procedures include mastectomy, and genital surgeries including hysterectomy/salpingoophorectomy, metoidioplasty with phalloplasty, vaginectomy, scrotoplasty, and implantation of erectile and testicular prostheses. Genital surgeries are typically not recommended until the patient has reached legal age of majority. Chest surgery in FTM patients can be considered earlier.[1]

## Outcomes

Treatment with pubertal suppression in transgender adolescents improves psychological functioning and decreases depressive symptoms, however it does not seem to eliminate gender dysphoria.[72] Long-term outcomes data from the Netherlands suggests that transgender persons treated with pubertal suppression, followed by cross-sex hormones and finally gender affirmation surgery in young adulthood yields positive outcomes with none regretting starting gender affirming medical treatments.[73] In a study primarily sampling from the US, FTM individuals reported diminished quality of life compared to cisgender males and females, however, those who have received testosterone report significantly higher quality of life compared to those who have not.[89]

More robust long-term outcomes data may be necessary for the WPATH and The Endocrine Society recommendations to be more fully adopted, embraced and refined. In addition, these interventions will remain "off-label" in the US until approved by the US Food and Drug Administration. That said, it is evident by the growing demand of these interventions and the increase in pediatric gender programs in the US, that gender affirming medical interventions for appropriately assessed patients has become the standard of care.

## Challenges and Barriers to Care

The National Transgender Discrimination Survey Report on Health and Health Care in 2010 surveyed over 6,000 transgender adults in the U.S. and U.S. territories and found that transgender adults experience discrimination by medical providers, with 19% of respondents reporting that they have been refused care due to their gender identity. Over a quarter responded that they have been verbally harassed in a medical setting and over half had to teach their provider about transgender healthcare. Over a quarter reported postponing or delaying needed either preventive care or care when they were sick or injured.[61] Transgender individuals who belong to racial and ethnic minorities experience more discrimination.[90] Finally, insurance company denial of transgender-related interventions remains a significant barrier to care.[12] There have been efforts to improve resident and medical student education and comfort with taking care of transgender patients,[10,91] including a recent report by the Association of American Medical Colleges on implementation of curricular changes.[92]

## Current Gender Management Programs in the US and Canada

A recent report provides descriptions and contact information for 35 gender programs in the US and Canada.[93] In addition to these programs, several other programs are known to exist by the authors. The descriptions of the various programs in this report makes clear that different centers have approached providing gender services to children and adolescents in diverse ways. For example, providers from the fields of pediatric endocrinology, adolescent medicine, gynecology, primary care, and nurse clinicians are working in these programs to provide hormonal interventions. Programs often employ mental health providers from the fields of social work, psychology and psychiatry to provide individual counseling, assessments, family therapy and/or group therapy. Some programs serve as a primary care medical home for patients, whereas others function as a consultative program.[93]

We suggest that other roles of multi-disciplinary programs could include: providing training programs for hospital staff and other members of the health system, advocating for changes to paper forms and the electronic medical record to make them more gender inclusive, providing education for medical students and trainees, promoting community partnerships, collaborating with and/or providing education to school systems, promoting research, and assisting with transition to adult care.

## Case Examples

### Patient 1

An 11-year-old biologic male presented to the pediatrician with concerns regarding gender identity. The child had been interested in stereotypically feminine toys and play from a very young age, and the parents had assumed that the child would grow up to be a gay man. However, more recently the child has clearly expressed a female gender identity to the parents. The child has declared herself to be transgender and insisted on use of female pronouns at home. The parents noted that school performance had suffered and the child has become withdrawn and depressed over the past year. The pediatrician referred the family to a mental health professional with experience in gender identity in children. After several sessions, the mental health professional confirmed a diagnosis of gender dysphoria and recommended referral to a medical clinic with experience in gender dysphoria. At the clinic, the child was found to be at SMR 2. After discussion of risks and benefits of intervention, the child and family elected to proceed with pubertal suppression. A bone age and DEXA were found to be normal for age, and 25-hydroxyvitamin D was slightly low. A histrelin acetate implant was placed and vitamin D supplementation was initiated. Pubertal suppression continued until age 14. By that time, the child had made a complete social transition, using a female name and pronouns at home and at school, and had been supported by ongoing therapy from her mental health professional. Oral 17β-estradiol was started and pubertal suppression with histrelin acetate was continued. The child proceeded through a normal female puberty on 17β-estradiol treatment. At age 18, she elected to have gender affirmation surgery including orchiectomy and vaginoplasty, at which point histrelin acetate was discontinued.

### Patient 2

A 10-year-old biologic female with characteristically male interests and behaviors became distressed with the development of breast budding. The patient also disclosed a male gender identity to friends, and then to parents. After a diagnosis of gender dysphoria was made by a mental health professional, the child was referred to a gender program. The clinic physician confirmed breast maturity rating 2, and after discussion with the patient and family, suppression of puberty was initiated using leuprolide acetate, administered every 90 days. The treatment halted progression of breast development. At age 15, after the child had made a complete social transition, testosterone enanthate was initiated, administered subcutaneously weekly at home. At age 19, the patient elected to undergo hysterectomy/salpingoophorectomy and leuprolide acetate was discontinued.

### Patient 3

A 15-year old biologic male with female affirmed gender identity presented to a gender clinic after being referred by their primary care physician and mental health professional for treatment of gender dysphoria. The adolescent was found to be at SMR 4. Goals of treatment were determined to be suppression of continued masculinization and promotion of feminization including breast development. The provider attempted to prescribe a GnRH agonist but it was rejected by the patient's insurance. The provider instead prescribed

norethindrone to suppress androgen production, spironolactone to inhibit androgen action, and 17β-estradiol to promote breast development and feminization.

### Patient 4

A 16-year-old biologic female presented to a gender clinic after receiving a diagnosis of gender dysphoria by a mental health professional. The teen was especially dysphoric with monthly menses, but the family was uneasy about committing to irreversible therapy with testosterone. Treatment with norethindrone 5 mg oral daily was initiated, and the monthly menses were suppressed, with resulting improvement in well-being. At age 18, the patient had made a complete social transition and elected to start testosterone, prescribed at 50 mg subcutaneous weekly, at which point norethindrone was discontinued without subsequent return of menses on testosterone monotherapy.

### Patient 5

A 12-year-old biologic male presented to the gender clinic after referral by a mental health professional. The child had been having dysphoric feelings about his male pubertal development, and was found to be at SMR rating 3. Treatment with a GnRH agonist was initiated. The child continued in therapy and by age 14 had developed a better understanding of their gender identity. The child accepts that they do not identity completely with a male or female gender identity, and begins to refer to themself as genderqueer. They prefer to be referred to using the them/they/their pronouns. After discussion with the family and mental health professional, the decision is made to withdraw the GnRH agonist medication and allow male puberty to progress with continued supportive counseling in place.

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**Key points**

- Children and adolescents with gender dysphoria are presenting for medical attention at increasing rates.
- Standards of Care have been developed which outline appropriate mental health support and hormonal interventions for transgender youth.

**Table 1**

Terminology Related to Gender Identity

| | |
|---|---|
| Gender identity | An internal feeling of one's gender as a boy or man, girl or woman, no gender, or a non-binary understanding of one's gender |
| Biologic sex | The genetic, anatomic, and hormonal determinants of sex classified as male or female, or indeterminate due to a disorder of sex development |
| Transgender | Having a gender identity which is not congruent with one's biologic sex |
| Cisgender | Having a gender identity which is congruent with one's biologic sex |
| Transsexual | A term most often used to describe a transgender person who is or has transitioned using hormones and/or surgical procedures |
| Gender non-conforming | Describes a person who behaviors, actions, or interests do not conform to the societal expectations based on their biologic sex |
| Gender role | The stereotypical role which members of each biologic sex are expected to play based on societal norms or expectations |
| Gender dysphoria | A DSM-defined diagnosis describing distress caused by a incongruence between gender identity and biologic sex |
| Agender | A gender identity characterized by feeling no identification with being a boy or man, girl or woman, or any other gender identity |
| Gender fluid | Gender identity which varies over time |
| Genderqueer | A term used by people who do not classify themselves using conventional gender distinctoins, but may instead identify as neither gender, both genders, or a combinatoin of male and female genders |
| Gender attribution | How an observer decides which sex or gender they believe another person to be |

**Table 2**

Medications used in the treatment of transgender adolescents

| ***Prevention of the development of unwanted secondary sex characteristics*** | | | |
|---|---|---|---|
| **Class of medication** | **Medication names** | **Mechanism of Delivery** | **Mechanism of action** |
| GnRH agonists | Leuprolide acetate<br>Histrelin acetate | IM injection<br>SC implant | Inhibition of the HPG axis |
| Progestins | Medroxyprogesterone acetate<br>Norethindrone | Oral or intramuscular injection<br>Oral | Inhibition of the HPG axis |
| Androgen receptor inhibitors | Spironolactone<br>Cyproterone acetate | Oral<br>Oral or intramuscular injection | Inhibition of testosterone action |
| *Promotion of the development of desired secondary sex characteristics* | | | |
| Class of medication | Medication names | Mechanism of Delivery | Mechanism of action |
| Testosterone | Testosterone enanthate<br>Testosterone cypionate<br>Other testosterone | IM injection<br>IM injection<br>Transdermal gels and patches | Activation of androgen receptors |
| 17β-estradiol | 17β-estradiol | Oral or transdermal patch most common; also available as IM injection and sublingual | Activation of estrogen receptors |

Abbreviations: IM: intramuscular; SC: subcutaneous; HPG: hypothalamic-pituitary-gonadal

Ex. 2





# Q & A: What does gender-affirming care mean for kids?

August 4, 2023

Author | Katie Whitney

When Daniel Shumer, M.D., was training as a pediatric endocrinologist, he saw the profound effect of compassionate, gender-affirming care. Patients and their parents came in looking nervous and scared, "some even seeming a little embarrassed to be talking about these topics," he says.

"Those same patients and families, when they were leaving the room, felt heard, relieved, proud, and hopeful." That's what inspired Shumer, who is associate professor of pediatrics and medical director of the Gender Services Program at C.S. Mott Children's Hospital, to become an expert in gender-affirming care. We talked with him about how to care for kids who are experiencing gender differences.

**What is the difference between sex and gender?**

First of all, everyone has a gender identity, which is how I know myself inside as a man or woman, boy or girl, or somewhere else on the gender spectrum. Gender identity is something you can't measure with a blood test or X-ray. It's only something a person can tell you about themselves from their lived experience.

When I hear the word "sex," to me that's a term that's trying to separate human beings into two categories, male and female. And it turns out that, while that seems simple, it's actually pretty complicated. We have chromosomes, hormones, and anatomy. A lot of times, all these things line up, but sometimes they don't.

In my opinion, gender is a component of sex. Someone's gender identity can help inform that person's sex. We know there are biological influences of gender identity, and even though gender identity is something we can't measure, that doesn't make it any less real or valid than something like chromosomal sex.

**What is the difference between gender and sexuality?**

Shumer
EXHIBIT NO. 2
FOR IDENTIFICATION
DATE: 4/2-24
Leisa Pastor, CSR, CRR

Sexual orientation refers to the people that a person finds physically or romantically attractive. Oftentimes we think of terms like straight, gay, or bisexual, as opposed to gender identity terms such as cisgender [which is when your gender aligns with your sex assigned at birth] and transgender. My mentor had a somewhat humorous way of explaining the difference: "Sexual orientation is who you want to go to bed with, but gender identity is who you wake up as."

**What is gender-affirming care?**

When I think of gender-affirming care, I think of it as a combination of support a patient might get from medical and non-medical interventions.

Not everyone with a difference in gender identity should be considered as having a medical problem or needing to see a doctor. When someone is facing challenges due to a difference between sex assigned at birth and gender identity, this is often referred to as gender dysphoria or gender incongruence.

When you ask someone to use a different name or pronouns, when you pick out clothes that match your gender and decide how you want to wear your hair, those are, in some ways, non-medical treatment options for gender dysphoria. On the other hand, there are medical treatments, including hormone therapy and even, potentially, surgical interventions.

**What are the risks and benefits of delaying puberty?**

For a subset of young people, going through puberty can be extremely challenging and can complicate their mental health situation.

Pubertal suppression can reduce distress, but it is reversible. We're allowing time for the child to get older and make a more balanced decision with their family about what comes next. Discontinuation of pubertal suppression would result in puberty. However, patients who have persistent identity that is aligned with the opposite gender from their sex assigned at birth who are treated with pubertal suppression could subsequently make a decision about hormones in later adolescence.

We're also really cautious about using medical interventions to treat dysphoria because it delays growth spurts and bone density accrual. These things will happen eventually once the medication is discontinued, but pubertal suppression does change the timing of the body's growth and development.

We balance those risks against potential benefits of delaying puberty for each individual. For some patients, withholding medical intervention could mean worsening distress, anxiety, depression, and potential suicidality. Every major medical association in the U.S. recognizes that gender-affirming care is safe and effective at treating gender dysphoria.

**What can you tell people, especially parents, who are struggling with understanding these concepts?**

I want to recognize that gender identity can be a challenging topic, especially for older generations. For transgender people, it helps to have a name or a face of someone we love to understand gender identity better. I think that's why the youngest generation of Americans is unfazed by these conversations.

There's nothing wrong with being excited to have a baby boy or baby girl and to be really proud of the newborn that you've created. What I would ask is that everyone has more openness and tolerance for the idea that there is diversity in how people identify. The majority of families won't have a child who has a difference in gender identity or sexual orientation, but it's important to let kids know from a young age, "Whoever you are and whoever you become, you will beloved and supported."

I want to acknowledge that being a parent is a hard job. Parents' feelings about gender difference are normal and valid. I also know that, even more important than any medical decision, a child knowing their parent loves

and supports them unconditionally is the most important predictor of that child's success. We know that LGBTQ+ youth who report high levels of rejection from parents do have a higher risk for depression, anxiety, drug use, and high-risk sexual behavior, than kids who report no or low parental rejection.

Meeting parents where they are and educating them about the meaningful impact of their support on their child's health and well-being can help motivate parents to be more supportive. Even a parent who is supportive enough to honor a child's name and pronouns but is not ready to discuss medical interventions is doing an amazing thing to help their child feel loved.

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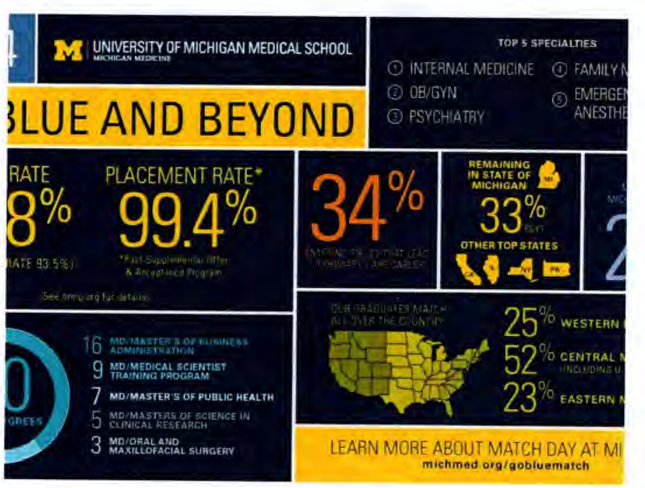


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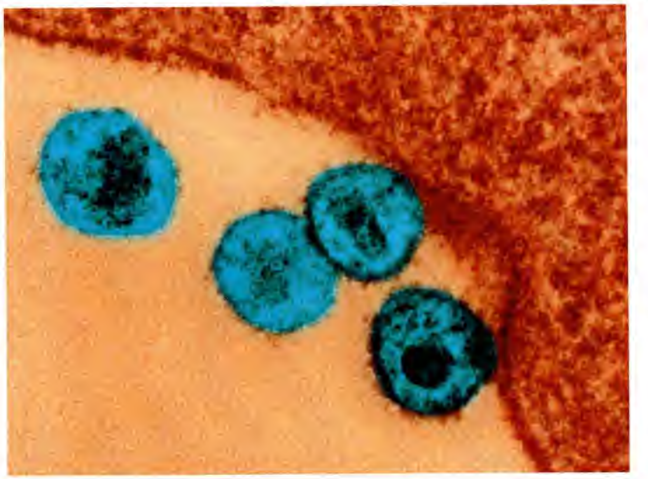

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Ex. 3

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Scientific Statement

Scientific Statement

# Considering Sex as a Biological Variable in Basic and Clinical Studies: An Endocrine Society Scientific Statement

**Aditi Bhargava,[1,2] Arthur P. Arnold,[3] Debra A. Bangasser,[4] Kate M. Denton,[5] Arpana Gupta,[6] Lucinda M. Hilliard Krause,[5] Emeran A. Mayer,[6] Margaret McCarthy,[7] Walter L. Miller,[1,8] Armin Raznahan,[9] and Ragini Verma[10]**

[1]Center for Reproductive Sciences and [2]Department of Obstetrics and Gynecology, University of California, San Francisco, CA 94143, USA; [3]Department of Integrative Biology & Physiology, University of California, Los Angeles, Los Angeles, CA 90095, USA; [4]Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122, USA; [5]Cardiovascular Disease Program, Monash Biomedicine Discovery Institute and Department of Physiology, Monash University, Clayton, Victoria, 3800, Australia; [6]G. Oppenheimer Center for Neurobiology of Stress and Resilience, Division of Digestive Diseases, University of California, Los Angeles, Los Angeles, CA 90095-7378, USA; [7]Department of Pharmacology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA; [8]Department of Pediatrics, University of California, San Francisco, CA 94143, USA; [9]Section on Developmental Neurogenomics, Human Genetics Branch, National Institutes of Mental Health, Intramural Research Program, Bethesda, MD 20892, USA; and [10]Diffusion and Connectomics In Precision Healthcare Research (DiCIPHR) lab, Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

**ORCiD number:** 0000-0003-1334-0517 (A. Bhargava).

**Abbreviations:** ACTH, adrenocorticotropic hormone; $AT_2R$, angiotensin type 2 receptor; BMI, body mass index; cAMP, cyclic adenosine monophosphate; CKD, chronic kidney disease; CRF, corticotropin-releasing factor; CVD, cardiovascular disease; dMRI, diffusion magnetic resonance imaging; fMRI, functional magnetic resonance imaging; FCG, Four Core Genotypes (model); GMV, gray matter volume; GPCR, G-protein coupled receptor; HPA, hypothalamic-pituitary-adrenal; KYN, kynurenine; LC, locus coeruleus; MIH, Müllerian inhibitory hormone; PAR, pseudoautosomal region; PKA, protein kinase A; PTSD, posttraumatic stress disorder; RAAS, renin-angiotensin-aldosterone system; rs-fMRI, resting state functional magnetic resonance imaging; sMRI, structural magnetic resonance imaging; UCN, urocortin.

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## Abstract

In May 2014, the National Institutes of Health (NIH) stated its intent to "require applicants to consider sex as a biological variable (SABV) in the design and analysis of NIH-funded research involving animals and cells." Since then, proposed research plans that include animals routinely state that both sexes/genders will be used; however, in many instances, researchers and reviewers are at a loss about the issue of sex differences. Moreover, the terms *sex* and *gender* are used interchangeably by many researchers,



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further complicating the issue. In addition, the sex or gender of the researcher might influence study outcomes, especially those concerning behavioral studies, in both animals and humans. The act of observation may change the outcome (the "observer effect") and any experimental manipulation, no matter how well-controlled, is subject to it. This is nowhere more applicable than in physiology and behavior. The sex of established cultured cell lines is another issue, in addition to aneuploidy; chromosomal numbers can change as cells are passaged. Additionally, culture medium contains steroids, growth hormone, and insulin that might influence expression of various genes. These issues often are not taken into account, determined, or even considered. Issues pertaining to the "sex" of cultured cells are beyond the scope of this Statement. However, we will discuss the factors that influence sex and gender in both basic research (that using animal models) and clinical research (that involving human subjects), as well as in some areas of science where sex differences are routinely studied. Sex differences in baseline physiology and associated mechanisms form the foundation for understanding sex differences in diseases pathology, treatments, and outcomes. The purpose of this Statement is to highlight lessons learned, caveats, and what to consider when evaluating data pertaining to sex differences, using 3 areas of research as examples; it is not intended to serve as a guideline for research design.

**Key Words:** brain-gut, cardiovascular disease, chromosome complement, gender, sex differences, steroid hormones

Sex is an important biological variable that must be considered in the design and analysis of human and animal research. The terms *sex* and *gender* should not be used interchangeably. Sex is dichotomous, with sex determination in the fertilized zygote stemming from unequal expression of sex chromosomal genes. By contrast, gender includes perception of the individual as male, female, or other, both by the individual and by society; both humans and animals have sex, but only humans have gender. Both sexes produce estrogens, androgens, and progestins; there are no male- or female-specific sex hormones, *per se*, although these steroids are present in substantially different levels in males and females. Sex differences are caused by 3 major factors—sex hormones, genes, and environment. To understand disease mechanisms and exploit sex differences in protection or exacerbation of diseases, one needs to determine the relative contribution of factors, including observer effect (1), causing sex differences. Here—using 3 broad research areas as examples—the roles of sex differences in brain anatomy, brain-gut axis, and cardiovascular disease are discussed. Contemporary brain imaging methods show age- and sex-related differences in brain size, global and regional gray matter volume, white matter connectivity, and neuroanatomic regulation of appetite and satiety; while these differences are seen in large population-based studies, there is tremendous individual overlap, but such group-level findings do not inform findings, physiology, or pathology at the individual level. Sex differences in disorders of the brain-gut axis, obesity, type 2 diabetes, and metabolic syndrome are caused by differential actions of brain-gut peptide and steroid hormones. The activation, signaling, and pharmacotherapy responses of the components of the hypothalamic-pituitary-adrenal (HPA) axis differ between the sexes. Heart and kidney functions are linked. Age, hormones, and sex biases seen in cardiovascular and chronic kidney diseases also differentially influence pharmacologic responses in men and women. Thus, sex differences pervade biology and medicine, and while not discussed in this Statement, must be considered in virtually all areas of biomedical research.

## Section I

### Sex Versus Gender

Much of the American public is surprisingly prudish about the word *sex*; it has now become commonplace to use the seemingly more genteel term *gender* when one really means *sex*. In *Moritz v Commissioner of Internal Revenue* (469 F. 2d 466 [1972]), Ruth Bader Ginsburg (subsequently, The Honorable Ruth Bader Ginsburg) argued against discrimination "on the basis of sex" not "on the basis of gender," thus clearly, knowledgeably, and presciently understanding that "sex" does not equal "gender." In a decision 48 years later (*Bostock v Clayton County*, 590 US, decided June 15, 2020), the United States Supreme Court separately ruled against discrimination on the basis of gender. *Gender* is often misused as a synonym for *sex*—for example, when filling out forms for various activities, we are routinely

asked to check a box labeled "gender," but the only available options are boxes labeled "M" and "F." But *sex* is not the same thing as *gender* and using these terms as equivalents obfuscates differences that are real and important in society in general and biomedical research in particular.

## Biological Sex: The Definition of Male and Female

*Sex* is a biological concept. Asexual reproduction (cloning) is routine in microorganisms and some plants, but most vertebrates and all mammals have 2 distinct sexes. Even single-cell organisms have "mating types" to facilitate sexual reproduction. Only cells belonging to different mating types can fuse together to reproduce sexually (2, 3). Sexual reproduction allows for exchange of genetic information and promotes genetic diversity. The classical biological definition of the **2 sexes** is that females have ovaries and make larger female gametes (eggs), whereas males have testes and make smaller male gametes (sperm); the 2 gametes fertilize to form the zygote, which has the potential to become a new individual. The advantage of this simple definition is first that it can be applied universally to any species of sexually reproducing organism. Second, it is a bedrock concept of evolution, because selection of traits may differ in the 2 sexes. Thirdly, the definition can be extended to the ovaries and testes, and in this way the categories—female and male—can be applied also to individuals who have gonads but do not make gametes.

In mammals, numerous sexual traits (gonads, genitalia, etc) that typically differ in males and females are tightly linked to each other because one characteristic leads to sex differences in other traits. The type of gonads is controlled by the presence of XX or XY chromosomes, and gonadal secretions in turn regulate formation of female or male reproductive tissues, and characteristics that differ in typical males or females. These characteristics include external genitalia, uterus and oviducts, sperm ducts, and secondary sexual characteristics such as facial hair and pitch of voice. However, many people cannot make either eggs or sperm, yet are recognized as female or male based on other physical characteristics; people who do not have either ovaries or testes are rare. For individuals that possess a combination of male- and female-typical characteristics, these clusters of traits are sufficient to classify most individuals as either biologically male or female. For example, a person with testes and a penis, who cannot make sperm, is usually classified as a biological male, as long as the person does not possess female features such as a vagina, ovaries, or uterus. Based on evidence presented, to define male and female individuals in general society, we expand the defining characteristics of sex to include nongonadal traits, as well as classical gonadal traits.

A simple biological definition of male and female, satisfactory to all people, is elusive. In human societies, the terms *female* and *male* can have several meanings, as they refer both to a person's biological sex and to their social roles. Most people learn to discriminate males and females from an early age, but often not based on biological traits (4). For example, behaviors such as pair-bonding, sexual activity, offspring defense and care, and mate/partner selection (5) involve complex interplay between sex steroid hormones and peptide hormones (oxytocin and arginine vasopressin); these behaviors are encouraged differently in women and men, which influences their role in the society and culture in which they live to behave as "females" or "males." While these factors have little impact on their biological sex, they can have profoundly different outcomes in the behavior and health of an individual. Biological sex is dichotomous because of the different roles of each sex in reproduction. For scientific research, it is important to define biological sex and distinguish it from other meanings.

## Sex Chromosomes and Biological Sex Determination

Among mammals and many other taxa, males are characterized as the heterogametic sex (6), having 2 different sex chromosomes, X and Y, whereas females are homogametic (XX). By contrast birds, many reptiles, and some other organisms have Z and W chromosomes (7). In these organisms, the female is the heterogametic sex (ZW) and males are homogametic (ZZ). Some adult fish and reptiles can also change sex in response to environmental factors (8, 9), and even the adult mouse gonad can undergo partial sex reversal when specific genes are deleted (10, 11). Human biological sex is often assessed by examining the individual's complement of sex chromosomes as determined by karyotypic analysis: males are XY and females are XX. Karyotypic sex is actually a surrogate for genetic sex, determined by the presence of the *SRY* gene on the Y chromosome (12, 13). However, karyotypic analysis may be misleading, as there are well-described 46,XX males (with testes). Most of these individuals carry a short segment of the Y chromosome that includes *SRY* transferred to an X chromosome, but up to 10% lack an *SRY* gene (14, 15). Similarly, there are 46,XY females, who have *SRY* but also have a duplication of *DAX1* (*d*osage-sensitive sex reversal, *a*drenal hypoplasia critical region, on chromosome *X*, gene 1) (16).

## Sex Determination and Sex Differentiation

In mammals, sex determination begins with the inheritance of XX or XY chromosomes, which are the only factors that are different in XX and XY zygotes. Thus, all phenotypic sex differences, including gonadal development, stem originally from the unequal effects of XX and XY

sex chromosomes. Phenotypic sex differences develop in XX and XY embryos as soon as transcription begins. The categories of X and Y genes that are unequally represented or expressed in male and female mammalian zygotes, which could cause phenotypic sex differences, fall into 3 main categories (17).

1. *Y genes causing male-specific effects.* These Y-linked genes do not have homologous genes on the X chromosome. The most important Y-linked gene is *SRY*, the testis-determining gene, which encodes the SRY transcription factor expressed during embryonic life in the bipotential gonadal ridge; SRY activates downstream autosomal genes such as *SOX9* to cause formation of a testis (18). In the absence of *SRY*, autosomal and X chromosome genes (*WNT-4*, *DAX-1*, *FOXL2*, *COUP-TFII*, and *RSPO1)* are activated to cause formation of an ovary (19-22). Both testicular and ovarian development are subject to active genetic regulation (12, 13, 16). Pathways downstream of *SRY* inhibit ovary-determining pathways, and ovary-determining pathways also inhibit pathways for testis development. Once the testes form, they secrete sex hormones that act widely throughout the body to cause male differentiation of nongonadal tissues. Other Y genes also have male-specific effects (for example, those required for spermatogenesis) (23, 24).
2. *X gene dosage or parental imprint.* Because XX nongermline cells inactivate one X chromosome (25, 26), it was long thought that both XX and XY cells have only one active X chromosome, with little inherent difference in expression related to the number of X chromosomes. The inactivated regions of the X chromosome are "coated" with large noncoding RNA transcribed from the X-inactive specific transcript (*XIST*) gene, part of the XIC (X inactivation center) located on Xq13 (27, 28). But some genes escape X inactivation (termed as *X escapees*), and therefore are expressed more in XX than XY cells, resulting in imbalance or incomplete dosage compensation (29). About 23% of human X-linked genes are more abundantly expressed in XX cells than XY cells in many tissues (30, 31). Recent evidence from mouse studies suggests that the inherent male-female difference in expression of X genes leads to significant sex differences in disease phenotypes. For example, sex differences in placental *Ogt* expression are associated with sex differences in prenatal vulnerability to stress (32). X escapee *Kdm6a*, a histone demethylase, contributes to sex differences in mouse models of bladder cancer (33), autoimmune disease (34), and Alzheimer disease (35). Similarly, variations in human *KDM6A* are associated with prognosis of bladder cancer or cognitive decline in female patients (33). The dose of another X escapee histone demethylase, *Kdm5c*, contributes to sex differences in adiposity and body weight in mice, and variations in *KDM5C* in humans are associated with body mass (36).

   Sex differences may also arise from genes in the pseudoautosomal regions (PARs) of the sex chromosomes, small regions of sequence similarity on the X and Y chromosomes that allow for X and Y chromosome pairing during meiosis. Both XX and XY cells have 2 PARs, implying equivalent effects of XX and XY PARs. Paradoxically, the process of X inactivation appears to spill over into the PAR and reduce expression on one X chromosome only in XX cells, leading to greater expression of PAR genes in XY cells compared to XX cells in the human transcriptome (30). A third potential source of X-linked imbalance stems from parentally imprinted genes in XX cells, which have one X chromosome from each parent and thus are influenced by any imprint on X genes from either parent. XY cells only receive imprints from the mother, and thus differ phenotypically from XX cells (37).
3. *XX mosaicism.* Female mammals are a mosaic of cells of 2 types: those expressing the X chromosome from the father (Xp), or from the mother (Xm) because of X inactivation (25). In contrast, XY individuals will lack this diversity within cell types in each organ because only one X (Xm) chromosome and only the maternal imprint of X genes will be expressed in each cell. The mosaicism in females means that in genetically diverse populations, the effects of disease-promoting X-linked alleles, inherited from one parent, will be muted in XX cells because half of the cells will have a different allele (38), and genomic imprints from each parent will only be expressed in half of the cells. In general, XX tissues are thought to have less extreme phenotypes than XY tissues, because the effects of extremely deleterious or beneficial alleles or imprints are buffered by the diversity of X alleles and imprints. For example, hemophilia A and hemophilia B (clotting factor VIII and IX deficiencies, respectively), are X-linked diseases that affect men, whereas most women are asymptomatic carriers.

## Sexual Differentiation Caused by Gonadal and Nongonadal Hormones

In mammals, the process of reproductive system development requires the action of hormones (peptide/gonadotropins and steroids) from the pituitary gland, the adrenal cortex, and the gonads. Testicular development leads to secretion of Müllerian inhibitory hormone (MIH, also termed anti-Müllerian hormone, AMH), a glycopeptide, and testosterone, which affects many sex differences in nongonadal tissues (39). In contrast to the fetal testis, the fetal ovary makes minimal steroid hormones

(40), and ovarian function is not needed for development of the female reproductive system, as evidenced by the normal female anatomy of individuals with Turner syndrome, who have 45,X gonadal dysgenesis. The pioneering work of Alfred Jost suggested that 2 classes of testicular hormones are involved in sexual differentiation. First, testicular androgens drive the differentiation of the fetal external genitalia from female morphology to that of the male and are required for the differentiation of embryonic Wolffian ducts into male internal reproductive structures (41, 42). Androgens, secreted by Leydig cells, are required for the differentiation of embryonic Wolffian ducts into male internal reproductive structures (epididymis, vas deferens, ejaculatory ducts, prostate, and seminal vesicles), and drive the differentiation of the undifferentiated external genitalia toward male morphology. Second, the testis produces locally acting MIH that causes involution of the Müllerian ducts, which would otherwise develop into the fallopian tubes, uterus, and cervix (43, 44).

It was long thought that only the involution of the Müllerian ducts was an active process, with the Wolffian ducts simply involuting in the absence of androgens. Recent evidence from mice indicates that Wolffian involution is also an active process controlled by the transcription factor COUP-TFII (22, 45), but the nature of any factors stimulating COUP-TFII remains unknown (22). Some aspects of gonadal differentiation are active throughout life, preventing ovarian follicle cells from transdifferentiating into "testis-like" cells (11). MIH is secreted by Sertoli cells and androgenic steroid hormones, usually testosterone, are secreted by Leydig cells. Testosterone and its more potent derivative dihydrotestosterone are responsible for the development of the male external genitalia (46). Androgens from adrenal glands and alternative pathway androgen biosynthesis in the human placenta can influence virilization of the developing fetus (47, 48). The adrenals of adult primates also produce abundant androgens, profoundly influencing phenotypes, so that not all sex steroids are gonadal (see Boxes 1 and 2). Although the term *sexual differentiation* is usually applied to the development of sex differences in genitalia and other organs such as the brain in the growing fetus; sex differences also occur later in life during the mini-puberty of infancy (49), puberty, the female menstrual cycle, menopause in women, and andropause in men. The actions of gonadal and nongonadal hormones as well as sex and autosomal chromosome gene products in adult people causes many sex differences in health and disease.

## Influence of Gonadal Steroid Hormones and Nongonadal Hormones in Brain Development

Differentiation of the brain by gonadal hormones is implemented during a restricted critical window, which is operationally defined by the onset of copious androgen

**Box 1.** Steroidogenesis in gonadal and nongonadal tissues

All biologically active sex steroids, whether gonadal or nongonadal in origin, are derived from cholesterol by the process of steroidogenesis. Two steroidogenic steps must be considered (for details see (50)). First, the cholesterol side-chain cleavage enzyme, P450scc (CYP11A1) initiates steroidogenesis by converting cholesterol to pregnenolone; expression of P450scc renders a tissue "steroidogenic," that is, able to make steroids de novo (51). The gonads, adrenals, and placenta express abundant P450scc and produce the familiar circulating endocrine steroids, but the brain, skin, and some other organs also express low levels of P450scc and produce steroids involved in paracrine actions. Brain steroidogenesis has been studied mainly in fetal rodents, with little information in other systems (52). Many nonsteroidogenic tissues (liver, kidney, fat, breast, heart) do not express P450scc but express other steroidogenic enzymes that modify steroids taken up from the circulation. Fat and breast express CYP19A1 (aromatase), permitting local production of estradiol from circulating 19-carbon (C19) steroids; this estradiol is important in breast cancer but is not a gonadal steroid. Similarly, prostate and genital skin express several enzymes leading to dihydrotestosterone, accounting for the failure of "androgen deprivation therapy" by gonadectomy in prostate cancer. Not all gonadal steroids are sex steroids, as both the ovary and testis secrete some "upstream" steroids that are precursors of the classic sex steroids. For example, dehydroepiandrosterone (DHEA) does not bind to sex steroid receptors, but it can be converted into testosterone and estrone. Second, synthesis of all sex steroids requires P450c17 (CYP17A1), which catalyzes 17α-hydroxylation and the 17,20 lyase activity that changes 21-carbon steroids to C19 precursors of androgens and estrogens. P450c17 is abundantly expressed in the gonads of all vertebrates and in the adrenals of most vertebrates other than rodents, but the rodent *Cyp17A1* gene is silenced by tissue-specific methylation (53). Consequently, rodents make only miniscule amounts of adrenal C19 steroids and also use corticosterone instead of cortisol as their glucocorticoid. In most mammals, P450c17 has low 17,20 lyase activity, so that their adrenals produce rather small amounts of C19 steroids, but primate P450c17 has abundant 17,20 lyase activity, generating abundant C19 androgen precursors (DHEA, DHEA-sulfate, androstenedione) (47, 48). Furthermore, production of these C19 steroids proceeds by different pathways in rodents and primates: primates favor the "Δ5 pathway," through DHEA, whereas rodents favor the "Δ4 pathway" through 17OH-progesterone (17OHP) (50). Primate adrenals also produce a true androgen, 11-keto-testosterone (54), profoundly influencing phenotypes (apocrine odor; female sexual hair). Thus, not all sex steroids are gonadal: ~ 50% of the circulating androgens in adult women are of adrenal origin.

**Box 2.** Gonadectomy and sex steroids

Many animal studies employ gonadectomy to eliminate the actions of sex steroids (estrogens, androgens, progestins). If using this approach, the investigator must consider whether nongonadal tissues will produce sufficient sex steroids to influence the study. The gonads produce most but not all circulating sex steroids; furthermore, some tissues produce steroids that act locally and do not enter the circulation, hence absence of a measurable steroids in blood does not ensure absence of its action in the target tissue. Both sexes produce all steroids and their metabolites, hence there are no male- or female-specific sex hormones, *per se*. In male mammals, testosterone release is highly pulsatile in nature (49, 55) and in laboratory mice, strain-dependent variations in androgen levels are reported (56). In female rodents, circulating levels of estradiol, testosterone, and DHT are highest in proestrus phase; a comprehensive analyses of sex steroids in intact and gonadectomized rodents can be found elsewhere (57). Circulating concentrations of testosterone in adult women are similar to those of boys in early puberty, and estradiol concentrations in men are similar to those in mid-cycle women, but the tenfold higher concentrations of testosterone obscure its effects. Rodents are widely used in research, but they differ from primates in several important aspects of steroidogenesis (see Box 1), and hence must be used with caution in studies seeking to model aspects of human physiology that might be influenced by steroids. These differences include: (i) In humans, substantial amounts of circulating sex steroids are bound to sex hormone–binding globulin (SHBG), whereas this carrier protein is not present in rodent circulation (58). (ii) Dehydroepiandrosterone (DHEA) and androstenedione, 19-carbon (C19) precursors for testosterone and estrone, that do not bind to sex steroid receptors, are secreted from the adrenal glands, the ovary and testis in humans, but not rodents (59). Thus, not all gonadal steroids are sex steroids. (iii) The rodent ovarian corpus luteum produces progesterone throughout pregnancy but in human pregnancy the corpus luteum involutes early in the second trimester, after which the placenta produces the progesterone needed to suppress uterine contractility, permitting term pregnancy. (iv) Adrenal-specific methylation of rodent *Cyp17A1* prohibits their adrenal synthesis of C19 precursors of sex steroids; however, changes in methylation status can occur under conditions of pathology. (v) As a further consequence of adrenal *Cyp17A1* methylation, rodents utilize corticosterone as their glucocorticoid, whereas almost all other vertebrates use cortisol. (vi) Rodent adrenals use high-density lipoproteins (HDL) taken up via scavenger receptor B1 (SRB1), as their principal source of cholesterol for steroidogenesis, whereas primates use low-density lipoproteins (LDL) taken up by receptor-mediated endocytosis. (vii) Several genes encoding steroidogenic enzymes are duplicated; rodents and primates differ in which copy(ies) of these genes are expressed: *CYP21; HSD3B, HSD17B, AKR1-3*. Such differences may affect laboratory results in unanticipated fashions. (viii) In rodents, nonsteroidogenic tissues such as the gut, liver, kidney, fat, breast, heart, thymus, skin, and the placenta have all been shown to make steroids. Thus, gonadectomy may eliminate most, but not all, circulating sex steroids, depending on the species being studied and may not reveal much about the paracrine effects of sex steroids present in the tissue(s) under investigation. Nonetheless, gonadectomy is an invaluable research tool that helps unequivocally confirm the influence of gonadal hormones in sex differences.

production from the fetal testis. Human fetal androgen production begins at 8 to 10 weeks postconception and in rodents is closer to parturition, at embryonic days 16 to 18, with birth following 2 to 4 days later. An important effect of this androgen surge is to masculinize the rodent brain. Steady but pulsatile release of the gonadotropins luteinizing hormone and follicle stimulating hormone from the pituitary gland support continuous steroidogenesis and production of sperm (60). In female rodents, the feminization of the brain proceeds in the absence of exposure to high levels of androgens or their aromatized byproducts, estrogens, a developmental strategy highly analogous to that used for masculinization of the gonads, reproductive tract, and secondary sexual characteristics, with the exception that estrogens are actively downregulated in male rodents. In human females, gonadotropins from the pituitary gland regulate ova development, induction of ovulation, and stimulation of estradiol and progesterone from the ovaries (49). An important feature of this developmental strategy is the existence of a sensitive period in female rodents (61). Male rodents must be exposed to high levels of androgens during the critical period; if exposure occurs too early or too late it will be ineffective at inducing masculinization. However, females are also sensitive to androgens during a restricted period of development, hence a sensitive period in rodents. In males, the critical period closes shortly after androgen exposure because the cellular and molecular processes of masculinization have been initiated and cannot be reversed; the train has left the station. In both primates and rodents this process is largely prenatal, but female rodents remain sensitive to androgen exposure into the first postnatal week. Injecting a newborn female rodent with androgens will initiate the process of masculinization, thus she is still sensitive. After the first week, the feminization process cannot be overridden by androgens and thus the sensitive period has closed. The existence of the sensitive period in females is useful as a research tool—it is important in understanding the potential impact of exposure to endocrine-disrupting compounds or other cellular agents of masculinization that act in an analogous manner to androgen exposure in modulating female brain development. There is evidence for a later sensitive

period for brain feminization mediated by small increases in estrogens (62); this topic warrants further investigation. The closing of the sensitive period in primates, especially humans, remains poorly understood, but it appears to end prenatally, similar to the critical period in rodents. The sources of androgens that females can be exposed to during the sensitive period include from: (i) experimental interventions; (ii) male littermates in animals; (iii) or human adrenals carrying genetic mutations in the steroidogenic pathway (as in congenital adrenal hyperplasia).

Given that the critical and sensitive periods for sexual differentiation are defined by the production and response to gonadal steroids, it is not surprising that steroids are the primary drivers of developmental origins of sex differences in brain (and probably other tissues) and behavior. But how do steroids achieve this? The first step in any investigation is often is to identify the active steroid metabolite(s). In rodents, circulating fetal testicular testosterone enters the fetal brain where it can serve as a direct precursor for estradiol synthesis via aromatase (*Cyp19A1*) (see Box 1). Fetal and adult neurons can aromatize testosterone to estradiol in a nonrandom distribution: neurons of the hypothalamus, preoptic area, and amygdala are particularly active for local estradiol synthesis, whereas the hippocampus and parts of the cortex, midbrain, and spinal cord are also active at a lower level (63). For most reproductive endpoints, it is the local actions of estradiol that drive neural phenotype toward masculinization, which to some seems counterintuitive, given that estradiol is so often referred to as a "female" hormone (64), and further highlights that it is impossible to completely eliminate the effects of sex steroids, especially in the brain, by simple gonadectomy (see Box 2). Developing rodent embryos sequester maternal estrogens by binding to circulating alpha-fetoprotein, which is present only during the critical/sensitive period; when it is genetically deleted, all the offspring are masculinized (65). However, in humans, sex hormone–binding protein, not alpha-fetoprotein, is the major serum glycoprotein that binds androgens and estrogens with an undetermined role in fetal sexual development (66, 67).

In rodents, there is abundant evidence that gonadal androgens are metabolized to estrogens in the brain and mediate "masculinizing" effects on the brain; similar evidence in primates is limited. In primates, the principal masculinizing agents are androgens, not estrogens, and although there is alpha-fetoprotein present in fetal circulation, it has a weak binding affinity for estradiol (68), and instead it plays a much broader role in brain and body development (69). The conclusion of no strong role for estrogens in humans is based on individuals with dysfunctional aromatase or androgen receptors. Males lacking aromatase still identify as men, while XY individuals with complete androgen insensitivity identify as women (70). The disparity between the principal differentiating hormones in primates versus rodents suggests that findings may not be easily extrapolated, and it is important to specify both the hormone and species under investigation. To discern whether the biological basis of sexual differentiation of brain and behavior differs between primates and rodents, one needs to identify mechanisms by which steroids transduce signals to modify the trajectory of the nervous system. While those mechanisms are incompletely understood, a few general principles are clear. First, there is no unified mechanism that applies broadly across the brain, with the exception that androgens and estrogens are the primary drivers of masculinization during a restricted developmental window. Similar masculinizing effects of testicular androgens may also occur during puberty (71). Second, all aspects of neural development are capable of being "organized" or programmed by sex steroids. This includes cell genesis, migration, myelination, dendritic and axonal growth and branching, synapse formation, synapse elimination, and neurochemical differentiation. Effects are not limited to neurons, with both astrocytes and microglia also exhibiting morphological sex differences. Third, each discrete brain region, nucleus, or subnucleus appears to have unique mechanisms of cellular masculinization. In some brain regions, such as the preoptic area, there are multiple separate mechanisms at play simultaneously. Sex steroids act in both paracrine and endocrine manners to influence structural development and function (72, 73).

## Biological Basis of Diversity in Sexual/Gender Development and Orientation

Given the complexities of the biology of sexual determination and differentiation, it is not surprising that there are dozens of examples of variations or errors in these pathways associated with genetic mutations that are now well known to endocrinologists and geneticists (74); in medicine, these situations are generally termed *disorders of sexual development* (DSD) or *differences in sexual development* (75). DSD includes genetic disorders in the sexual determination pathway (76), disorders of steroidogenesis (50, 77), disorders of steroid hormone action, especially androgen insensitivity syndrome (78), and less well-defined "developmental field defects" (79), such as Mayer–Rokitansky–Küster–Hauser syndrome (80). The study of genes and factors underlying DSD and the diagnosis and management of the various forms of DSD is a complex and rapidly evolving area of endocrinology: clinical management is complex (81) and requires both contemporary molecular genetics (82) and well-integrated interdisciplinary care (83).

Gender includes perception of the individual as male, female, or other, both by the individual and by society. *Gender identity* is a psychological concept that refers to an individual's self-perception; while associations between gender identity, neuroanatomic, genetic, and hormone levels exist, a clear causative biological underpinning of gender identity remains to be demonstrated. Both animals and human beings have biological sex, but only humans have evident self-awareness that allows them to express gender; self-awareness in animals has not been investigated in this context. Gender also includes differences that males and females experience in their social and physical environments, which can have differentiating effects on the sexes. Human social environments are poorly modeled in laboratory animals and thus animal studies are usually limited to addressing sex differences. For centuries, the concept of male and female did not distinguish between biological sex differences and those caused by consistent differences in the environments. Thus *sex differences* are those caused by biological factors, whereas *gender differences* reflect a complex interplay of psychological, environmental, cultural, and biological factors (Fig. 1).

At birth, individuals are assigned a sex or gender ("natal gender"), almost always based on the appearance of the external genitalia. In most individuals, the various biological determinants of sex are consistent with one another, and this biological sex is also consistent with the individual's self-perception—the sex and gender are concordant. However, a substantial minority of people who do not have DSD have some degree of variation in their self-perception of their gender, which may differ from their biological sex; this is usually termed *gender incongruence* (84). The term *gender disorder* has been replaced with the term *gender dysphoria* which describes the distress that an individual might feel as a consequence of having gender incongruence. *Transgender* (often called *trans*) refers to individuals who do not identify themselves as being of their natal gender, whereas *cisgender* (*cis*) people do not experience gender incongruence (85). Readers are also referred to Endocrine Society's 2017 Clinical Practice Guideline and Transgender Health Fact Sheet (84). Estimates of the prevalence of male-to-female transgender individuals among general populations range from 0.5% to 1.3% and estimates for female-to-male transgender individuals range from 0.4% to 1.2% (85). State level population-based surveys indicate that 0.6 % of US adults (25-64 years of age) and 0.7% of adolescents and young adults (13-24 years of age) identify as transgender. Other studies of US high school

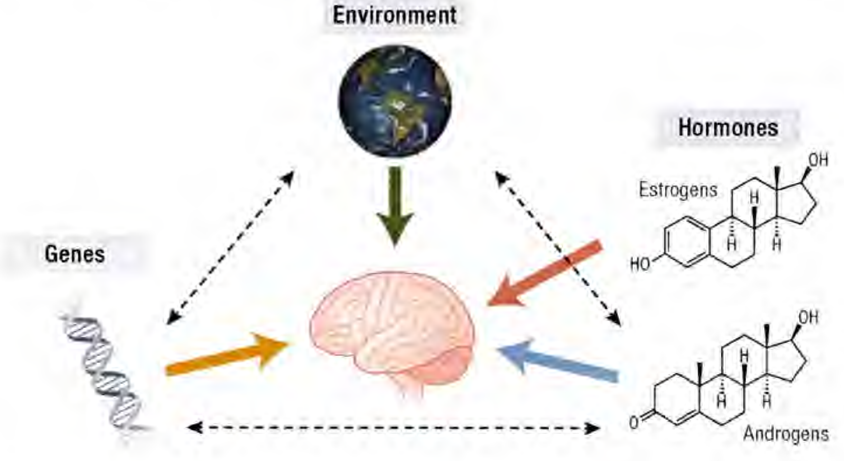


**Figure 1.** Simplified view of the factors influencing sex differences in the brain. Three broad groups of factors influence the sexually dimorphic brain, as indicated by the broad, colored arrows. 1) Genes and genetic factors that influence the brain include both those on sex chromosomes and autosomes, and include both the DNA itself (represented by the classic double helix) but also chemical modification of DNA (eg, methylation) and modifications of proteins associated with DNA to form chromatin, including histones, and also changes in proteins that bind to DNA. 2) Hormones clearly influence sexual dimorphism in the brain; these are represented by the principal sex steroids, estradiol and testosterone, but also include other steroid and protein hormones (progestins, MIH, oxytocin, prolactin, etc). 3) The environment includes a wide spectrum of influences, including perinatal nutrition and familial support, socioeconomic and demographic factors, intrinsic factors of brain development, age, and gender, and larger environmental factors, such as education, profession, and societal expectations (the "gendered environment"). In addition to each class of factor influencing the brain (bold arrows), the human brain also reciprocally influences each of these groups of factors. Furthermore, each group of factors influences the other, as represented by the dotted arrows. Some examples include: the environment influences genes via epigenomics and genes influence the environment by population sizes and domains; the environment influences hormones by seasonal variations and the actions of xenobiotics, and hormones influence the environment by promoting reproduction and consumption of foodstuffs; genes directly influence hormones by regulating their production and action, and many hormones, including all steroid hormones, regulate gene transcription.

students suggest a prevalence of 1.8% to 2.7% of being gender nonconforming or transgender (86-88). However, several factors may influence reported prevalence of gender dysphoria: (i) small sample sizes; (ii) differences in assessment techniques leading to incomplete ascertainment of gender dysphoric individuals; (iii) unwillingness of some individuals to respond fully and honestly, especially in older studies or studies deriving from locales where gender incongruence is a social taboo; (iv) differences in the subjects ages. *Sexual orientation*, not to be confused with gender identity, refers to the group of persons to whom an individual is sexually attracted; both cisgender and transgender individuals may be hetero-, homo-, or bi-sexual (89).

Although gender is strongly influenced by environmental and cultural forces, it is unknown if the choice to function in society in male, female, or other role(s) is also affected by biological factors (89-91). A general issue is that the association of sex, gender, or sexual orientation with specific brain structures, or with other biological variables, does not establish whether the biological variables are causes or consequences or noncausal correlates of the behavioral characteristics or function of the individuals studied. Three areas of biological difference have been studied fairly extensively: neuroanatomy, genetics, and hormones. Studies have reported differences in the hypothalamic INAH3 nucleus in men vs women and in homosexual vs heterosexual men (92, 93). Although initially controversial, others have confirmed sex differences in INAH3 numbers, not in size or densities, whereas no evidence for sexual dimorphism of any other INAH structures are reported (94). Studies in people with gender dysphoria found that the phenotypes of specific brain structures, such as the bed nucleus of the stria terminalis, of transgender women and transgender men differ from cisgender men and women, with partial, but incomplete sex reversal of sexually dimorphic structures (95). Brain networks involved in one's body perception, (pregenual anterior cingulate cortex, temporo-parietal junction, and fusiform body area) differ in individuals with gender dysphoria compared with cisgender individuals (96-98). Neuroimaging shows that testosterone treatment resulted in functional and structural changes in brain areas associated with self-referential and own body perception (99). Transgender men have thicker medial prefrontal cortex than cis men. Testosterone treatment does not change prefrontal cortex thickness in transgender men, but it has other effects on cortical thickness, connectivity, and fractional anisotropy (99).

Genetics may play a role in gender identity (100): monozygotic twins have 39% concordance for gender dysphoria (101). Attempts to identify specific genes governing gender identity have been plagued by small numbers of subjects and low statistical significance; no specific gene has been reproducibly identified. However, such studies have suggested associations with genes encoding steroidogenic enzymes and sex steroid receptors, and it is generally agreed that androgens play an important but not determinative role. For example, many 46,XX individuals with severe virilizing congenital adrenal hyperplasia (steroid 21-hydroxylase deficiency) are exposed to intrauterine testosterone concentrations typical of those in normal male fetuses and consequently have severely virilized external genitalia; nevertheless, most have a female gender identity, but about 5% to 10% of such individuals have gender dysphoria, an atypical gender identity (89, 102, 103), or atypical sexual orientation and gender behavior (104, 105). Similarly, about half of 46,XY individuals with defects in androgen synthesis who were raised as females revert to a male gender role (106). The biological underpinnings of sexual orientation and gender identity are apparently related but are not the same (107). Thus, there is ample but incomplete evidence for biological substrates—neuroanatomic, genetic, and hormonal—for gender orientation, making this an important area of ongoing research.

## Hormonal Versus Sex Chromosome Effects

Sex differences are caused by 3 major factors—sex hormones, genes on sex chromosomes/autosomes, and environment (Fig. 1). To understand disease mechanisms in both sexes and exploit sex differences in protection or exacerbation of diseases, it is important to determine the relative contribution of each of these factors in causing sex differences (17). Many sex differences caused by gonadal hormones have been discovered by measurements of sex steroids and gonadotropins during human development, and in animals by similar measurements or by interventional methods, such as gonadectomy, hormone administration, or the expression of synthetic enzymes or receptors in transgenic mice. Sex steroids play an integral part in many physiological processes (Box 1). Whereas the gonads are the major site of sex steroid synthesis, the adrenals, placenta, brain, and skin can also initiate steroidogenesis, and steroid-modifying enzymes are found elsewhere, especially in liver and fat, permitting synthesis of sex steroid hormones in multiple other sites (50). Thus, animal gonadectomy may provide information about endocrine effects of gonadal steroid hormones but cannot address tissue-specific paracrine effects (Box 2). Moreover, gonadectomy cannot mimic low pre-pubertal levels or physiological conditions in which hormone levels decrease, such as aging or menopause. Manipulations of human gonadal hormones are routinely used in contraception and in the management of sex steroid–dependent cancers (eg, breast, prostate). When

a sex difference is discovered in human disease, and modeled in animals, the investigation of possible hormonal causation of the sex difference is usually the first option considered.

To detect effects of sex chromosomes that cause sex differences, one can compare people who have differences in their sex chromosomes, revealing effects of X or Y chromosome number (108-110). These results strongly suggest direct sex chromosomal contributions to sex differences in cell function. Comparison of brains of XY patients with complete androgen insensitivity (who are phenotypically female), with brains of control XY males and XX females, suggests that cortical thickness and functional connectivity between the limbic regions and the cortex are influenced not only by testosterone actions, but by sex chromosome factors as well (111). However, changes in the sex chromosome ploidy also alter gonadal hormones, so it can be difficult to isolate sex chromosome effects not mediated by gonadal hormone effects. Circulating human embryonic/fetal sex steroid concentrations are poorly characterized, and the tissue concentrations are almost totally unknown. Another approach is to use mice to identify genes on the X or Y chromosome that act outside of the gonads to cause sex differences, and then seek evidence that the orthologous human genes cause human sex differences. Controlled experiments are possible in which XX or XY mice with comparable gonadal hormones can be compared. A frequently used model is the Four Core Genotypes (FCG) model, in which the testis-determining mouse *Sry* gene is deleted from the Y chromosome (creating the $Y^-$ or "Y minus" chromosome) and inserted as a transgene on chromosome 3 (*Sry+*) (Fig. 2 and Box 3) (112). The utility and limitations of these models have been extensively discussed (113, 114).

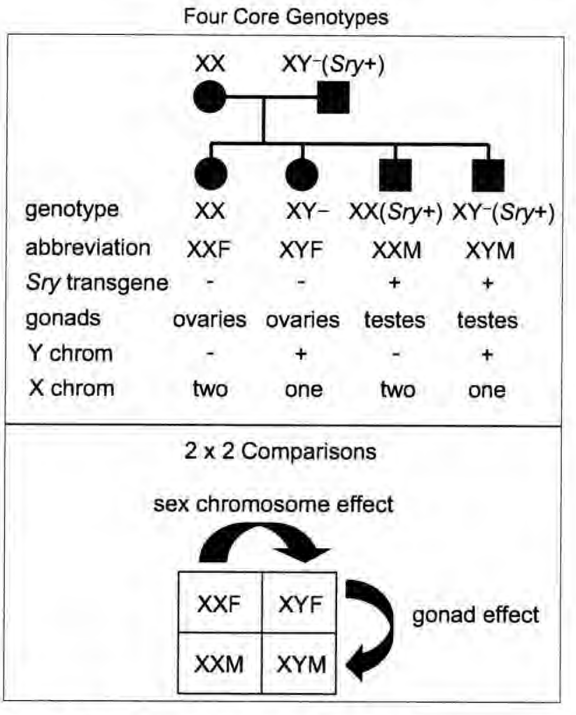


**Figure 2.** Schematic diagram of the Four Core Genotypes mouse model. The testis-determining gene *Sry* is deleted from the Y chromosome, producing the $Y^-$ chromosome. An *Sry* transgene is inserted onto chromosome 3. Thus, the type of gonad is no longer linked to the sex chromosomes. The model produces XX and XY mice with *Sry* and testes, and XX and XY mice without *Sry*, with ovaries. Sex differences in phenotype can be attributed to an effect of gonadal hormones, comparing mice with ovaries and testes, or to an effect of sex chromosomes, comparing XX and XY mice with the same type of gonad. [Modified with permission from Arnold AP & Chen X. *Front Neuroendocrinol*, 2009; 30(1) © Elsevier Inc. (112)].

## Considering Sex and/or Gender as Variables in Health and Disease

Women and men differ in many physiological and psychological variables. It is important to establish the mechanisms causing such differences in health and disease, and to consider sex-related variables in studies of human health and disease. These variables include, but are not limited to, sex- and gender-related factors. The inability to control all variables in human studies means that it may be impossible to determine the relative roles of environment and biology in causing a difference between women and men, when both types of variable can influence the trait. Furthermore, while "gender expression/behavior" can be observed, "gender identity" can only be known by what an individual states. Thus, gender identity, *per se*, cannot be studied in animals. In human studies, it is unethical to selectively manipulate specific biological and environmental variables, and most currently available data derive from studies comparing groups of men with groups of women. It is therefore difficult to disentangle the specific contribution of sex-related genes, hormones, gender-related variables, and other variables that contribute to being female or male. Because sex has long been defined by gonadal type, the list of sex-influencing factors has been primarily associated with gonadal hormones, especially estrogens, progestins, and androgens (121). However, some phenotypic sex differences develop before the gonads differentiate as testes or ovaries (122), so other factors also contribute to sex differences (123) but are seldom considered.

Sex is an essential part of vertebrate biology, but gender is a human phenomenon; sex often influences gender, but gender cannot influence sex. Studies of animal physiology must consider sex as a variable (124), with sex steroids (of both gonadal and nongonadal origins), sex chromosomes, and other factors contributing to sex differences in many physiologic processes. Similarly, studies of human physiology and disease must also consider sex for the same reason (125) and its disorders must

**Box 3.** Investigating sex chromosome complement versus gonadal hormones in health and disease: the four core genotypes (FCG) model

The FCG model allows for discriminating hormonal vs sex chromosome effects in animals. Gonadal males ($XY^-$(*Sry+*)), bred to XX gonadal females, produce 4 types of offspring: $XY^-$ and XX mice with the *Sry* transgene and testes, and $XY^-$ and XX gonadal females lacking the *Sry* gene (Fig. 2). Thus, it is possible to compare XX and XY mice with the same type of gonad, in 2 separate comparisons. Differences between XX and XY are attributed to effects of sex chromosome genes acting on nongonadal tissues. To determine if this sex chromosome effect is caused by X or Y genes, a second model is studied, the XY* model (113, 114). This model produces genotypes that are similar to XO, XX, XY, and XXY. An effect of number of X chromosomes is discovered by comparing XO and XX, or XY and XXY. An effect of the Y chromosome genes is discovered by comparing XO and XY, or XX and XXY. These mouse models have been used to demonstrate sex chromosome effects causing sex differences in a wide variety of phenotypes and disease models, including brain and behavioral phenotypes, metabolism, autoimmune, cardiovascular and pulmonary diseases, Alzheimer disease, aging, and cancer (35, 113, 115). These models have facilitated discovery of several disease phenotypes in which the number of X chromosomes contributes to sex differences (116), and a smaller number of sex-biasing effects of Y genes (117). Sex chromosome effects occur in the same disease systems alongside sex-biasing effects of gonadal hormones, such that the 2 effects can synergize to increase the amount of sex difference, or counterbalance each other to reduce a sex difference. Moreover, genes encoded on the Y chromosome can have gene-specific effects, and/or effects that overlap with those of X genes (118). In the cardiovascular system and associated physiological/disease states, sex chromosomes and gonadal hormones can have opposing effects. Estrogens generally protect from cardiac ischemia/reperfusion injury and other cardiovascular diseases, reducing disease in female relative to male mice. However, studies of ischemia/reperfusion injury in gonadectomized FCG mice reveal that the XX sex chromosome complement is associated with worse outcomes, relative to XY (119). In another study, sex chromosome effects in angiotensin II-induced hypertension showed that arterial pressure was greater in gonadectomized XX mice than in gonadectomized XY mice (120). Sex chromosome complement also influences the development of abdominal aortic aneurysms, fat metabolism and adiposity, plasma lipids and lipoprotein levels (particularly HDL-C) (115)).

also consider gender. However, human gender is a spectrum from feminine to gender-neutral to masculine, and also likely includes individuals who do not fit readily on a simple linear continuum (84). Studies addressing the endocrine care of transgender youth during the time of their potential gender transition (84, 89) find that they have a higher prevalence of stress-associated mental health disorders such as depression and anxiety, which can be ameliorated by gender-affirming endocrine treatment (126). It is essential to recognize these sex and gender differences as our health care systems endeavor to develop "individualized medicine."

Despite the fact that biological sex is such a fundamental source of intraspecific variation in anatomy and physiology, much basic and clinical science has tended to focus studies on one sex (typically male). Few studies have done side-by-side testing for sex differences at baseline and in experimental models of human diseases (127-129). Studies in laboratory animals that manipulate biological (eg, genes and hormones) and environmental variables (eg, housing conditions, diet, physical activity, etc) demonstrate that many variables can affect sex-related aspects of an animal's physiology. However, laboratory rodents may show male-female differences caused by different housing conditions, which could be misinterpreted as being caused directly by biological differences without environmental mediation. In studies concerning animal behavior, the sex and gender of the researcher conducting behavioral measures may also influence outcomes (130). Thus, for reproducibility and proper interpretation of the data, at the minimum, it is important to state the precise housing conditions, anesthetics, analgesics (different effects in sexes), doses, surgical manipulations, diet, sex, strain, species, and age of animals used, as well as sex/gender of the researcher(s) performing experiments.

Having laid the foundation for several factors that contribute to sex versus gender, this Statement will use 3 areas of research as examples (not as a literature review) where human and animal sex differences are well known. First, sex differences in specific brain regions of healthy men and women are increasingly being documented along with differences in brain connectomes; these will be discussed in detail in Section II. Second, stress-related pathophysiologies are known to affect twice as many women as men. However, few studies systematically include study designs to ascertain function or mechanisms that may be similar or different between males and females. Hormones and signaling pathways that contribute to sex-specific differences in stress-based pathophysiologies will be discussed in Section III. Similarly, sex differences in manifestation of cardiovascular and renal diseases are well recognized and will be discussed in Section IV.

## Section II

### Developmental Origins of Sex Differences in Brain Anatomy, Function, and Behavior

Sex differences in the human brain are a topic of intense popular and scientific interest. Several scientific observations motivate the search for sex differences in brain structure

and function. First, the act of sexual reproduction requires that the male and female animals show qualitatively different reproductive behaviors. The stereotyped emergence of these reproductively critical and sexually differentiated behavior reflects biologically programmed (or "innate") sex differences in the organization of those brain circuits that support the motivational and consummatory phases of copulatory behavior (131). Second, the fact that males and females make different biological investments in reproduction—eg, the risks of pregnancy in mammals are borne entirely by the female—sets up sex differences in the behavioral strategies that optimize reproductive fitness (132). Sexual selection based on sex-biased behavioral strategies is predicted to drive the evolution of sex differences in those brain circuits that are responsible for sexually selected behaviors. Third, males and females can show consistent sex biases in broader behavioral domains beyond those that directly relate to reproductive strategies. In our own species for example, there are highly consistent sex differences in the prevalence of physical aggression and violence (both male-biased) (133), as well as extensively documented sex differences in risk for different mental disorders (134).

In this section, we will first describe the main neuroimaging techniques commonly used in comparisons of brain anatomy, connectivity, function, and subnetwork organizations. We then review the key aspects of sex-biased brain anatomy and connectivity that have been revealed by these techniques; sex differences in stimulus-based or task-based functional magnetic resonance imaging (fMRI) studies are not addressed here. Next, we discuss specific disease states that appear to have different outcomes in the 2 sexes due to baseline differences in the "connectome" and animal models used in neuroimaging. Finally, we will address some important caveats and controversies in the field of brain imaging.

## Brain Imaging Techniques

Modern neuroimaging methods make it possible to characterize diverse aspects of brain structure, function, and connectivity in vivo. This large toolbox of methods has been used to examine sex differences in brain organization at several levels of analysis. These techniques aim to analyze, map, and visualize regional and inter-regional (connectomic) features of the brain at macroscopic (systems-level) and mesoscopic (neural circuit architecture) levels in order to illuminate brain organization in health and disease (135). Of note, cellular-level details are beyond the resolution of most in vivo brain imaging techniques.

Sex differences in global and regional brain anatomy can be measured in vivo using structural magnetic resonance imaging (sMRI). Several considerations have made sMRI an especially popular technique in the study of brain sex differences in humans. First, sMRI allows a quick and spatially comprehensive screen of the entire brain that can quantify thousands of morphometric properties simultaneously in vivo across a large number of individuals. These characteristics not only facilitate testing for sex differences outside defined regions of interest, but also allow longitudinal measurements that can track the emergence of brain sex differences over development (136, 137). Second, because sMRI considers structure rather than function, it can leverage evolutionary conservation of the basic mammalian brain plan (138), and it is therefore particularly well-suited for cross-species investigation of sex differences in humans and animals. Thus, a critical role for sMRI research in the study of brain sex differences is to screen for brain regions that can then be prioritized for closer analysis using more resource-intensive assays that are typically applied in a regionally selective manner.

Complimenting sMRI, other in vivo neuroimaging techniques such as diffusion MRI (dMRI), resting state functional MRI (rs-fMRI), and fMRI provide unprecedented insights into tissue microstructure and brain connectivity. fMRI maps brain circuitry based on stimulus- or task-based brain functional responses. In contrast, rs-fMRI, by measuring changes in blood flow in the brain generated by signals dependent on blood-oxygen-levels, helps explore the brain's functional organization by providing insights into intrinsic brain activity without requiring participants to be trained in specific tasks, thereby eliminating task performance as a confounder (139, 140). dMRI measures the differential patterns of water diffusivity in biological tissue revealing details of tissue microstructure, especially in white matter (141). Fiber tractography on dMRI enables mapping the fiber architecture of the brain, and subsequently, the network organization of the brain through structural connectomes (142-144). A brain connectome is an extensive map of the white matter structural or functional connections of the brain, created using dMRI or rs-fMRI (145). Modeling efforts, such as the Human Connectome Project, and the use of connectome-based predictive modeling, have provided an integrative, in-depth, and multilevel understanding of the structural and functional connectivity (regions that get coactivated) of the neuronal networks (146, 147).

## Sex Differences in Global and Regional Brain Anatomy

It is well established that men have an average total brain volume that is approximately 10% greater than that of women (148, 149). A similar sex difference in average

human brain volume (~8%) appears to be present at birth (150) and is sustained throughout childhood and adolescence (151). The sex differences for total brain volume also hold for the 2 main subdivisions of brain tissue—gray matter and white matter—despite these 2 brain compartments following very different developmental trajectories (151, 152) (Fig. 3).

The robust sex difference in brain volume identified through human sMRI research cannot be fully explained by the fact that brain volume is positively correlated with height (average height is greater in men that in women). Statistical control for body size diminishes, but does not remove, sex differences in total brain volume (149), and boys also show greater average brain volume than girls during early adolescent development, at a time when girls are taller than boys (153). Thus, available literature supports a consistent picture in which there is overlap between the distribution of brain size in men and women, but the mean of this distribution is significantly greater in men than women. The medium effect size of sex on brain volume exists above and beyond sex differences in stature. However, it is important to note that no known functional sex differences associate with the sex difference in overall brain size. Sex differences in overall brain size, and their developmental timing, are both theoretically and methodologically important when considering: (i) whether neuroanatomical sex differences are conserved across species; (ii) whether there are sex differences in regional brain anatomy above and beyond sex differences in overall brain size; and (iii) whether there is concordance between sex differences in brain size and any observed associations between brain size and putative biological causes of sex differences, such as gonadal or sex chromosome status (see below).

The patterning of sex differences in behavior and mental illness risk across the lifespan suggest that sex differences in human brain organization are likely to vary across different brain sub-systems or regions, and potentially also across different developmental periods. Structures in human gray matter compartments mediate neural computation and information processing—in contrast to axon-rich white matter compartments that are primarily involved in connectivity between different brain regions (see "Sex Differences in Brain Network Organization: The Brain Connectome," below). Here, we focus on sMRI studies that have tested for sex differences in regional gray matter volume (regional GMV) after controlling for sex differences in overall brain size. Regional GMV sex differences that survive statistical correction for total brain volume variation are of special interest because they exist beyond global sex differences in brain size. We emphasize GMV rather than other morphometric properties of the brain such as cortical thickness, sulcation, or the shape of subcortical structures (144, 154), because GMV provides a common metric that can be examined across cortical and subcortical structures, with equal applicability to humans and mice. Independent large-scale human sMRI studies in biobanks have identified a reproducible pattern of sex differences in regional GMV using sample sizes that are

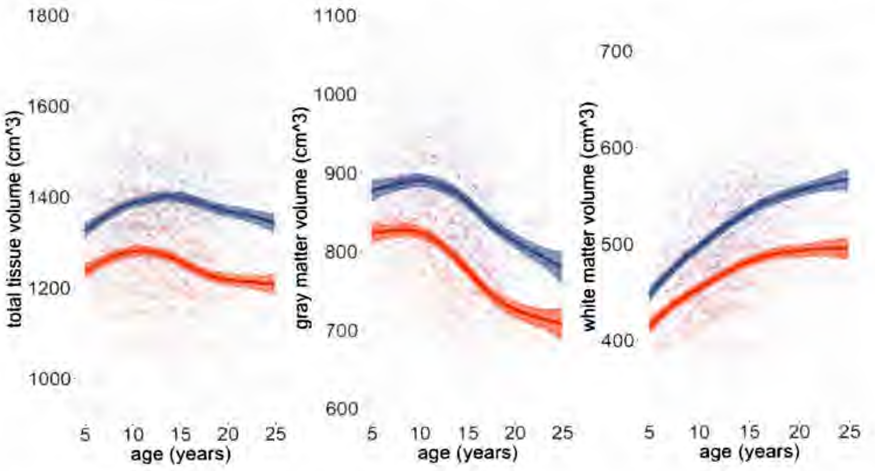


**Figure 3.** Developmental trajectories for total brain tissue volume, gray matter volume, and white matter volume in men and women over Development. Person-level data are shown for women (red) and men (blue) as points, with lines linking measures from the same person over time. Note the large interindividual variation in volumes within each sex, and the overlap of these distributions, between the sexes. Superimposed on these person-level data are group-level best fit volume trajectories (bold lines with shaded 95% confidence intervals). The developmental window covered is 5 to 25 years of age. For all plots, there are statistically significant sex differences in both trajectory shape (ie, sex differences in the tempo of volume change, $P < 0.00001$), and trajectory "height" (ie, sex differences in absolute volume across ages, $P < 0.00001$). [Adapted with permission from Giedd JN et al. *Neuropsychopharmacology*, 2015; 40 © Springer Nature (153)].

significantly larger than those used in earlier work (148, 149, 155). A structural neuroimaging study involving >2000 individuals demonstrated that higher regional expression of sex-linked genes was coupled with greater GMV in men relative to women (155). These studies, by different laboratories, using different datasets and different techniques for sMRI analysis, find a largely overlapping regional pattern of GMV sex differences after correction for sex differences in total brain volume. These independent replications of regional sex differences in GMV are also in agreement with meta-analytic studies (156). Together, these studies show that, in adulthood, regional GMV is (on average): (i) greater in women than men within superior parietal, dorsolateral frontal, and anterior cingulate cortices; and (ii) greater in men than women within occipital, fusiform, and parahippocampal cortices as well as the amygdala and putamen. Furthermore, while these studies lack temporally resolved developmental maps of male-female differences in regional GMV throughout the brain, there is extensive evidence from focused studies of particular structures that neuroanatomical sex differences can vary dynamically over development, such as observed with amygdala volume and shape (156).

The rapidly expanding body of sMRI research on regional GMV sex differences in the murine brain shows important overlaps and differences with findings from human studies (137, 157). These murine sMRI studies—which are most commonly conducted ex vivo at a spatial resolution of <100 μm throughout the whole brain—have been able to confirm the identification of all classically sexually dimorphic nuclei of male-biased volume from prior histological research, including the bed nucleus of the stria terminalis and medial amygdala (137, 157). These brain regions play a predominant role in modulating social and goal-directed behaviors, pain, and cardiovascular control, all of which are conserved among mammalian species and subject to sexually dimorphic outcomes. By allowing a full-brain screen, murine sMRI has also newly identified a reproducible set of regions with greater GMV in females, including the cerebellar cortex, ventral thalamus, and somatosensory cortex (137, 157). Furthermore, a longitudinal sMRI study in mice found that the set of regions with male-biased GMV can be detected by early postnatal life (with some accentuating over puberty), whereas regions of female-biased GMV in murine adulthood appear to emerge in adolescence (137). To date, there are no studies that formally seek to compare the spatiotemporal patterning of regional GMV sex differences in humans and mice, although existing work already suggests some potential homologies, including foci of greater cerebellar cortex GMV in females vs males by adulthood (137, 148) and the adolescent accentuation of male-biased amygdala volume (158, 159). An important technical challenge in assessing the degree of anatomical homology between regions of sex-biased brain anatomy in humans and mice is that most of the best-established and histologically validated foci of sex-biased brain volume in mice (eg, bed nucleus stria terminalis, medial preoptic nucleus of the hypothalamus) are hard to image in humans due to their small size and intrinsic tissue contrast properties.

## Sex Differences in Brain Network Organization: The Brain Connectome

The structural or functional brain network is represented by a "connectome," wherein the structural or functional connectivity between coactivated regions is encoded either through fiber tracts or functional co-activations (160). These connectomes can be studied at the level of subnetworks like visuospatial, auditory, cognitive control, or macro-scale level through global measures of network segregation, integration, and efficiency, to obtain functional associations (161).

A study of 949 individuals (aged 8-22 years; 428 males and 521 females) showed that on average, there are significant differences between the sexes in their structural connectomes (Fig. 4) (162). On average, men had greater within-hemispheric connectivity, as well as enhanced network segregation, whereas between-hemispheric connectivity and network integration predominated in women (Fig. 4A), but these differences were most prominent during adolescence (Fig. 4B-4D). However, an opposite trend was seen for cerebellar connections, which developed differently between human males and females in adolescence and adulthood. The structural connectivity findings were consistent with a behavioral study conducted on the parent cohort (the above-mentioned imaging study was performed on a subset of participants), with women outperforming men on attention, word and face memory, and social cognition tasks, and men performing better on spatial processing and motor and sensorimotor speed tasks (163). An analysis of the Human Connectome Project rs-fMRI data identified age and sex as independent variables that contributed to differences in functional connectivity (164). In brains of men, functional connectivity was more clustered locally in all lobes, except in the cerebellum, whereas the brains of women showed a higher clustering coefficient at the whole-brain level. Thus, brains of men were classified as more segregated and brains of women as more integrated, which agrees with the structural connectivity findings (162). In connectomes, the identification of subnetwork properties (165) can reveal how the complex functional and behavioral repertoire emerges from the simultaneous processes of segregated neuronal clusters and their

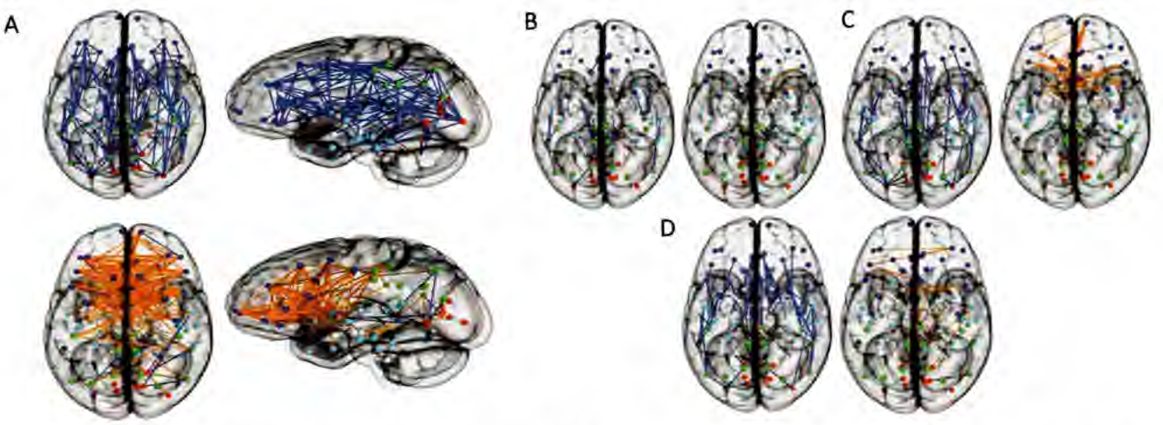


**Figure 4.** Sex differences in structural connectomes across development. Connectomes representing the white matter structural connectivity in the brain, with nodes indicating the brain regions and edges between the nodes representing the structural connectivity between the nodes. Node colors representing respective brain regions are as follows: dark blue, frontal; cyan, temporal; green, parietal; red, occipital; white, subcortical. The depicted edges shown are those that survived permutation testing at $P = 0.05$. **A,** shows increased intrahemispheric connectivity in men (Upper, in blue) and increased inter-hemispheric connectivity in women (Lower, in orange) on average. **B-D:** Connectivity differences shown in A separated by age groups are shown: **B,** under 13 years, **C,** adolescent (13-18 years), and **D,** young adults (18-22 years). Left image: Men/Boys; Right image: Women/Girls. [Adapted with permission from Ingalhalikar M et al. *Proc Natl Acad Sci U S A*, 2014; 111(2) © National Academy of Sciences (163)].

integration during complicated cognitive tasks (166, 167). Consistent with the behavioral findings on sex differences, men had increased connectivity between motor and sensory (auditory) systems, along with increased connectivity in the fronto-parietal and cingulo-opercular systems that are traditionally associated with complex reasoning and control, whereas women had higher connectivity between reward, memory, and sensory (auditory) systems (163, 168). Better spatial skills in men and improved memory and social cognition skills in women have been reported in behavioral literature (169, 170).

It is important to point out that observed group-level differences in brain structure, function, or connectivity in men and women may reflect the influence of several extraneous factors. For example, in a set of elegant studies, brains of men were imaged to ascertain the contribution of performing complex spatial navigation tasks as part of their daily work on gray matter volume. These studies found that posterior hippocampi of London taxi drivers were significantly larger compared with controls (171), although the work did not address sex differences. Driving a taxi in London before the era of digital maps/navigation systems required extensive training and learning to navigate complex routes before being given a license to operate. In a subsequent study, comparison between London taxi drivers and bus drivers matched and controlled for age, education, intellectual, and stress levels, as well as years of driving experience, showed that taxi drivers had greater GMV in the posterior and less volume in the anterior hippocampi compared with bus drivers (172). Interestingly, years of navigation experience associated with hippocampal volume in taxi drivers alone, but they were significantly worse at acquiring or retrieving novel visuo-spatial information than bus drivers. Importantly, no differences in other GMV, including the caudate nucleus, were found between the taxi and bus drivers; the caudate nucleus is associated with a myriad of cognitive and emotional functions. These studies illustrate brain plasticity and that professional work and years of performing certain tasks can result in brain structural, volume, and connectivity differences that may have little to do with sex or gender per se, but more with training, social environments, and behaviors. In other studies, GMV changes were greater in professional musicians, or after induced training (juggling for 3 months), and in early bilinguals, and white matter volume changes were found in adults learning a second language, irrespective of sex, when reported (173-176). These findings suggest that brain structure retains its plasticity and controlling for factors other than sex or gender are key in interpreting data on structural volumes and associated functions.

The above-mentioned existing datasets did not collect the requisite information on self-report of gender, thereby precluding retrospective analysis of gender in these cases. As identifying correspondence between behavioral scores and the regions that are involved in the manifestation of that behavior remains challenging, analyses of subnetworks pertaining to functional and behavioral domains can help elucidate a brain-behavior correspondence. The detailed description of sex differences in brain organization at the group level, and concerted efforts to specify

the role of sex-biased biological factors in shaping such sex differences, is of fundamental importance (177) and also provides a crucial adjunct for indispensable studies on environmental and wider societal contributions to sex-biased brain development. Such studies should be undertaken jointly using structural and functional connectivity. These studies elucidate the various ways in which sex differences in brain microstructure and connectivity can be investigated.

## Sex Differences in Structural and Functional Brain Regions in Obesity

The hypothalamus has long been known as the "center" where peripheral and neural signals converge in the regulation of food intake and energy homeostasis in both sexes. Advances in neuroimaging studies have helped identify activation of several distinct brain regions comprising brain networks in response to eating in men and women. Behavioral and sociocultural factors may play a role in the observed sex differences in ingestive behaviors, appetite, and cravings related to obesity (178). Women report higher prevalence of maladaptive ingestive behaviors such as binge eating, food cravings, and "food addiction," and the lifetime prevalence of disordered eating behaviors are about 3 times higher in women than in men (179, 180). Women also experience episodes of food cravings of greater intensity (181, 182), and greater frequency (183-185), and are less able to suppress food cravings than men (184, 186). Despite the wealth of data indicating that women experience disproportionately higher rates of food cravings, stress eating, and eating disorders than men, the reasons for these differences are incompletely understood (184, 187).

Regulation of food intake entails both homeostatic and nonhomeostatic factors (188). Homeostatic regulation balances energy needs with energy consumption, whereas nonhomeostatic regulation—in particular hedonic regulation and food addiction—involves reward-seeking behaviors that drive humans and animals to consume food beyond their metabolic needs, leading to the development of obesity (189-191). These findings have directed attention toward the extended reward system in obesity-related research, which consists mainly of basal ganglia regions and is involved in dopamine signaling and addiction-like behaviors (192). The extended reward system is composed of 6 interconnected brain networks—salience, central autonomic, basal ganglia, somatosensory, executive control, and emotional regulation (192).

Functional MRI studies have found that, in response to food images, obese individuals show greater activation than normal-weight individuals in regions associated with reward anticipation, dopamine signaling, and addiction-like behaviors (193-196). Greater activity in brain regions of the extended reward network may drive obesity-related behaviors, such as greater responses to food odors and food consumption (197-199). Recent meta-analyses have further supported the role of the brain in disrupting the balance between energy consumption and expenditure. This combination of increased activity in regions associated with reward-driven behaviors and decreased activity in regions moderating top-down control of appetite may lead to consumption of excess calories (188).

Furthermore, sex-specific activations in response to food intake have been observed in cognitive, emotional, and reward-related regions (200-202). For example, obese men had greater activation than obese women in the supplementary motor area, precentral gyrus, fusiform gyrus, and inferior parietal lobule, which are associated with motor control, visuospatial attention, and responding to salient new or alerting stimuli (203). In this same study, obese women showed greater activation than obese men in the caudate and parahippocampal gyrus, regions implicated in reward processing and memory (203). Using graph theory to define the underlying architecture of brain structural connectivity obtained from diffusion tensor imaging, sex differences were observed in the topological measures of centrality (which determine the degree of information flow in specific brain regions) in regions of reward and salience networks in women, and in reward and sensorimotor networks in men (204). Resting state fMRI studies have found sex differences and commonalities in body mass index (BMI)-related connectivity associated with specific defined regions of interest in the reward network (205). For example, women had increased associations between BMI and increased connectivity in the in right globus pallidus and bilateral putamen. In men, BMI was associated with increased connectivity in the medial frontal cortex. A study of sex differences in response to visual and auditory food cues found that women experience greater activation in lateral and dorsolateral prefrontal and parietal cortical regions involved in cognitive planning and executive guidance and evaluation of behavior, compared with men (202). When viewed together, these studies highlight the importance of investigating sex differences in obesity-related alterations in the core and extended reward networks.

Although many single-sex studies of fMRI and obesity have been published, with the majority having all-female subjects, few studies have specifically investigated sex differences in brain function and structure in obesity. Despite the literature supporting sex differences in the brain, including in regions implicated in reward behaviors and energy homeostasis, few comprehensive reviews of sexually dimorphic brain signatures related to obesity have

been performed. A recent meta-analysis using an activation likelihood estimation approach to evaluate comparisons in functional responses to stimuli by obesity and by sex revealed differential sex- and BMI-related activations in reward anticipation and response, in shaping food-related memories, and in generating top-down control of appetitive processes. Together, these findings have important implications for sex-specific obesity treatments.

## Models to Study Sex Differences in Normal Brain Structure and During Pathophysiology

Studies of sex differences offer important considerations for personalized medicine. The prevalence, clinical presentation, and symptomatic progression of many neurological and psychiatric disorders are remarkably different between the sexes. In addition to common X-lined mental retardation syndromes, men have a greater prevalence of neuropsychiatric disorders such as autism, attention-deficit/hyperactivity disorder (ADHD), and Tourette syndrome (206), whereas women have a greater prevalence of mood and eating disorders (207, 208). From the perspective of developmental disorders, the differences in the developmental trajectories of the sexes perhaps represent different vulnerabilities of maturing brain circuitry, leading to differences in symptoms, onset, and severity of neurological disorders. There are also sex differences in the risk factors, average age of onset, and prevalence of late-life dementias, as well as cerebrovascular disease (209). Additionally, in traumatic brain injuries, where the network organization of the brain is affected by the injury, such as the corpus callosum region, sex differences in inter-hemispheric connectivity and brain subnetworks may influence the impact of injury, and hence subsequent recovery. Thus, sex differences in brain connections are crucial to identify, as they may elucidate mechanisms in disease risk and potential treatment and recovery (210).

Most models of sex-biased mammalian brain development are based on experimental data from rodents (now largely from mice, but previously also from guinea pigs and rats). One of the most systematic dissociations of gonadal and chromosomal contributions to sex-biased anatomical brain organization in mammals is provided by a recent sMRI study of adult mice from the FCG model (112, 211). By combining sMRI with behavioral assays, these studies determined the contribution of sex chromosomes and gonads to adult mouse brain structure and function (211). This study revealed: (i) an effect of sex chromosomes on regional GMV in the cerebellar cortex and olfactory bulb; and (ii) an effect of gonads on regional GMV in the parietotemporal cortex and the bed nucleus of the stria terminalis. Some of these effects overlapped with regions of normal sex differences in murine GMV (eg, cerebellar cortex and bed nucleus of the stria terminalis), and some brain regions were anatomically sensitive to both effects (basal forebrain and periaqueductal gray matter). Sex-chromosome effects on regional gray matter anatomy have also been reported by complementary sets of sMRI studies in both mice and humans that compare groups of euploid individuals with groups carrying X-chromosome aneuploidy (157, 212). Finally, in both mice (137) and humans (155), the spatial patterning of sex differences in regional GMV in adulthood appears to be preferentially aligned with the spatial patterning of sex-chromosome gene expression—which points toward a potential role of sex-linked genes in the establishment of maintenance of regional GMV sex differences. These studies emphasize the need for integrative models that view biological contribution to sex-biased brain development as a developmental dance of coordinated influences from both gonads and sex chromosomes.

## Caveats and Critiques Relating to Neuroimaging of Brain Sex Differences

While several sMRI studies apparently establish that there are highly reproducible male-female differences in regional gray matter volume after controlling for variation in total brain size in humans, this conclusion should be considered in the light of several important caveats and critiques to avoid misinterpretation. First, all sMRI phenotypes that show reproducible and statistically significant sex differences also show a considerable overlap between men and women. This overlap is illustrated by total brain volume: total brain volume averages 10% greater in men than women, but many women have a total brain volume above the 30$^{th}$ centile for male brain volume, and many men have a total brain volume below the 30$^{th}$ centile for female brain volume (149). Sex differences in brain structure and organization are present across the lifespan and vary based on age, so inferences should be drawn cautiously. Thus, while total brain size shows a robust mean difference between men and women, an individual's total brain volume is a weak predictor of biological sex. These 2 facts arise because biological sex is only one source of variation in brain size (149), and other factors/variables that influence total brain size are unknown and/or hard to model statistically (Fig. 1). By extension, because sources of anatomical variation can differ between brain regions—the same individual can have GMV values that appear to be "sex-typical" in one region, but "sex-atypical" in another (when typical and atypical are defined by an individual's percentile position relative to the distribution of population-level trait variation in each sex) (213). This interpretation offers one

potential explanation for the observation that an individual brain can show varying degrees of GMV "sex-typicality" in different brain regions (relative to the population distribution). Alternative explanations have been proposed, including regional variations in programs of sex-biased development such that one individual's brain may be considered a "mosaic" of male and female parts regardless of their chromosomal and/or gonadal sex (213).

Second, although sex differences in regional GMV are highly reproducible in humans and mice, these meso-anatomical sex differences *cannot* be assumed to correlate with behavioral sex differences. The functional relevance of neuroanatomical sex differences is hard to establish experimentally in humans, but correlations between anatomical and behavioral sex differences could be modeled in humans using several feasible study designs. To date, however, very few studies have directly tested for such structure-function correlations in humans (161), and this is an important priority area for future research. Several other challenges will need to be addressed in future work for any given sex-biased sMRI phenotype, including which aspects of behavior to measure and how to consider properly all possible configurations of brain-behavior association in 2 groups (eg, varying intercepts and/or regression slopes across groups). Moreover, some sex-biased sMRI phenotypes, such as trajectories of anatomical change, can only be estimated from group-level data, which complicates comparisons with interindividual variation in behavior. More fundamentally, however, regional GMV sex differences may be useful for understanding the brain basis for sex-biased behavior without GMV variation itself being the behaviorally relevant marker. For example, sex differences in mean regional GMV may help to define brain circuits that subserve sex-biased behaviors through their molecular, cellular, or connectivity features rather than through their volume *per se*. It is also important to entertain the possibility that sex differences in the anatomical organization of a given brain system may actually serve to equilibrate function between the sexes despite each sex having a categorically different genetic starting point.

Third, in addition to the functional considerations above, full understanding of a given sex bias in regional brain anatomy requires a mechanistic account that can link observed anatomical sex differences back to specific genetic and/or environmental factors that differ between men and women. It is usually impossible to disentangle biological sex differences from those which could be the result of environmental influences during development, differences in gender, and in sexual orientation (Fig. 1). Strict causal tests for mechanistic models of sex-biased brain development are very hard to achieve in humans, although several informative approaches have been pursued including: (i) modeling sMRI data using normative variation in hypothalamic-pituitary-gonadal axis maturation or function (214); (ii) applying sMRI methods to cohorts undergoing gender-reassignment (215); and (iii) studying how sMRI features differ between typically developing groups and those affected by medical disorders involving the sex chromosomes (eg, sex chromosome aneuploidies) or sex steroids (eg, androgen insensitivity, congenital adrenal hyperplasia) (215, 216). However, the opportunistic and correlational nature of these approaches places considerable limits on the inferential power of mechanistic studies of human sex-biased brain development. Moreover, as challenging as it is to study chromosomal or gonadal factors in humans, it is even harder to address empirically the many plausible hypotheses about the potential for experiential and societal influences to differentially shape brain development in both sexes (121) or genders.

## Section III

### Sex Differences in Molecular Mechanisms Underlying Brain-Gut Disorders

The brain and the gut communicate with each other in a bidirectional way through parallel and interacting channels, involving immune, endocrine, and neural signaling mechanisms (217). The brain is able to modulate gut permeability, motility, intestinal transit, and microbial function via the autonomic nervous system (217), and the gut in turn sends signals to the brain to modulate behavior, in rodents (218). This brain-gut communication is especially critical in mediating stress responses and in stress-based disorders. In psychiatric and other neurological diseases, there are notable sex differences that point to different underlying neurobiological mechanisms in men vs women (219-221). Despite their clear documentation, these sex differences have largely been ignored, in order to develop broadly applicable pharmacotherapies that come at a considerable cost, especially for women's health (222, 223). Sex biases in psychiatric risk are particularly instructive as they are developmentally patterned in a manner that is highly reproducible across different cultural settings and historical epochs: early-onset neurodevelopmental and gut disorders are more prevalent in boys than girls, while the opposite sex-bias is seen for adolescent-emergent mood disorders (134, 224). Brain-gut disorders are more prevalent in women than men, but this may be due to underreporting by men due to social stigma associated with several of these

disorders. The etiologies and risk factors for several brain-gut disorders differ between the sexes, yet study designs include predominantly male sex. In this section, we discuss the possibilities that shared and distinct mechanisms operate in males and females resulting in similar as well as distinct manifestation of symptoms for a given disease/disorder.

### Sex-Related Differences in Obesity

Although prevalence rates for obesity are at unprecedented levels in all ages (225) and are almost equal in men and women (except when stratified by race or ethnicity) (226), recent surveys indicate an increase in the incidence of obesity in adults and sex differences in the associations between weight, physical health, and psychosocial functions (227, 228). Sex differences in body fat distribution have also been observed (178, 229), with women showing an increased propensity to gain total body fat, especially subcutaneous abdominal fat, whereas men tend to have more visceral adipose fat (230), which is associated with higher risks of type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease (231). Most clinical trials do not report sex differences related to health outcomes or treatment responses, but a few existing reports suggest women are less likely to complete treatment, tend to lose less weight than men, have a greater number of unsuccessful attempts to maintain weight loss resulting in the well-known "yoyo" diet phenomenon, and have limited responses to pharmacological treatments (225). Obesity-related studies in humans and rodents have expanded in scope to not only focus on structural and functional brain differences between obese and lean male and females, but also include investigations into the bidirectional signaling associated with the brain-gut microbiome axis (232, 233). In obese individuals, changes in the relative abundance and gut microbial diversity have been linked to changes in metabolism, insulin resistance, inflammation, and fat deposition (234). The importance of the intestinal microbiome to human health has been of interest over the past few decades, with multiple studies now linking the microbiome to energy homeostasis, immune function, and development of obesity and metabolic syndrome (235-237), even though few studies have addressed causality.

Not only does the brain-gut axis demonstrate changes in obese individuals, but evidence also highlights differences in the microbiota based on sex hormones (238). More recently, the effect of sex hormones on the composition of the gut microbiota has been explored, with differences seen in the microbiota between men and women during various stages of human development and maturation (238). These sexually dimorphic microbiome signatures are likely to contribute to differences in susceptibility to autoimmune and metabolic diseases between the sexes. Studies performed in immunocompromised mouse models have shown delayed onset and lessened severity of type 1 diabetes in female mice who receive male microbiota transplants; testosterone activity and androgen receptor signaling was essential for this protection (239, 240).

These sex-specific differences in the microbial communities persist throughout adult development, with murine models demonstrating the role of testosterone in orchestrating these divergences in host selection of microbial communities (240). In rodents, males exhibit lower microbiome variability relative to females, likely due to the pulsatile nature of estrogens (240). Human studies comparing the microbiome of twins also revealed more divergences in microbial composition in opposite-sex versus same-sex twins (241). When the cecal contents from adult male mice is transferred into female mice, metabolomic profile changes and masculinization of the hormonal profile results, suggesting the gut microbiota's influence on sex-specific metabolic and behavioral phenotypes (239, 242).

Circulating estrogens in the body are metabolized by the liver and undergo methylation, hydroxylation, and conjugation reactions to produce metabolites that affect host metabolism (243). Certain metabolites are excreted through the bile and are further processed by microbial enzymes in the distal small and large intestine. Certain microbial species secrete beta-glucuronidase, an enzyme that deconjugates biliary estrogen metabolites and allows for its reabsorption into the bloodstream to act on distal sites through binding of estrogen receptors (244). Dysbiosis and decreased microbial diversity result in decreased production of absorbable estrogen metabolites. This mechanism has been implicated in pathologies associated with low circulating estrogens, such as obesity, metabolic syndrome, cardiovascular disease, and cognitive decline in women (245, 246); however, estrogen replacement therapy does not reverse these conditions (247). Growth hormone similarly contributes to sexually dimorphic responses in the above-mentioned diseases (248). In addition, estrogens modulate inflammatory pathways driving disease processes such as nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes (249, 250). More specifically, estrogens regulate adipokines and lipopolysaccharides, which respectively are adipocyte-derived hormones and endotoxins that have been associated with type 2 diabetes (251). Adipokines play a role in metabolic homeostasis as well as in mediating the beneficial and detrimental effects of inflammation (252). The androgen- and estrogen-dependent regulation of adipokines, including leptin, resistin, adiponectin, and visfatin, provides a possible mechanistic link between metabolic disorders (obesity,

atherosclerosis, insulin resistance) and autoimmune dysfunction. The estrogen-microbiome axis can provide a potential avenue for a sex-specific approach to combating metabolic disorders and highlights the bidirectional interaction of estrogens and microbial communities in the pathogenesis of disease processes.

Although the exact signaling mechanisms underlying the communication within the brain-gut-microbiome axis remain incompletely understood, tryptophan metabolites have been implicated as important signaling molecules (253). The most extensively studied tryptophan metabolite is serotonin (5-HT), a molecule with diverse roles in both the gastrointestinal tract (ie, peristalsis, secretion, and absorption) and the central nervous system (ie, mood, pain modulation, behavior, sleep, and ingestive and cognitive functions) (254). Tryptophan also acts as a precursor to the kynurenine (KYN) family of molecules (255). In obesity, the KYN pathway is preferentially activated and may contribute to immune-mediated inflammation, which may drive inflammation-associated changes to the extended reward network described in previous brain studies, particularly changes involving the amygdala and lateral orbitofrontal cortex (256-259). KYN may also modulate signaling within the brain-gut-microbiome axis through downstream neuroactive metabolites, such as kynurenic acid and quinolinic acid, functioning as N-methyl-D-aspartate (NMDA) antagonists and NMDA excitotoxins, respectively (260). Sex differences have been reported in these metabolite products in obese individuals, with lower tryptophan levels but elevated KYN and KYN/tryptophan ratios in women with high BMI compared to men with high BMI (256, 261, 262).

## Sex Differences in Stress-Based (Patho) Physiologies

Epidemiological data reveal that the majority of psychiatric disorders occur at different rates in men and women. For example, men are more likely to suffer from attention-deficit/hyperactivity disorder (ADHD), whereas women are more likely to suffer from major depression and posttraumatic stress disorder (PTSD) (219, 263-265). Even when the rates of disorders are similar, their presentations can differ. Schizophrenia, for example, is only slightly more common in men than women, but men develop schizophrenia at an earlier age and present with more negative symptoms, such as social withdrawal and lack of motivation. (224). In the case of bipolar disorder, rates are similar between the sexes, but women more often have more rapid cycling and mixed episodes and they report higher comorbidity with eating disorders and PTSD, whereas men report higher comorbidity with alcoholism (266). Not only does the risk and presentation of psychiatric disorders vary between men and women, but there are differences in treatment responses. For example, the efficacy of antidepressants differs between the sexes: men respond better to tricyclic antidepressants, whereas women respond better to selective serotonin reuptake inhibitors (267, 268). These findings implicate neurobiological sex differences in contributing to disease. In support of this idea, recent studies using animal models are beginning to uncover molecular processes that can bias males and females toward different pathology. Findings from some of these basic research studies will be highlighted here as examples of how including sex as a biological variable can inform our understanding of the etiology of stress-based disorders, as well as guide the development of better treatments.

While there are sex differences in rodent studies in the structure and the size of certain brain regions that can contribute to sex differences in behavior (211), imaging studies that focused on sex differences in cortical thickness and gyration suggest a role for these brain regions in humans as well. In adolescent girls, cortical thinning in the right temporal regions, the left temporoparietal junction and the left orbitofrontal cortex is faster than in boys (154). In contrast, changes in cortical folding were only found in one cluster of the right prefrontal region, suggesting that the mechanisms underlying changes in cortical thickness and gyrification in adolescents are distinct. Sexual dimorphism in the developmental course of the cortical maturation, which coincides with the onset of puberty, might explain sex differences in the age of onset and clinical presentation of many psychiatric disorders (154). Recent evidence has revealed that molecular sex differences in the brain are more widespread than initially thought and such seemingly small-scale differences can have a large impact on physiology and behavior (269). Neurons typically communicate with each other via neurotransmitters and neuropeptides, which are released from a presynaptic neuron and travel across a synapse to bind to receptors on the postsynaptic neuron to exert downstream cellular effects. There are sex differences in production and release of many neurotransmitters and neuropeptides that can result in behavioral changes. In other instances, sex differences in these systems are compensatory, leading to similar behavior endpoints via different mechanisms. For example, both male and female juvenile rats play, but the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) into the lateral septum mediates juvenile play only in female rats (270). There are also sex differences in receptors that can influence how these neurochemicals affect their downstream targets. For instance, dopamine 1 (D1) receptors, which belong to the family of G protein-coupled receptors (GPCRs), in the nucleus accumbens, are necessary for social

withdrawal in female but not male California mice (271). The function of GPCRs is often complex and they can induce different downstream effects depending on their conformation and location. Sex differences can occur at each level of receptor function, in some cases altering physiology differently in male vs female rodents. Sex differences in GPCR signaling are particularly important to consider, especially given that GPCRs are the most studied drug target family for a myriad of indications; in fact, 34% of all US Food and Drug Administration (FDA)-approved drugs are targets of GPCRs (272). As an example of the myriad of sex differences that can be mediated by receptors, we will use the corticotropin-releasing factor 1 and 2 ($CRF_1$ and $CRF_2$, respectively) receptors that facilitate responses to stress, exhibit sexually dimorphic expression pattern, are modulated by both estrogens and androgens, and have been relatively well characterized in both sexes (273, 274).

Upon perception of stress or perturbation of homeostasis, CRF is synthesized in the paraventricular nucleus and released from the median eminence of the hypothalamus into the pituitary portal circulation, which in turn stimulates the synthesis and secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary into the general circulation. ACTH acts on the adrenal cortex to stimulate the synthesis and release of glucocorticoids and other steroids. This activation of the HPA axis in the classic "flight or fight" response by the CRF system is present in all mammals. The mammalian CRF family comprises 4 agonists, CRF and 3 urocortins (UCN1-3); and 2 known class B GPCRs, $CRF_1$ and $CRF_2$. While $CRF_1$ and $CRF_2$ share ~68% identity at the amino acid level (275), they perform distinct functions; CRF binding to $CRF_1$ initiates stress responses by activating the HPA axis, whereas UCN1-3 binding to $CRF_2$ brings systems back to homeostasis (274). Not surprisingly, perturbations in the components of the CRF family impact several organs and lead to brain-gut disorders, type 2 diabetes, metabolic syndrome, cardiovascular, and reproductive diseases, among others (274). There are sex differences in CRF's endocrine effects. In female rats, higher levels of CRF mRNA in the paraventricular nucleus are reported that associate with the estrous cycle (276, 277) and are reviewed elsewhere (274). Perhaps as a compensatory response, CRF binding protein, an endogenous protein that sequesters CRF thus preventing its bioavailability, is expressed at higher levels in the pituitary of female compared with male mice (278). In humans, there is evidence for increased CRF receptor sensitivity at the level of the pituitary of women relative to men, because peripherally administered CRF, which acts at the pituitary, increases ACTH to a greater degree in women (279).

During stress, CRF is also released centrally into many brain regions, where its neuromodulatory effects coordinate cognitive and behavioral changes to promote stress coping (280). There are sex differences in the way these brain regions respond to CRF that are largely due to sex differences in CRF receptor signaling (274). For example, there is greater $CRF_1$ receptor binding in the basolateral amygdala in female rats (281). In contrast, binding of the $CRF_2$ receptor subtype, which is involved in stress recovery, is greater in the central nucleus of the amygdala in male rats (281). It is unknown precisely how these sex differences affect behavior, but given that the amygdala is critically involved in fear, it is likely that these receptor sex differences differently alter fear processing in males and females. In the brain, $CRF_2$ is most abundant in the bed nucleus of the stria terminalis, a region that regulates sexual behavior and stress-related functions (282, 283). Promoters in genes for $CRF_1$ and $CRF_2$ receptors harbor estrogen and androgen responsive elements and show tissue-specific modulation by sex hormones (284, 285). The sexually dimorphic expression pattern of these receptors at normal physiological states and during stress or disease pathology are summarized in a recent review (274).

Sex differences in $CRF_1$ receptor signaling have been identified in the noradrenergic-containing nucleus of the locus coeruleus (LC) and these differences have important implications for understanding disease vulnerability (273). The LC-noradrenergic system regulates levels of arousal such that higher levels of norepinephrin are associated with greater levels of arousal (286-289). Stressor exposure causes CRF to be released into the LC, which speeds up LC neuronal firing, increasing norepinephrin release (290, 291). Activation of this system during an acute or moderate stressor is thought to be adaptive, because it is important to be alert during a stressful event. However, if this system is activated inappropriately or persistently it can lead to hyperarousal that contributes to agitation, restlessness, impaired concentration, and sleep disturbance. Hyperarousal is a key feature of PTSD and reported in a subset of depressed patients (292, 293). Similar sex differences in spatiotemporal expression of $CRF_2$ and its ligands are found in humans with gut disorders, where they could contribute to differences between males and females in vulnerability to brain-gut disorders (127, 294).

There are sex differences in $CRF_1$ receptor signaling in the LC that increase female sensitivity to CRF. In the LC, CRF receptors primarily couple to Gs to initiate signaling through the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling pathway (295-297). Sex differences in $CRF_1$-induced cAMP-PKA signaling are linked to greater coupling of the $CRF_1$ receptor to Gs in females compared to males (298). This sex difference in

coupling of Gs may indicate that the $CRF_1$ receptor has a different conformation or binding partner in females vs. males, permitting different proteins to preferentially bind in each sex. Further support for this idea comes from studies demonstrating that, in male rats, acute swim stress increases the binding of a different protein, β-arrestin2, to the $CRF_1$ receptor, and this effect is not observed in female rats (298). The increased β-arrestin2 in male rats likely contributes to the greater $CRF_1$ receptor internalization in stressed males (298). When taken together, these findings suggest that $CRF_1$ receptors preferentially signal through different pathways in males (small GTPases) and females (cAMP-PKA) (299). This difference in signaling could alter physiology and disease risk. In fact, sex differences in $CRF_1$ receptor signaling in cortex were linked to increased Alzheimer-related pathology, including increased tau phosphorylation and amyloid β signaling in female compared with male mice (300). Few studies investigate sex differences in GPCR signaling, but it is likely that sex differences in GPCRs are also found in receptors other than CRF and that these differences could confer vulnerability and resilience to many diseases.

In human studies, single nucleotide polymorphisms in the CRF receptor gene (*CRHR2*) are associated with negative emotions in patients with irritable bowel syndrome (IBS) (301). Immune cells secrete $CRF_2$ in extracellular vesicles that circulate in the plasma and associate negatively with disease severity scores in IBS-diarrhea patients (294). Single nucleotide polymorphisms in *CRHR2* are also associated with lifetime PTSD in women (302) and with type 2 diabetes (303). The prevalence of type 2 diabetes and insulin resistance is greater in men (304). Epidemiological studies have shown that men with high levels of self-reported perceived stress have a 1.4 higher odds ratio of developing type 2 diabetes during a 10-year follow-up period and are at 2-fold higher risk of developing diabetes than women with similar levels of reported stress (305). In agreement with human data, male mice lacking functional stress receptors (*Crhr2*$^{-/-}$) and haploinsufficient (*Crhr2*$^{+/}$) mice have worse glucose and insulin tolerance, microvesicular hepatic steatosis, and dyslipidemia than female *Crhr2*$^{-/-}$ or C57BL/6 male and female mice in a high-fat diet–induced model of diabetes (129). Female *Crhr2*$^{-/-}$ mice had significantly greater brown adipose fat mass on high-fat diet than C57BL/6 female or male mice of either genotype, suggesting greater thermogenic responses that might be protective. However, the mouse study did not address whether steroid hormones contributed to changes in adipose mass or function. Thermogenesis in brown adipose tissue in humans in response to a meal or cold stress suggests that women have greater thermogenic responses than men and that these responses correlate positively with progesterone levels, but negatively with cortisol levels (306). Thus, analyzing data from both sexes provides insights into sex-specific mechanisms that regulate physiological processes in both sexes.

In colonic tissues of pediatric patients with Crohn's disease, subcellular localization of $CRF_2$ differs between boys and girls (127). Furthermore, lack of $CRF_2$ revealed several sex-specific signaling pathways and differential degree of inflammatory responses in male and female mice (127). Treatment with UCN1, a high-affinity agonist for both CRF receptors, rescued *Crhr2*$^{-/-}$ male mice from colitis-induced mortality, whereas UCN1 treatment increased mortality in *Crhr2*$^{-/-}$ female mice (127). Both diabetes and Crohn's disease show sex differences in disease prevalence and outcomes, yet most animal studies use male sex to delineate mechanisms. Analysis of the data by segregating the 2 sexes can reveal significant insights into distinct and shared mechanisms and factors that exist at baseline and during disease. For example, sex differences exist in the etiology of pancreatitis: alcohol and tobacco predominate in men, whereas idiopathic and obstructive etiologies predominate in women (307), yet to date only a few studies have used both sexes to study mechanisms involved in pancreatitis. While both males and females develop pancreatitis in animal models, when administered identical doses of the pancreatic stressor caerulein, C57BL6 female mice show less severe pancreatitis and histological damage than male mice (128). Lack of $CRF_2$ rendered female mice more susceptible to caerulein-induced pancreatitis compared with male *Crhr2*$^{-/-}$ mice (128), with both male and female *Crhr2*$^{-/-}$ mice exhibiting similar levels of total histological damage (128). Detailed analysis of components contributing to histopathological damage showed that female C57BL6J mice have less necrosis, zymogen granules, and vacuolization than male mice with pancreatitis, but they have similar levels of edema and neutrophil infiltration as male mice (128). This data segregation allowed isolation of factors that differentially contribute to histological damage, which otherwise would be lost, if grouped together in this analysis. Taken together, these data support a role for the CRF receptors, product of an autosomal gene and regulated by steroid hormones to bring about sex-specific cellular signaling and function.

## Sex Differences in Pharmacotherapy of Stress-Based Diseases

Sex differences in GPCR signaling are also relevant for pharmacology. Biased ligands can shift signaling toward

β-arrestin pathways and away from G-protein-mediated pathways based on how they bind to the GPCR (308). These biased ligands are being designed with the hope of providing more targeted therapies with fewer side effects (308, 309). Understanding sex differences in signaling and how such differences contribute to changes in physiology can inform the development of these biased ligands. For example, a $CRF_1$ receptor ligand that biases signaling through β-arrestin pathways may be useful for treating hyperarousal symptoms or reducing the progression of Alzheimer disease, especially in women. An idea for such a compound would never have come about if women were excluded from preclinical and clinical studies on $CRF_1$ receptor function.

The idea of using $CRF_1$ antagonists to treat depression, PTSD, and irritable bowel syndrome has been around for decades, but these compounds were ineffective in several clinical trials (222, 310). Sex differences in $CRF_1$ and $CRF_2$ receptor signaling may also explain the failure of different selective $CRF_1$ antagonists as treatments for these disorders. While there are likely many reasons for their failure, critical ones could be sex differences in their target, association of CRF receptors with different binding partners in female versus male cells, or heteromerization of CRF receptors (311-313), all of which can result in altered signaling. The consistent efficacy of $CRF_1$ antagonists in reducing anxiety-like and depressive-like behavior in rodents and nonhuman primates was established in studies primarily conducted in male animals (222, 314-317). In a study in which females were included, local blockade of $CRF_1$ receptors in the dorsal raphe with an antagonist reduced anxiety in male but not female mice, highlighting sex differences in efficacy (318). Yet these compounds developed primarily in male rodents were tested in clinical trials with participants of both sexes or only in women. Notably the only $CRF_1$ antagonist study that had success in reducing depressive symptoms, NBI-34041, was conducted only in men (222, 319). The approach of developing compounds in male animal models is not unique to $CRF_1$ antagonists and has been common practice (222). Collectively, these studies suggest that a failure of certain therapeutics may result from ignoring sex differences in their targets. Sex differences in targets are not well known because most preclinical studies use only male rodents (320, 321). Excluding females in the drug development stage particularly impacts women's health. Indeed, it is likely that some compounds deemed ineffective in male rodents would work in females, yet such compounds never would have a chance to make it to market, because of testing exclusively in male subjects. Moreover, the fact that most drugs are designed using males also likely contributes to the higher rates of adverse drug reactions in women compared to men (322).

Including both sexes in mechanistic studies is critical for developing drugs that work efficaciously in both sexes (see Box 4). Latent sex differences can also impact drug development: a compound targeting a mechanism in men may not work in women. As the field moves forward, we may find that sex-specific therapeutics based on understanding latent sex differences are required to truly improve patient outcomes. In sum, there are observable sex differences in behavior that extend beyond reproductive function. Molecular sex differences in several organs, such as the gut and the central nervous system, play a key role in driving these functional and behavioral differences. Moreover, even when function and behavior are consistent between the sexes, the underlying processes can differ. Thus, including both sexes in preclinical molecular studies guiding drug development is key for improving the health of men and women.

## Section IV

### Sex Differences in the Cardiovascular-Renal System

Cardiovascular disease (CVD) is the major cause of premature death in both sexes worldwide, although women generally develop CVD 10 years later than men (328). In 2016, ~18 million people died from CVD, representing ~30% of all deaths worldwide (329). There are marked sex differences in CVD and renal disease. For example, women are protected from heart disease during the reproductive years but are more likely to die in the first year following a cardiovascular event than males (330). Most heart conditions, including myocardial infarction, Takotsubo syndrome, and cardiac arrythmia, exhibit sex differences in symptoms and severity (331). Chronic kidney disease (CKD) is more prevalent in women but, once established, progresses more rapidly in men (332). However, this female advantage is lost after menopause. These sex differences in cardiovascular and renal disease have long been overlooked and underappreciated. The clinical presentation, the response to pharmacotherapies, standard care practices, and the underlying pathophysiological mechanisms differ in women compared to men. Furthermore, lack of understanding of sex differences in mechanisms underpinning cardiovascular and renal disease has led to poorer outcomes in women than in men. A major problem is that mechanistic preclinical studies in animal models have largely been conducted in males (333). Yet, it has become increasingly clear that sex differences

**Box 4.** Sex differences in pharmacokinetics and pharmacodynamics of drugs

Thalidomide, a sedative that was prescribed to many pregnant women to relieve pregnancy-associated nausea, was first sold in Germany (without a prescription) in 1957; it had been tested in animals and in men, but not in women. It was soon noted to cause multiple birth defects, most notably phocomelia (arrested limb development) and postnatal deaths. Fortunately, it was never approved in the United States, but thousands of children were affected around the world. In 1962, the US Congress passed the Kefauver-Harris Drug Amendments Act requiring manufacturers to prove a drug is both safe and effective (323). Consequently, the US Food and Drug Administration (FDA) recommended against drug testing on women, particularly those of child-bearing age, until the early 1990s. To date, most treatment guidelines are based on results from clinical trials conducted on middle-aged men. Dosage, pharmacokinetics, and pharmacodynamics data for women (and children) are lacking for most drugs. Activities of cytochrome P450 (CYP) enzymes show significant sex differences in drug metabolism in Phase I clinical trials (324). Gastric enzymes involved in oxidative degradation such as alcohol and aldehyde dehydrogenases are significantly more active in men than in women resulting in higher bioavailability of ethanol in women versus men. In Phase II trials, glucuronidating enzymes and some efflux transporters have been shown to be more active in men then in women. Together with estrogens and androgen that alter transmembrane transporters, these processes contribute to efficacy of metabolism in both Phase I and II. Drugs used for treatment of cardiovascular disease, such as angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers, diuretics, the aldosterone blocker eplerenone, antiplatelet agents, and oral antithrombotic medications, all show sex differences in efficacy and safety (325, 326). Over-the-counter nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen are more effective in men than women; there is more liver toxicity with acetaminophen use in women, whereas opioids and benzodiazepine work better in women. While some sex differences in metabolic clearance for statins and beta-blockers are known for these frequently prescribed drugs, dosing and adverse event monitoring in routine clinical practice is inadequate. Alosetron, a serotonin receptor 3 antagonist, is approved for treatment of severe irritable bowel syndrome–diarrhea symptoms in women, as it is largely ineffective in men (327). These findings emphasize that women and men take divergent routes (molecular mechanisms and signaling pathways) to reach the same destination (normal function or diseased state), with paths often intersecting. In the era of personalized medicine, there is no one-size-fits-all therapy, and considering sex-specific outcomes in pharmacokinetics and pharmacodynamics of drugs as well as clinical guidelines is warranted to ensure efficacy and safety of medications.

are apparent in all endocrine systems, which are modified by sex chromosomes and sex hormones, with temporal actions across the lifespan.

## Blood Pressure Links Cardiovascular and Renal Diseases

Cardiovascular and renal diseases are linked by the relationship of each to arterial pressure (Fig. 5). The cardiovascular system determines arterial pressure, with the heart generating cardiac output and the blood vessels determining total peripheral resistance. The kidneys contribute by regulating extracellular and intravascular fluid volume, and hence blood volume, and venous return. It is established that CVD leads to chronic kidney disease (CKD) and that CKD leads to the development of CVD. For example, following a myocardial infarct, cardiac output declines and arterial pressure falls causing the kidney to vasoconstrict and retain extracellular fluid, with the effect to increase venous return and normalize cardiac output. However, this has the unwanted effect of placing further stress on the failing heart. Conversely, kidney failure causes fluid retention and hypertension (334). Thus, cardiovascular and kidney function are intertwined, as are the endocrine systems that regulate organ function; including the renin-angiotensin-aldosterone system

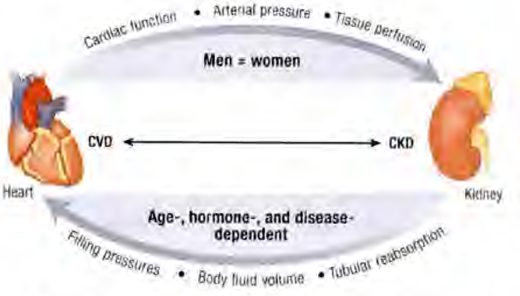


**Figure 5.** Heart and kidney functions are linked. Sex differences exist in many aspects of heart and kidney function at baseline and in CVD and CKD, as shown. Both organs feed-forward and influence each other's function. Genes, hormones, and age are some known factors that modulate this relationship in a sex-specific manner. Abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease.

(RAAS), the endothelin system, atrial natriuretic peptides, vasopressin, and glucocorticoid and mineralocorticoid hormones. There is an increasing recognition that there are fundamental sex differences in each of these systems. For example, aldosterone contributes to obesity-induced CVD with a greater impact in females than males (335). However, further research is required to fully elucidate the sex differences present in each endocrine system and how these impact disease development and progression.

## Sex Differences in Arterial Pressure and Hypertension

Hypertension is a major risk factor for cardiovascular and renal disease. Over the lifespan there are age- and sex-related differences in arterial pressure. The majority of the data are derived from cross-sectional studies, but a few powerful studies have tracked arterial pressure over decades within a population (332, 336-339). Arterial pressure increases in both men and women with age, although the slope of the relationship is different between men and women. Sex differences in arterial pressure emerge during adolescence and are maintained throughout adulthood until women reach menopause (336, 337, 339). Arterial pressure is ~5 to 10 mmHg greater in men than age-matched women during the reproductive years (340-342). Postmenopause arterial pressure rises steeply in women regardless of race, ethnicity, or country of origin (340-342). One of the most striking characteristics of hypertension is that the prevalence and severity is lower in premenopausal women than in age-matched men. The prevalence of hypertension is ~10% in young premenopausal women, ~50% in postmenopausal women and by the age of 75 years almost ~80% of women are hypertensive (342-344).

Nonhuman mammalian species also display sex differences in arterial pressure. Arterial pressure in adult females is lower in normotensive dogs, sheep, rabbits, rats, and mice as compared with adult males (338, 345). Furthermore, in rodents, rabbits, and sheep, females of reproductive age are protected against the development of hypertension, such that arterial pressure increases significantly less in females than in males, in settings of disease (338). Thus, sex differences are present in the pathophysiology of cardiovascular and renal diseases. Yet, the mechanisms underlying the sexual dimorphism of arterial pressure in men and women as they age are poorly understood. However, extensive evidence indicates that sex hormones likely contribute to the regulation of arterial pressure through their actions on endocrine systems.

## Sex Differences in Endocrine Control of Arterial Pressure and Kidney Function

There are subtle differences in most endocrine actions between men and women. It is not the maximal response of each system but rather the slope of the response that is altered. In this manner, a system responds maximally in a hemodynamic crisis (eg, hemorrhage) but in a sex-specific manner to lesser challenges. For example, a greater dose of the vasoconstrictor angiotensin II is required to increase arterial pressure in female than male mice (346). Consistent with this finding, the same dose of angiotensin II caused a greater reduction in renal blood flow in men than women, with the suggestion that this was an angiotensin type 2 receptor ($AT_2R$) mediated effect (347). In rodents, females of reproductive age have a greater $AT_2R$ to angiotensin type 1 receptor ($AT_1R$) ratio than males, which contributes to the reduced pressor response to angiotensin II (348). This has been indirectly demonstrated in women, in studies examining forearm vascular resistance responses to $AT_2R$ blockade (349). The $AT_2R$ also mediates a leftward shift in the pressure natriuresis-diuresis relationship, an effect that is greater in female than male mice (350). In women, indirect evidence also indicates a more pronounced role for the $AT_2R$ in the regulation of renal blood flow responses to angiotensin II (347). This is linked to differential expression of components of the RAAS in males and females, which have been demonstrated in most mammalian species, including humans (351). In the context of the above example, estrogen interacts with the glucocorticoid response element on the X-linked *AGTR2* gene, to increase $AT_2R$ expression in females (352). In addition, there are sex differences in human aminopeptidase A, aminopeptidase N, and angiotensin-converting enzyme 2 levels, responsible for generation of the angiotensin peptide fragments, angiotensin III, and angiotensin-(1–7), which have a high affinity for the vasodilatory $AT_2R$ and Mas receptors, respectively (353-356). Lastly, there are marked and important sex differences in the production and function of aldosterone, although this has only recently been started to be examined (335). Thus, in females the RAAS is balanced toward the protective depressor RAAS arm, which at the lower physiological range may prevent arterial pressure increasing to the same extent as in males. However, this delicate balance may be lost in women after menopause and in the situation of metabolic syndrome.

Other vasoconstrictor systems also have sexually dimorphic actions. Endothelin-1 causes vasoconstriction via the endothelin type A receptor ($ET_AR$), and vasodilation and sodium excretion via the $ET_BR$. Testosterone increases $ET_AR$ and estrogen increases $ET_BR$ expression, which contributes to the differential control of arterial blood pressure and renal function between the sexes (357). Vasopressin, with important roles in circulatory and water homeostasis, is affected by age and sex. Urinary concentrating ability declines with age, but more steeply in women. Young men produce more concentrated urine than women, in part due higher plasma arginine vasopressin levels and greater vasopressin type 2 receptor expression in the collecting ducts of the kidney in males (358, 359). Renal vasopressin type 2 receptor expression declines with age in association with a reduction in maximal urine concentrating ability (358, 359). Interestingly, aldosterone signaling via mineralocorticoid receptors is associated with increased CVD risk and is

enhanced in obese women (another example of how the RAAS is differentially modulated in females), which has been linked to leptin-induced endothelial dysfunction (360, 361). Moreover, evidence in rodents indicates that sodium reabsorption along the length of the renal tubule is sexually dimorphic, with reabsorption shifted to the later segments in females compared to males. This was associated with greater sodium epithelial channel expression, under the control of aldosterone, in the collecting duct, which could also contribute to the increased cardiovascular and renal risk associated with aldosterone in females (362). Finally, oxytocin, relaxin, and prolactin, which are traditionally known for their roles in pregnancy, have differential cardiovascular and renal actions in nonpregnant female and male rodents (348, 363, 364). Thus, evidence points to sex differences in endocrine control of extracellular fluid homeostasis and vascular function, which likely contribute to age- and sex-related disparities in renal and cardiovascular disease risk. Further studies are warranted to understand this complex issue more fully. In particular, it is important to take into account the subtle effects within the physiological range that counterbalance function of each hormonal system, rather than examine the impact of pharmacological doses which can mask sex differences in responses.

## Cardioprotective Mechanisms in Women Sustain a Healthy Pregnancy

The cardioprotective mechanisms that predominate in women during the reproductive years enable the extensive hemodynamic adaptations required to meet the metabolic demands of the developing fetus and a successful pregnancy. During a normotensive pregnancy, blood volume increases and cardiac output increase by ~30% to 50%, but arterial pressure declines due to marked peripheral vasodilatation (365, 366). The associated renal vasodilation accommodates an increase in glomerular filtration rate to process the additional blood volume, but an increase in vasopressin type 2 receptor expression enables increased tubule reabsorption of sodium and water. However, in women with preeclampsia, a pregnancy-induced form of hypertension, these cardiovascular adaptations are perturbed. Accumulating evidence now indicates that women with a history of pregnancy-associated hypertension have a 2- to 5-fold increased risk of CVD in later life (367). Understanding the mechanisms underpinning this dysregulation of vascular function in pregnancy-related hypertension may lead to the identification of new therapeutic targets for the treatment of cardiovascular disease in both sexes. For example, relaxin, which is known best for its role in pregnancy but is also produced in males, plays roles in the regulation of renal function, blood pressure, and tissue fibrosis (363). Thus, it is a mistake to assign hormonal systems a specific role as most have wide-ranging tissue-specific pleiotropic effects.

## Sex Hormones and Sex Chromosome Complement in CVD

Sex hormones contribute to sexual dimorphism in endocrine control of the cardiovascular system, with evidence suggesting that there is a "sweet spot" for both testosterone and estradiol, as unusually high or low levels of either promote disease (368-370). This has been the cause of apparent discrepancies in the literature. In particular, this remains a problem in animal studies in which the dose of estrogen used to study the impact of estrogen replacement in aged or gonadectomized models varies widely (~1000-fold), as does the route or length of administration; none of which accurately reflect the cyclic pattern of in vivo production. This lack of rigor into investigation of the effects of sex hormones in preclinical models likely contributes to the controversy that surrounds hormone replacement therapy for the prevention of CVD risk. Despite extensive evidence that hormone replacement therapy is cardioprotective, the negative results of the Women's Health Initiative Trial effectively halted the use of hormone replacement therapy (371). Certainly, high-dose estrogen can increase blood pressure and cardiovascular risk in women (372). However, continued investigation supports the use of hormone replacement therapy in subsets of women, and further work in this area is required (373). In contrast, in men with low testosterone, beneficial cardiovascular effects are seen with testosterone replacement (374). In women with polycystic ovary syndrome, high testosterone levels are associated with elevated blood pressure (374). Dose-ranging studies are required to delineate these effects.

The sex chromosomes may have a direct impact on sex differences in the physiology and pathophysiology of the cardiovascular system and cardiovascular risk, independent of sex hormones. Human sex chromosome aneuploidies, such as Turner and Klinefelter syndromes, suggest that sex chromosome abnormalities can carry an increased risk of CVD. Women with Turner syndrome have around a 3-fold greater mortality and reduced life expectancy relative to the general population (375-377). CVD is a leading cause of increased mortality in Turner syndrome (375-377). Congenital cardiac anomalies, hypertension, coarctation of the aorta, diabetes, ischemic heart disease, and stroke are commonly associated with this condition (378). Similarly, men with Klinefelter syndrome have a high cardiovascular risk profile (379, 380), and an increased risk of

mortality from cardiovascular disease (381, 382). However, observations from studies in individuals with sex chromosome aneuploidies are complicated by confounding factors, including abnormal gonadal sex hormone levels associated with gonadal failure. Thus, it is very difficult to distinguish between hormonal versus genetic mechanisms and cardiovascular risk in these human conditions.

Experimental approaches, such as the FCG mouse model discussed in "Section I," and Box 3 can discriminate between hormonal and sex chromosome effects in cardiovascular disease (115). Beyond genes on the sex chromosomes, there are sex differences in autosomal gene expression, which can be both organ or cell specific (383). In the kidney and the heart, hundreds of rat and human genes are regulated differently between the sexes (384-386). This disparate expression is triggered by sex hormones in ~30% of cases, with the other 70% linked to sex chromosome and microRNA dimorphisms (384, 385). For example, sex differences have been reported in the expression of nitric oxide synthase, tyrosine hydroxylase, and sodium channels in the rodent heart and kidney (332). However, few studies to date have compared gene expression and the effect on the proteome between the human sexes, and further studies are required.

### Sex Differences in Pharmacotherapy for Cardiovascular and Renal Disease

Men and women respond to disease differently: kidney diseases progress faster in men than women, kidney transplants from women to men tend to fail more frequently than the reverse, and the effects of diabetes on the kidney differ between the sexes (387-392). Furthermore, symptoms and mechanisms of heart failure differ between the sexes (393). This suggests that sex-specific treatments for CKD and CVD could be required. There is currently little evidence to suggest that men and women respond differently to current treatments for hypertension (394). In large part, this is because clinical trials have lacked statistical power to take this into account. It will be difficult to achieve such an outcome for drugs that have already received FDA approval. However, some treatments are more frequently prescribed, without any basis in evidence (395). There are also marked differences in pharmacokinetics and pharmacodynamics (see Box 4), leading to more frequent adverse drug reactions in women, related to differences in drug clearance and breakdown (396). Therefore, sex should be taken in account for new treatments seeking approval in the future. When women are considered, important and unexpected sex differences are observed in almost every aspect of cardiovascular and renal function in health and disease. Further research is required to fully understand these differences, and in turn to guide the development of sex-specific treatment guidelines for CVD and CKD.

## Section V

### Challenges for the Future of Sex Differences Research—Areas Requiring Special Attention

Sex differences exist in anatomy, behavior, and physiology across the animal taxa. By extension, because of these innate differences, sex differences exist at molecular and cellular levels in mechanisms that underlie these processes. Despite concerted efforts by the Office of Research on Women's Health and the Organization for the Study of Sex Differences in educating researchers about the distinction between sex versus gender, the indiscriminate use of the word "gender" continues to pervade scientific literature. The sex of established cultured cell lines is another issue; in addition to aneuploidy, chromosomal numbers change as cells are passaged and are dependent upon the tissue of origin (397, 398), but this aspect is beyond the scope of this Statement. Not surprisingly, sex differences are seen in etiology, prevalence, and outcomes in a myriad of human diseases that range from psychological and autoimmune to gastrointestinal, cardiovascular, renal, and reproductive; SARS-CoV-2 causes more severe COVID-19 disease in men than in women despite similar infection rates (399-401). Besides genetic makeup (predisposition), extraneous factors, such as the socioeconomics, demographics, education level, profession, age, and the environment, greatly influence an individual's health; COVID-19 disease outcomes especially highlight the contribution of these extraneous factors in health disparities. Factors such as the endocrine-disruptive chemicals can disproportionately affect one sex over the other; regardless, whether favorable or adverse effects are present in one or both sexes, the effects would impact trans and cisgender persons, and hence these sex-specific effects should not be overlooked or underestimated (402). Some human studies addressing sex differences take these factors into account, whereas others are more selective. Many studies of disease pathways are sensitive to levels of gonadal steroid hormones, which contribute to sex differences. In human studies, unless gender information is explicitly collected or available, the study deals with biological sex, not gender. Use of sex and gender interchangeably deemphasizes the importance of studying gender as an independent variable.

In animals or experimental models of human diseases, effects of estrogens have been investigated more often than effects of progestins and androgens, which should

be corrected. Paradoxically, female sex is often excluded from experimental design on the basis that: (i) the estrus cycle will interfere with data interpretation; (ii) mechanisms that operate in the male sex will operate in the female sex and thus only need to be confirmed in females; (iii) metabolic demands are similar between the sexes; (iv) the X chromosome in males and females is subject to similar regulation; and (v) autosomal genes will be subject to equal variance between the sexes. The same studies often ignore the diurnal cycling nature of testosterone in males; testosterone levels in male rodents can show more day-to-day variability than estrogen and progesterone levels in females. Other steroid hormones, such as glucocorticoids, that show circadian rhythm and whose levels differ between the sexes also influence gene expression and function. In rodents but not primates, sex differences in secretion of growth hormone result in sexually dimorphic hepatic metabolism of drugs and xenobiotics (403). In rodents, endocrine disruption can have transgenerational effects on male and female reproductive systems (404). Since changes in hormone levels and gene expression are dynamic, can be localized, and are spatiotemporally distinct, no one study design or condition can be used as a gold standard. Animal housing and handling conditions can also create sex differences, and thus any experimental design and data interpretation should take these variables into account. If sex-segregated data does not differ for the aspects under study, then data can be pooled from the 2 sexes and reported accordingly.

Studies in animal models have just begun to uncover unequal effects of the sex chromosomes in XX vs XY cells, so we expect further discoveries about such effects in the future. Once genes that cause sex differences are discovered in animals, the findings generate new hypotheses and rationalize human studies to determine whether the same gene also creates sex differences in humans. That question can be studied by the methods of human genetics, relating genetic variation to disease incidence and outcome. Without the animal studies, however, it is difficult to understand detailed molecular mechanisms. It is also important to remember that no single rodent or animal model can capture the complexity of any human disease, but each model provides valuable insights into one or another major aspect of disease. If different etiologies of a given disease share mechanisms, then mimicking the precise conditions that initiate human disease may not be critical.

The study of sex chromosome effects is in its infancy and has focused on proving that sex chromosomes play a role and finding the genes responsible for the effects. So far there has been little effort to understand how these factors interact with steroid hormones to cause sex differences. If both types of factors cause differences in disease incidence, are they affecting the same or different downstream pathways? Do their effects converge, or do they independently affect different mechanisms that each influence a complex disease? Do male-biased factors (hormones, Y-chromosome genes) act synergistically to induce a male-specific state, or do they counteract each other to reduce the difference between males and females (123, 405)? Are the diverse sex-biasing factors changing in their effects across the lifespan, leading to changes in the type or amount of sex difference at different ages?

When studying sex differences in animal models of human diseases, it is important to first understand and elucidate differences at baseline in gonadally intact animals. As pointed out earlier, steroidogenic enzymes are also present in nongonadal tissues, especially the brain, thus it is not entirely possible to eliminate effects of sex steroids from all tissues. Moreover, tamoxifen-inducible *Cre* recombinase used to routinely perform lineage tracing and gene inactivation studies in mice has its own problems (406, 407) that are largely ignored and can further confound sex-specific data analysis; tamoxifen antagonizes actions of estrogen receptor-β and inhibits expression of over 70 genes (408), but the contribution of these tamoxifen-regulated genes on study results and outcomes is never accounted for and requires careful consideration. Before mechanisms behind sex differences in physiology and disease can be elucidated, a fundamental understanding of sex differences that exist at baseline, is needed.

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## Additional Information

***Correspondence:*** Aditi Bhargava, PhD, Professor, Department of ObGyn and Center for Reproductive Sciences, 513 Parnassus Avenue, HSE1635, Box 0556, UCSF, San Francisco, CA 94143, USA. Email: Aditi.bhargava@ucsf.edu

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Ex. 4



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# Serving Transgender Youth: Challenges, Dilemmas and Clinical Examples

**Amy C. Tishelman, Ph.D.**[1], **Randi Kaufman, Psy.D.**[1], **Laura Edwards-Leeper, Ph.D.**[2], **Francie H. Mandel, LICSW**[3], **Daniel E. Shumer, M.D.**[3], and **Norman P. Spack, M.D.**[1]

[1]Boston Children's Hospital and Harvard Medical School

[2]Pacific University School of Professional Psychology

[3]Boston Children's Hospital

## Abstract

Historically, many gender variant individuals have lived in a chronic state of conflict between self-understanding and physical being, one in which there was a continual misalignment between others' perceptions of them and their internal self-perception of gender. Only recently have professionals from mental health and medical realms come together to provide services to these youth. This paper describes an innovative program: the first mental health and medical multidisciplinary clinic housed in a pediatric academic center in North America to serve the needs of gender variant youth. We describe our model of care, focusing on the psychologist's role within a multidisciplinary team and the mental health needs of the youth and families assisted. We highlight clinical challenges and provide practice clinical vignettes to illuminate the psychologist's critical role.

### Keywords

transgender; gender dysphoria; gender non-conforming; youth; adolescent

## Introduction

Historically, many gender variant individuals have lived in a chronic state of conflict between self-understanding and physical being, with a continual misalignment between others' perceptions of them and their internal self-perception of gender. Only recently have professionals from mental health and medical realms come together to provide services to youth and, hopefully, some validation. As with other newly evolving fields of study, initial interventions were applied without the benefit of much research or precedent for guidance, and at times in an atmosphere of professional division (see Drescher & Byne, 2012, for a summary of continued controversies).

Correspondence regarding this article may be sent to the lead author at: Amy C. Tishelman, Ph.D., Boston Children's Hospital, 333 Longwood Avenue, 6th Floor, Endocrinology, Boston, MA 02115.

The Gender Management Services-Disorders of Sexual Development Program (GeMS-DSD) evolved due to the dearth of available services for two distinct populations: a) youth with Disorders of Sexual Development (DSD) and b) gender variant youth. DSD refer to biological conditions in which anatomic sexual development is atypical (Houk, Hughes, Ahmed, & Lee, 2006) whereas gender variance refers to gender expression and/or identity inconsistent with prevailing societal expectations and norms (Kulick, 1999). The term transgender typically refers to those individuals for whom genotype and phenotype are mismatched. Therefore, biologically male children may self-identify as female and vice versa, or youth may not fit neatly into either category. This paper will focus on the gender variant group served by GeMS-DSD. We highlight clinical challenges, and provide clinical vignettes to illuminate the psychologist's critical role. Please refer to the online supplemental materials for further description of terms relevant to gender, sex and sexuality, and a summary of suggested psychosocial evaluation recommendations.

The development of the GeMS-DSD Program was made possible because the initiative of an endocrinologist with prior expertise treating transgender adults, and a strong passion to assist gender variant youth without access to care. As with any novel program, a vision and a sense of possibility are essential aspects of effective action. With a strong belief in the need for such a program in a multidisciplinary hospital setting, the GeMS-DSD service was developed, partially dependent upon the persuasive abilities of the founding physicians, but also within the structure of an institution that encouraged care for underserved youth and with clinic directors and hospital administrators who fostered innovation. The GeMS-DSD program became the first multidisciplinary mental health and medical program housed in a pediatric academic center in North America to serve youth with DSD or gender variance, and has forged a path for the development of other clinics in the United States. Many mental health professionals, medical students, pediatric house officers, endocrine fellows, and staff endocrinologists have participated in our program.

## Program Development

The development of GeMS-DSD was a shared effort, requiring extensive multidisciplinary collaboration. Consultation was sought from urology, endocrinology, medical ethics, genetics, neonatology, gynecology, psychology, and hospital administration. When the program opened, it was co-directed by a pediatric urologist with expertise treating children with DSD and a pediatric endocrinologist, working in tandem with a psychologist to provide evaluations and services for gender variant youth and their families. The remainder of the discussion will focus on the gender variant group in the GeMS program, with an emphasis on the crucial role of psychologists within this multidisciplinary team.

In order to develop our mental health protocols, our hospital supported the GeMS psychologist receiving training in Amsterdam from Peggy Cohen-Kettenis, PhD and her team, pioneers in assessing and treating transgender youth. The purpose of the trip was to learn and adapt the Dutch protocol for use in the United States. The Amsterdam group opened the first specialized gender identity clinic for children and adolescents in 1987 (deVries & Cohen-Kettenis, 2012) and have published numerous studies based on their protocol and interventions (e.g., Delemarre-van de Waal & Cohen-Kettenis, 2006; deVries,

Steensma, Doreleijers, & Cohen-Kettenis, 2011; Wallien & Cohen-Kettenis, 2008; deVries & Cohen-Kettenis, 2012). During the training trip, the GeMS psychologist and endocrinologist participated in the first international Adolescent Gender Identity Research Group Meeting. Psychological measures were selected collaboratively for clinics to use in the evaluation of transgender youth, based on shared experience with this population, while each clinic adapted and added measures as needed for individual sites.

When opened, the GeMS clinic was flooded with inquiries from families, not only from the local region, but also from across the nation and internationally. Notably, before the GeMS program existed, the demand for services was largely invisible. In addition, children and families struggled to identify resources (many of which were predominantly non-existent) without the aid of trained professionals, while sometimes coping with significant and multifaceted psychosocial challenges. These could include a range of issues such as managing family responses, including anxieties and discord related to atypical gender expressions and/or disclosures of children; managing peer, school and other social circumstances in contexts that were often less than accepting; and managing mental health issues. Numerous articles have been published outlining similar multifaceted issues gender nonconforming children and families may face (e.g., Dreger, 2009; Ehrensaft, 2007; Malpas, 2011; Menvielle, 2012). In response to the increasing volume of cases a social worker joined the team to conduct pre-screening telephone intakes, aid families in finding resources, and to help develop written clinic protocols in collaboration with the psychologist.

## Clinic Practice

The GeMS program, based on the model of care first developed and shaped in Amsterdam, continues to be adapted over time in response to new developments in the field and service demands. Our protocol relies on existing guidelines and standards for working with transgender individuals developed by various disciplines. For example, the World Professional Association for Transgender Health (WPATH) Standards of Care (Coleman et al., 2011), the Endocrine Society Guidelines (Hembree et al., 2009), the Report of the American Psychological Association (APA) Task Force on Gender Identity and Gender Variance (2009; http://www.apa.org/pubs/info/reports/gender-identity.aspx), and the American Counseling Association Competencies for Counseling with Transgendered Clients (2010) each offer valuable recommendations for working with the transgender population. Generally, these guidelines and standards are similar in that they all recommend supporting transgender individuals in their affirmed gender identity, which often includes assisting in medical interventions that will help make the individual's body congruent with their affirmed gender. The APA Task Force report (APA, 2009) states support for the "efficacy, benefit, and medical necessity of gender-transition treatments for appropriately evaluated individuals…" (p.67), a statement consistent with the goals of the GeMS team.

Nevertheless, many of these guidelines do not focus on issues specific to transgender youth. The Society for Adolescent Health and Medicine (2013) has issued recommendations for promoting the health and well-being of lesbian, gay, bisexual and transgender adolescents, and the American Academy of Child and Adolescent Psychiatry (2012) has published practice parameters addressing gay, lesbian, bisexual, gender nonconforming and gender

discordant children and adolescents. The APA also published a helpful and accessible pamphlet regarding gender identity and gender expression, with some information about transgender youth (http://www.apa.org/topics/sexuality/transgender.pdf). They note that "it may be helpful to consult with mental health and medical professionals familiar with gender issues in children" (p. 3), while also emphasizing that "identifying as transgender does not constitute a mental disorder" (p. 3) and that "it is not helpful to force the child to act in a more gender-conforming way" (p. 3). This position is aligned with our gender affirming approach to care (see Hidalgo et al., 2013 for an elaboration of a gender affirming model) which views gender variations as part of an expected diversity, and not pathology. Mental health challenges may emerge related to cultural and social responses to a child or co-exist with gender non-conformity. Consistent with much literature (e.g., Hidalgo et al., 2013; Steensma, McGuire, Kreukels, Beelman & Cohen-Kettenis, 2013; Wallien & Cohen-Kettenis, 2008) we view gender as sometimes fluid over time, recognizing that not all gender non-conforming children fit neatly into male or female identities, and that gender identity (internal sense of self) and gender expression (outward expression of gender) may modify over time. Members of the GeMS team have played a role in the development of standards and guidelines, including as a member of the active APA Task Force to develop guidelines for psychological practice with transgender and gender non-conforming clients.

As time has elapsed, and our clinical expertise has developed, we have advanced to a more flexible, individualized approach to care than was utilized at the clinic's inception, which may evolve further with increasing research to inform best practices. Within our current model we continue to prioritize evaluation and treatment, mental health and readiness for medical treatment, but allow for a variable structure and account for the unique circumstances of the youth and family. Therefore, the model set forth below is adaptable, serving as a guide for care as opposed to an inelastic protocol. Clinical discretion and family needs are prioritized, as deemed appropriate by the psychologist working within a multidisciplinary team. In addition, as the field evolves, our future practices may vary from those delineated. However, we anticipate that our fundamental approach will endure, and can be described as the intertwining of mental health and medical expertise, each informing the other to best assist families and youth.

## Intake

The initial telephone intake, conducted by a GeMS clinical social worker, includes gathering a substantial amount of information and allows the parent and/or guardian the opportunity to tell their story to a knowledgeable professional, often for the first time. The information includes reasons for concerns about gender variance, current crises, and developmental, medical, and mental health history. Other services include support, psycho-education, explanation of protocols, outside referrals and scheduling a clinic appointment when appropriate. We believe that it is imperative for a qualified and experienced clinician to be the first point of clinical contact to set the roadmap for future care, and to act as an identified trusted individual to whom the family can turn. The intake frequently plants the seeds of hope, providing relief for families who have been enduring the stress of a situation for which they have had little preparation, often within a context of isolation. A description of the patient population presenting in GeMS through the year 2010 indicated that the mean age at

intake was approximately 14, with a slight preponderance of genotypic female to male patients, many of whom (approximately 44%) presented with a significant psychiatric history (Spack et al, 2012).

It is important to note that the earliest we medically treat children is when puberty has just begun, medically defined as Tanner Stage 2 (Marshall and Tanner, 1969, 1970). A youth's chronological age is less relevant than their biological development and a cognitive level necessary to adequately assent to treatment. However, we do not accept new patients for treatment older than eighteen.

In the case of younger children who are not yet approaching puberty, guidance is often sought for gender related challenges, in which case we provide psycho-education, and offer referrals for families to receive supportive mental health counseling. These services may assist the youth in clarifying their gender identity, and help youth and families navigate the many anticipated and unanticipated issues they may confront, including whether or not to initiate a social transition (presenting in social settings as the affirmed gender). Children may experience anxiety and depression, often secondary to the social and familial ramifications of their gender questioning and/or atypical presentation, and a mental health professional with relevant expertise can be tremendously helpful.

When a child is seeking services closer to puberty, our current model typically recommends three to six months of psychotherapy. For some children who feel a compelling sense of urgency in light of impending physiological changes, this recommendation may be modified, especially when complicating factors are absent and the child is well supported. This aspect of the model reflects our recognition that many youth and/or parents seeking services in our clinic are in the early stages of gender exploration and consideration of medical intervention options, and need a safe forum in which to learn more about the issues involved, and treatment available. Further, we have found psychotherapy exceedingly helpful for treating co-occurring mental health issues and for exploring the child and/or adolescents' thought processes, family functioning, strengths and support systems. In addition, psychotherapy enables a deeper exploration of the child's Gender Dysphoria (GD), the range of gender expression and gender identity questioning, and whether the subjective experience fits more into a model of binary identity (e.g., male/female) versus a fluidity of gender and gender nonconformity. Mental health intervention can also support problem-solving regarding the medical and social challenges that lie ahead. It helps facilitate discussion between families and other support systems (schools, extended family, religious/sectarian community affiliates) as next steps are contemplated. Many authors also have noted the importance of mental health services (e.g., Bernal & Coolhart, 2011; Menvielle, 2012; Turek, 2011). Drescher & Byne (2012) emphasize that "the majority of adolescent persisters do well when they receive family and professional support for early interventions" (p. 504). Therefore, GeMS patients are asked to continue working with their outside mental health provider during the course of medical treatment in our clinic.

One of the purposes of the puberty blocking medical intervention (described below) is to buy time for the adolescent to continue exploring gender identity issues without the added stress of a puberty that is inconsistent with their self-identity. In our view, it is often

unrealistic to expect an adolescent to sort through the myriad of issues related to gender variance without the help of a professional. Many of the challenges adolescents face regard the reactions of others to their gender identity and/or expression, but can also include gender-related questioning and confusion (see Cohen-Kettenis, Steensma & de Vries, 2011, for an interesting discussion of psychological interventions for adolescents with GD).

## Psychological Evaluation

The goals of evaluation, conducted by a licensed psychologist, are to further understand the child and family's needs, and to inform medical treatment interventions. Before initiating the evaluation, we typically request a letter from the child's outside community therapist composed with the aid of a guide we provide. The therapist is asked to address their understanding of the patient's gender identity history, including length of time the patient has had gender questioning feelings, how long he/she has been living in the role of a different gender (if at all), and how persistent his/her identification with a different gender has been, if ever, over the course of time. The letter includes the therapist's impression of the patient's supports, the therapist's perception of other mental health issues or developmental concerns, and finally, the therapist's perception of benefits/drawbacks related to medical intervention.

Assuming that the therapist's letter is generally supportive of medical intervention, following review by our mental health clinicians, we move forward with an on-site psychological evaluation. This evaluation consists of extensive interviews of youth and families, and measures of anxiety, depression, self-concept, behavioral and social functioning, autism spectrum disorder (ASD), and gender identity. With consent, outreach is often made to collateral informants, and we review relevant documents (e.g., neuropsychological evaluations), as appropriate.

In the clinical interview, we address what the youth and parents hope to accomplish from the evaluation, family and developmental history, school and academic history, mental health and medical history, substance use, and trauma history. We gather an extensive gender history including the youth's subjective experience of gender across time, gender presentation, gender role expression, and sexual orientation. Considerable attention is paid to factors that make these cases more complicated, such as patients presenting with features of ASD, severe psychiatric concerns (e.g., suicidality, self-harming behaviors, psychosis, violence and aggression, and history of abuse/trauma), and/or complicated family factors (e.g., divorced parents, unsupportive family members). We assess support structures and strengths, familial attitudes about non-traditional gender roles and sexual orientation preferences, religious, cultural and ethnic background, and additional individual and family stressors. The youth's age at first signs of GD or disclosures is always noted; families may be caught off guard when their children first disclose gender questioning close to adolescence or after the onset of puberty, and often the evaluations of these youth and families are particularly complex.

Consistent with psychological evaluations in general, the rationale for numerous measures and methods of information gathering is to obtain the most authentic and comprehensive clinical picture possible. This is particularly critical, given that the results and clinical

formulation play the primary role in deciding whether to move forward with a potentially life-changing medical intervention for the adolescent. We synthesize and interpret the information obtained, and use the evaluation as a way to understand the youth and family's state of mind, ambivalences, and overt and covert pressures. We also want to ensure that, to the extent possible, a youth's cultural and social environment will support their chosen gender identity and provide a safety net as they move forward. A full clinical report is written that integrates the information, and provides a formulation and recommendations. The team psychologist then meets with the family to review this information. Medical interventions that often follow are either in the form of puberty blockers, and/or cross-sex hormone therapy, described below.

As noted above, continuing psychotherapy for youth is typically recommended by our protocol. At times we recommend family treatment and/or support groups to help with the family's adjustment to their child's transition. The GeMS team then remains in contact with community providers as clinical care dictates. In addition, youth treated in our program return for regular clinic visits, meeting with both mental health and medical team members, in order to provide continuity of care and further assist adolescents and family members as needed.

## Medical Intervention

Medical intervention with transgender youth in GeMS occurs under the auspices of a subdivision within the Endocrine Department. In brief, as alluded to above, with children who have recently begun puberty, puberty-blocking hormones are often prescribed. These are administered in the form of subcutaneous implants in the upper arm, which last two to three years, or monthly injections. These treatments are not routinely covered by health insurance in the United States and may range in cost from $120 to over $1,000 per month. Other medical services, laboratory tests, and sometimes cross-sex hormones may be covered by insurance.

In the absence of pubertal blockers, biological males with affirmed female identities may experience significant growth, permanent facial hair and vocal changes, and intolerable erections. A voice that has deepened cannot be raised through hormone therapy, and requires difficult and expensive speech therapy, in order to affect a higher voice. Similarly, without such intervention, biological females who identify as male may experience menstruation and breast development; the latter can only be modified through surgery. Nevertheless, an adolescent who has initiated puberty blockers can decide to terminate the intervention and allow physiological changes to occur as they would have, had the medical intervention never been initiated.

Only with an older adolescent, typically around age sixteen, are irreversible interventions initiated, and only after psychotherapy and a careful psychological evaluation has taken place. In this way, we try to ensure that an adolescent is not ambivalent, and that these interventions are well thought through and understood without coercion from others, and with full consent. When these conditions are met, an adolescent may be placed on cross-sex hormones (estrogen for genetic males and testosterone for genetic females), to facilitate a more complete transition into that individual's affirmed gender. When natal puberty has

been previously blocked, the cross-sex hormones are even more effective in rendering a more gender consonant, "typical" presentation. For male-to-female (MTF) patients, treating with pubertal suppression in early puberty followed by estrogen in later adolescence causes enhanced breast development, vocal quality consistent with the affirmed gender, no development of a protruding larynx or "Adam's Apple", absence of male-typical facial or body hair, and diminished masculinization of the body frame and facial bones. For female-to-male (FTM) patients, pubertal suppression in early puberty followed by treatment with testosterone later in adolescence leads to development of facial and body hair, deepening of the voice, masculinization of the body frame and facial bones, no need for mastectomies, and no menarche (see Delmarre-van de Waal & Cohen-Kettenis, 2006 and Shumer & Spack, 2013 for further information).

A common scenario is for GeMS to recommend puberty blockers, when the youth and/or the parent may feel that it would be best to start cross-sex hormone therapy instead. The delay of puberty, rather than the immediate onset of the puberty of choice (utilizing cross-sex hormones) is sometimes difficult for the youth or family to accept. This is an area where we currently have little research to guide us, and the decision of whether to block puberty, or instead move forward with an affirmed gender (i.e., cross–sex hormones) must be weighed carefully. Aside from the irreversible nature of cross-sex hormone initiation, this intervention has significant ramifications for fertility, while puberty blockers do not (Lazar, L, Meyerovitch,, de Vries,, Phillip & Lebanthal, 2014).

Anecdotally, we have found that the GeMS evaluation has been invaluable by providing information to guide subsequent psychosocial and medical decision-making. In general, adolescence is marked by a search for identity and personal transformation, and at times impetuous decision-making. Given the implications of social transition and medical intervention, coupled with the developmental challenges of identity consolidation, we feel the need to progress with care and forethought, to ensure that all interventions proceed safely, to minimize medical and psychosocial contraindications or complications, and to make sure it is the appropriate timeframe for intervention. We also want to ensure that the child/adolescent who may be gender variant does not feel compelled to choose a gender (male/female), when in actuality they may not fit into a typically recognized gender identity. Nevertheless, these considerations always need to be balanced by the very real physiological ticking clock, especially for the younger child on the verge of a puberty that they deeply want to avoid.

## Challenges and Dilemmas of Psychosocial Practice

### Child and Family Expectations

When confronted by a gender variant child, a parent may be caught very much off guard, with no ability to rehearse the best response to such an unanticipated circumstance. In addition, for a parent, it may feel like a loss of the daughter or son to whom they became so bonded. Moreover, some families are aware of their child's GD in early childhood while others are surprised to learn about it when their child is in their teens. Both instances carry particular emotional impact for families. Many parents are resilient and loving in the face of these challenges, but may experience an understandable drive for rapid certainty and

solutions. We have also encountered parents who are resistant to accepting this diagnostic picture, and believe their child's gender variance is a phase, or a manifestation of some other psychological issue that can be resolved, thus resolving the gender variance. Unfortunately, the problems and issues that often exist for gender variant children and their families are nuanced and indeterminate, and the resolutions may evolve through a time consuming process without a known end. This can add to the stress and consequent pressure to "solve" the issues (see Bernal & Coolhart, 2012, Dreger, 2009, Menvielle, 2012 and Turek, 2011 for further discussion of family issues).

It can be particularly challenging when two parents or guardians with legal custody are in dissent about how to proceed, especially in contentious divorce situations when communication is minimal or hostile, yet medical consensus needs to be reached. Typically, our program requires consent of both parents before medical treatment can go forward and mental health and/or medical clinicians may need to be proactive in trying to resolve disputes with sensitivity.

## Psychosocial Considerations

Any number of psychological, social and cultural factors can impinge upon youth and their family, and influence decision-making, expectations and emotional reactions. The Report of the APA Task Force on Gender Identity and Gender Variance (2009) summarizes some of these factors, including general behavior problems, peer related problems and other mental health issues. Below we outline some of the common issues we have encountered in our work.

Not infrequently, children and adolescents are involved in meaningful activities, which will be likely impacted by a gender transition. Prominent among these are youth sports teams, which are typically grouped by gender. Adolescents are often loath to lose these areas of gratification, along with the opportunity for social bonding. Other hobbies and interests that are often impacted include dancing, theatre, cheer leading and sleep-away camp, and children and families may be unable to forecast how they will weather these transitions. Therefore, a child may face the dilemma of losing the opportunity to sustain an ability or talent they value in order to live in a gender they embrace.

A youth's environment and culture is essential to consider when evaluating treatment options. Ideally, the family and community should provide every child safety, love and solace, and the support a gender questioning child and/or adolescent needs (as any youth does) to thrive into a healthy maturity. However, such youth often struggle for acceptance within their families and communities. We know from prior research (Dean, et al., 2000; Fitzpatrick, Jones, & Schmidt, 2005; Gibson & Catlin, 2011; Grossman & D'Augelli, 2007; Hass, et. al., 2010; Spack et al., 2012) that many children with GD become deeply anxious and depressed, and resort to suicide attempts. Others are at risk of leaving home and living a life with high costs and risks, including of exploitation, abuse, and as victims of violence, while obtaining hormones illicitly without the oversight of a qualified medical professional.

Even when families and children seek professional service and care, external factors beyond their control can impede access. Many geographic areas still lack basic services for children

with GD, and traveling for access to medical care is not always an option for families living within modest means. Furthermore, schools and religious institutions vary in level of comfort dealing with transgender children, and may not have the understanding or training to navigate the complexities of their transgender student or member's needs. Learning to deal with social issues such as bullying and isolation, and practical issues such as bathroom and locker use, requires open and honest dialogue with experts familiar with gender issues; not all communities are able or willing to avail themselves to this kind of discussion.

One positive outgrowth of the Internet and widespread coverage of transgender issues is mainstream access to information about gender variance and dysphoria. Families can become much less isolated by accessing on-line social networks and organizations such as Parents, Families, and Friends of Lesbians and Gays (PFLAG), even when there is not a chapter in their vicinity. However, the increased availability of differing professional standards and practices can sometimes also confuse families, who may specifically seek out professionals who seem open to providing services desired by the patient or parents, even if they are inconsistent with typical practice standards. This could result in circumventing the input of mental health professionals, or providing irreversible intervention for a young or ambivalent child.

## Mental health

Sadly, we know that transgender youth are at risk for anxiety, depression, self-harm, suicidal ideation, psychiatric hospitalizations, homelessness, exploitation, and abuse (Dean, et al., 2000; Fitzpatrick, Jones, & Schmidt, 2005; Gibson & Catlin, 2011; Grossman & D'Augelli, 2007; Hass, et. al., 2010Grossman & D'Augelli, 2007; Spack et al., 2012). In addition, the spectrum of issues that can present in any child or adolescent can present in gender variant youth, including history of trauma, oppositional defiant disorder/conduct disorder, and learning disabilities. These youth may do poorly in school, and/or have difficulty with socializing, and negotiating the normal developmental challenges of adolescence. Optimally, a pubescent child and adolescent should be stable, safe, and supported in advance of receiving medical interventions such as puberty blockers or cross-sex hormones. Yet, for many, medical intervention is an antidote for some of their mental health problems. This poses a dilemma for the clinician, who may be averse to going forward with medical intervention, but feel compelled to do so in case that is the critical step needed to jump start a child's recovery. Such intervention should only take place once the crisis of active suicidal ideation, behavior and/or self-harm has receded, and following a full psychosocial evaluation if it had not taken place already, as well as with close monitoring to ensure that the child is safe and that the dangers continue to remit. Delays can be particularly difficult and contribute to a child's distress because of the limited physiological time frame. At the very least, psychological services should help to ensure adequate support systems before any medical intervention occurs, and puberty blockers can buy time and allow for a child to make thoughtful decisions about his or her gender.

Finally, there appears to be a higher than expected incidence of co-occurring GD with ASDs based on clinical experience as well as research, although more empirical study needs to be completed (e.g. deVries, Noens, Cohen-Kettenis, van Berckelaer-Onnes, & Doreleijers,

2010; Drescher, 2012; Spack et al, 2012). Very often adolescents on the autism spectrum know they are different from peers, but have only recently identified gender identity as a factor contributing to this divergence. Sometimes they and their families believe that a gender transition will solve all problems, and/or latch on to gender as the sole reason they are unlike their peers. Similarly, parents may believe that the GD is a manifestation of the ASD, and resist treatment. Parents of youth on the autism spectrum may be concerned that their child's intense focus on gender is a fleeting concern, particularly if their child has a history of transitory preoccupations. When children with an ASD are evaluated, it is often more difficult to discern the degree of gender variance given the relatively concrete and binary thought processes and communication patterns that typify this population. A child with an ASD already has challenges in social realms and is faced with an additional unique and complex set of social circumstances. A comprehensive evaluation should help sort through these issues and it may be necessary to move forward cautiously. However, it is our opinion that treatment not be withheld indefinitely as these youth experience the same biological time constraints characteristic of all pubescent individuals, and therefore need to receive optimally timed interventions to the extent possible.

## Service Gaps and Evolution of Practice

Watching clinical services grow is rewarding, especially when they translate into more contented and peaceful lives for youth and their families. Nevertheless, evidence-based practices are aspirational when a new field emerges with no guiding clinical precedent. Controversies among providers in the mental health and medical fields are abundant. Drescher & Byne (2012) and Stein (2012) provide excellent discussions of issues of consensus versus continued controversies. These include differing assumptions regarding whether early intervention with gender variant youth can encourage desistance, and whether that is an appropriate practice. Other areas of debate include the age at which children (or adolescents) should be encouraged or permitted to socially transition; whether cross-sex hormones and surgery should be offered to youth, and if so, at what age; whether parental consent be required for these medical interventions; and whether mental health involvement be required, including psychological evaluation, prior to each stage of medical intervention. These issues are complex and providers in the field continue to be at odds in their efforts to work in the best interest of the youth they serve. Addressing each of these controversies goes beyond the scope of this paper; however, the GeMS team continues to stay abreast of these issues and actively participates in ongoing discussion and research (see Schwartz, D., 2012; Ehrensaft, D., Minter, S.P., 2012; Zucker, K.J., Wood, H., Singh, D., & Bradley, S.J., 2012; and Shwartz, D., 2012 for discussions of some of the issues and differing viewpoints).

An important priority going forward is to develop research to enhance our understanding of what typifies this population of children, and their developmental course and patterns, and to examine the long-term outcomes of treatment. The field needs to better comprehend which children are most likely to have a life-long and persistent identification with a different gender than the one they were assigned versus those who cease to self-identify as transgender over the course of time. Although some information is available (e.g., American Psychiatric Association, 2013; Steensma, McGuire, Kreukels, Beekman, & Cohen-Kettenis, 2013; Zucker, Wood, Singh, & Bradley, 2012) much more research in this area is needed.

Other high priority areas for systematic examination include the effects and side effects of various medical interventions, especially given that they are initiated with youth who may be on a lifetime course of hormone treatment, and psychosocial outcomes for youth who receive medical intervention during adolescence.

Finally, we can only report on children with access to services; youth may not have access because of geography and lack of availability, lack of financial means, and/or because of social structures that do not support them. As noted earlier, these children are at risk to be exploited, to be runaways, street youth and sex workers, and to self-medicate and self-harm. Prevention and outreach, to shelter at-risk youth from damaging and avoidable traumas, and to improve access to mental health services, should be one of the highest priorities for health care providers.

## Clinical Case-Composites

**The following represent composites, not actual cases, to serve as examples of how GeMS has addressed common clinical scenarios**

### Case Scenario # 1: Early Puberty

**Referral Information:** M. is a 10 year old Black natal female who identifies as male. He and his parents came to the clinic stating a desire to initiate puberty blockers to avoid feminizing.

**History:** Although only 10, M's pediatrician had put his pubertal development as Tanner Stage 2 (pubertal), and he was developing breasts. He had been living as a boy at school and elsewhere for two years, and was quite concerned that his pubertal changes might alert others to his natal gender, and was very also very assertive about his desire to avoid the onset of menstruation. He had been in therapy for two years, and was also being treated by a psychiatrist for anxiety symptoms. His therapist had written a letter in support of M living in his affirmed gender.

**Psychological Evaluation:** The formal psychological evaluation indicated that M had a longstanding identification as male, which emerged in his early preschool years, as well as ongoing GD, which predominantly took the form of anxiety. His anxiety diminished, according to him and his family, as well as his therapist and psychiatrist, as he transitioned socially and began to live and be treated as a male at home and at school. Information from school revealed that he was viewed as normal and high functioning in all areas. As an example of a response to gender-related questions, M stated that he was not a transgender boy, but just a regular boy. M did report significant anxieties related to social situations, as well as to bathing and bathroom situations. M resides with two biological parents who were both supportive and in accord with pursuing medical treatment, although they reported that it initially had been difficult for them to accept his social transition.

**Recommendations:** Given his long-standing history of GD, positive adjustment at school, the consistency of data obtained from the his psychiatrist, psychologist, both parents and himself, the GeMS team recommended puberty blockers as well as continued psychological treatment to help diminish his anxiety and problem-solve social situations as they may arise.

Continuing follow-up with the GeMS psychologist indicated that his anxiety diminished as his impending puberty was forestalled, with strong acceptance for his affirmed gender from his family and others.

### 2. Case Scenario # 2: Parent: Adolescent Conflict

**Referral Information:** E. is a 17 year old Hispanic natal male who came to the clinic with her parents, who immigrated to the United States soon after E's birth. E was hoping to be able to be treated with puberty blockers and female hormones, while her parents were unified in believing that psychotherapy could resolve her GD, and were hoping to have this confirmed by a psychological evaluation.

**History:** E's parents were invested in her remaining male, partially due to the elevation of male status in their traditional culture. Reportedly, E. had been interested in receiving care for her gender dysphoria for several years prior to the current appointment, to avoid the onset of pubertal changes she was already experiencing. However, her parents had been resistant. She had been in therapy with a psychologist for many years, and her therapist was instrumental in helping to persuade her parents to bring her to the clinic.

**Psychological Evaluation:** The evaluation revealed that E had identified as female since the age of 5, including using female pronouns, attempting to wear female underwear, playing with traditionally female toys, and identifying with female characters during pretend play. At present, E wore female clothing and had grown her hair, but appeared androgynous due to a deep voice and some light facial hair. She was generally assumed to be male at school and elsewhere, although her closest friends used her female name and pronouns at her request. The psychological evaluation revealed a strong cross-sex identification as female, and mild depression.

**Recommendation:** Puberty blockers were recommended, with possible cross-sex hormones in about six months. The psychologist spent considerable time with E's parents and with E, reviewing the results of the evaluation, and the basis for the recommendations. E's parents were distressed during discussion to learn that there was some urgency to proceed quickly, believing incorrectly that medical intervention could reverse pubertal changes. The treatment recommendations also included family therapy, to facilitate positive communication within the family and provide support and psycho-education for E's parents. We also recommended a continuation of psychotherapy for E., to help her adjust to personal and social changes, provide support, and to help her cope with family discord. E. continues to be seen by the psychologist in our clinic for consultation, and is adjusting well to the initiation of hormone treatment.

### Case # 3. Ambivalence and Mental Health Complexity

**Referral Information:** L. is a 16 year old White European American natal female who presents as male, and has chosen a male name and male pronouns. He has been in therapy since the age of 8, and was initially evaluated and put on pubertal blockers in our clinic at age 13. His mother called the clinic requesting that L. be considered for cross-sex hormones.

L. was not seen for a full evaluation as he is an ongoing patient in our service, but for a screening related to his mother's request that cross-sex hormonal treatment be initiated.

**History:** L. was adopted at the age of 1, and his early history is not known. He has been diagnosed with depression, anxiety, and Conduct Disorder. He has a history of self-harm related to depression, academic pressure, and of being bullied in school. His social, academic, and emotional functioning tends to be poor, and he is emotionally and behaviorally dysregulated, with periods of rage at school and at home, and some known drug use. He was recently suspended at school for cheating and for provoking physical altercations. His mother believes that cross-sex hormones would alleviate his distress and dysregulation.

**Psychological Screening:** L's therapist, when contacted with the family's consent, indicated that L. appears ambivalent about his affirmed gender, and therefore did not believe that cross-sex hormones should be initiated. Other aspects of our evaluation also suggested ambivalence on L's part. Although he ultimately agreed with his mother that he should start testosterone, he began the evaluation by suggesting it was "too early" to start them. In addition L. reported that he binds his breasts on occasion (1 × per week) to present convincingly as male, but mostly does not, and that he has been involved in an ongoing heterosexual romantic relationship as a male. He stated that this relationship has been very gratifying, and indicated concern about losing his girlfriend when he started testosterone. Although he stated that he wants to be viewed as male, L also stated that he did not look forward to the changes that testosterone would cause.

**Recommendations:** Given that L was initially resistant to the initiation of cross-sex hormones, and that his mother initiated the consultation, along with L's ambivalence about the changes that testosterone would precipitate, cross-sex hormones were not recommended at this juncture. Instead, we recommended that L. continue to sort out his desires in his therapy relationship, while also addressing some of his other concerning behavioral and mental health issues. We also recommended family therapy, as it appeared that parental anxieties and pressures may have been impacting L's choices. We agreed to consult with L and his family again in 3 to 6 months.

### Case #4.: Autistic Spectrum Disorder

**Referral Information:** B. is a 12 year old White European American natal male, Tanner stage 1, who has been increasingly presenting as female for approximately six months to one year. She and her parents presented in our clinic seeking an evaluation and recommendations for treatment.

**History:** B. was diagnosed with high functioning ASD at the age of 7, after experiencing social difficulties for several years. Although intellectually bright, B. has not done well in school. B. spends much of her spare time on the computer, investigating various subjects and reporting the details to her parents. Her parents worry about her poor academic progress and her socialization, and she has been in treatment since her initial diagnosis. B. disclosed

that she was a girl to her therapist and her parents 6 months earlier, after increasing depression and suicidal feelings.

**Psychological Evaluation:** The evaluation revealed that B. strongly identified as female. B. stated that this feeling had begun within the past year at the start of the school year. Her parents indicated that they would support her if she were truly transgender, but expressed concern that B. may be unhappy socially and using a transgender diagnosis as a means to attempt to resolve her social isolation, and as a result of self-hatred. They also expressed concern that B.'s identification as female is a passing phase, similar to other passing phases/ obsessions she experienced throughout her life, rather than an enduring identification, and that B had limited understanding of the impact of changing genders. B.'s therapist was unsure of whether B. should be treated with hormones yet, expressing similar concerns to her parents. School reports indicated that B. was sometimes taunted by peers, apathetic about schoolwork, often inattentive, and increasingly isolated. All data consistently indicated depression and anxiety.

**Recommendations:** Because of the complexities of B.'s situation, including a relatively recent identification as female, and limited social understanding, we recommended continued psychotherapy and monitoring of her GD, with treatment addressing her depression and anxiety, without immediate medical intervention. We also recommended that her therapist consult with her school to problem-solve solutions to isolation and bullying, and interventions to increase gratifying activities for B. outside the home. We recommended a psychiatric consultation for possible psychopharmacological intervention as well, and a return visit in 3 months to monitor B's progress and her gender identification in light of the new interventions.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Ex. 5



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# Autistic Traits in Mothers and Children Associated With Child's Gender Nonconformity

**Daniel E Shumer, MD**[1], **Andrea L Roberts, PhD**[2], **Sari L Reisner, ScD**[3,4], **Kristen Lyall, ScD**[5,6], and **S Bryn Austin, ScD**[2,7,8]

[1]Division of Endocrinology, Boston Children's Hospital, Boston, MA, United States

[2]Department of Social and Behavioral Sciences, Harvard School of Public Health, Boston, MA, United States

[3]Department of Epidemiology, Harvard School of Public Health, Boston, MA, United States

[4]The Fenway Institute, Boston, MA, United States

[5]Department of Nutrition, Harvard School of Public Health, Boston, MA, United States

[6]Department of Public Health Sciences, University of California - Davis, Davis, CA, United States

[7]Division of Adolescent/Young Adult Medicine, Boston Children's Hospital, Boston, MA, United States

[8]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

## Abstract

We examined relationships between autistic traits in children, mothers, and fathers and gender nonconformity (GNC) in children using data from the Nurses' Health Study II (NHSII) and the Growing Up Today Study 1 (GUTS1). Autistic traits of mothers, fathers and children were measured using the Social Responsiveness Scale (SRS). GNC in children was measured using questions from the Recalled Childhood Gender Identity/Gender Role Questionnaire. In multivariable analyses increase in child's SRS score was associated with increased odds (OR 1.35; p=0.03) of being in a higher GNC category. Increase in maternal SRS score was also associated with increased odds (OR 1.46; p=0.005) of the child being in a higher GNC category. Paternal SRS scores were not related to child's GNC category.

## Keywords

autism spectrum disorder; gender; gender identity; gender nonconformity; transgender

*Gender nonconformity* (GNC) refers to having a gender expression that is not conforming to one's sex, such as a girl preferring to play with toys generally considered masculine (K. J.

**CORRESPONDING AUTHOR INFORMATION:** Daniel E. Shumer, MD, Boston Children's Hospital, Division of Endocrinology300 Longwood Ave., Boston, MA 02115, Phone: 617-355-7476; Fax: 617-730-0194, Daniel.shumer@childrens.harvard.edu.

Shumer
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Leisa Pastor, CSR, CRR

Zucker & Wood, 2011). This concept is related to but distinct from *gender identity*, which refers to the internal sense of one's gender as man/boy or woman/girl, and *gender dysphoria*, which denotes incongruence in one's sex and gender identity, causing significant distress (American Psychiatric Association, 2013; Shechner, 2010). Both GNC and gender dysphoria describe individuals with characteristics and internal feelings diverging from typical gender norms.

There have been case reports suggesting co-occurrence of gender dysphoria and ASD (Gallucci, Hackerman, & Schmidt, 2005; Kraemer, Delsignore, Gudelfinger, Schnyder, & Hepp, 2005; Landén & Rasmussen, 1997; Mukaddes, 2002; Perera, Gadambanathan, & Weerasiri, 2003; Robinow, 2009; Tateno, Tateno, & Saito, 2008). A Dutch study reported a 7.8% prevalence of ASD in a gender dysphoria clinic (de Vries, Noens, Cohen-Kettenis, van Berckelaer-Onnes, & Doreleijers, 2010), much higher than expected based on the prevalence of ASD in the general population, estimated to be approximately 1% (Baird et al., 2006). Similarly, children seen at a US hospital-based pediatric neuropsychology program with ASD were found to be 7.6 times more likely to express gender variance compared to non-referred children as reported by their parents on the Child Behavior Checklist. Gender variance was assessed on the Child Behavior Checklist via a single item question, "wishes to be of opposite sex" with options "not true," "somewhat or sometimes true," and "very true or often true" (Strang et al., 2014).

Neither gender development nor the etiology of ASD are well understood. However, prenatal hormonal exposures, specifically androgen exposure, may influence both gender development (Berenbaum & Beltz, 2011; Hines, 2011) and development of ASD (Baron-Cohen, 2002; Baron-Cohen et al., 2011; Knickmeyer et al., 2006; Voracek, 2010). Genetic causes of autism have been described, with about 25% of children with autism carrying an autism-related genetic variation (Miles, 2011). Genetics have also been implicated as a contributing factor in gender dysphoria in a twin study demonstrating increased concordance of gender dysphoria among monozygotic twins compared to dizygotic twins. However, the study did not investigate specific genes or hormonal exposures in its evaluation (Heylens et al., 2012). ASD traits and diversity of gender identity could also be related in a more complex polygenetic or epigenetic fashion. The co-occurrence may be better framed as an example of *neurodiversity*, a term used by some in the ASD literature to frame autism symptoms and other neurologic symptoms as personality differences existing in a distribution as opposed to pathology (Jaarsma & Welin, 2012; Kapp, Gillespie-Lynch, Sherman, & Hutman, 2013). In addition, postnatal environmental factors, such as the social relationship between the parent and infant (Fausto-Sterling, 2012; pp. 408-9) and cognitive learning about parental expectations and societal norms (Martin, Ruble, & Szkrybalo, 2002) may influence gender development. As suggested by de Vries (2010), the social rigidity present in children with ASD may lead to inflexibility with regard to gender and contribute to co-occurrence of ASD and GNC.

To date, there have been no national cohort studies examining the co-occurrence of autistic traits in children and GNC, nor have there been studies examining autistic traits in parents and the gender development of their offspring. The current study aims to fill these empirical gaps by analyzing data from two related US national cohorts, the Nurses' Health Study II

(NHSII) and the Growing Up Today Study 1 (GUTS1). We hypothesized that young adults with more autistic traits would have higher degrees of recalled GNC in childhood. We also postulated that mothers and fathers with higher autistic traits would have offspring with higher GNC.

## Methods

NHSII is a prospective cohort initially consisting of 116,430 female nurses, the majority of whom are white, originally from 14 populous US states when the study began in 1989. Since baseline, the nurses have been followed biennially with mailed questionnaires. Details of the NHSII cohort have been reported in Solomon et al. (1997). GUTS1 is a prospective cohort study of children who are the offspring of female nurses participating in NHSII. Participants of NHSII who had children ages 9–14 years in 1996 were contacted and asked for permission to enroll their offspring. The initial cohort in 1996 consisted of 16,882 participants. Follow-up questionnaires were sent annually or biennially (Field et al., 1999).

In 2005, NHSII participants were asked if they had a child diagnosed with autism, Asperger's syndrome, or another autism spectrum disorder. In 2007, follow-up mailings were sent to mothers of the 756 ASD cases and 3,000 controls. Following exclusions, 2,144 participants were included in this nested case-control study; details have been described (Lyall, Pauls, Spiegelman, Santangelo, & Ascherio, 2012).

As part of the nested case-control study, participants completed the Social Responsiveness Scale (SRS) (Constantino & Gruber, 2005, 2012). The SRS is a 65-item questionnaire that assesses social functioning, reciprocal social interaction, and restrictive or stereotypical behaviors associated with ASD. It has been validated against the gold standard Autism Diagnostic Interview-Revised (ADI-R) with excellent agreement and has been shown to be stable over time and unrelated to age and IQ (Constantino et al., 2003). The mothers of index children (cases and controls) were sent SRS questionnaires regarding the index child.

The SRS was also used to assess the social functioning of the index children's parents; forms rating the fathers' social functioning were completed by the mothers, and forms rating the mothers' social functioning were completed by the children's fathers or a close relative. In total, SRS for 2,128 index children, 1,247 mothers and 1,629 fathers were collected (Lyall et al., 2014). SRS questionnaires were scored according to the scale's instructions. The scale is designed to have a mean score of 50 and standard deviation of 10 for the general population, with higher scores representing more autistic traits (Constantino & Gruber, 2005, 2012).

In the 2005 and 2007 GUTS1 questionnaires, four questions were included from the Recalled Childhood Gender Identity/Gender Role Questionnaire asking about recalled behaviors during childhood, up to age 11 years (K. Zucker et al., 2006). GUTS1 participants ranged in age from 19 to 27 years in 2007 (mean age = 22.7 years). The GNC-related questions asked about recalled GNC from childhood: media characters imitated or admired, roles taken in pretend play, favorite games and toys, and feelings of femininity or masculinity. Response options ranged from "always women or girls"/"very feminine" to

"always boys or men"/"very masculine" on a 5-point Likert-type scale. A GNC score was calculated by averaging scores from the four questions (Cronbach's $\alpha = 0.78$). In order to identify participants with moderate nonconformity and more extreme nonconformity, GNC scores were grouped into three categories: scores below the median, scores above the median but below the top decile, and scores above the top decile. This method of score grouping has been used in other GUTS1 publications because the relation between GNC and health outcomes appears to be non-linear, with strongest associations found in the top 10% of nonconforming children (A. L. Roberts, Rosario, Corliss, Koenen, & Austin, 2012; A. L. Roberts, Rosario, Slopen, Calzo, & Austin, 2013; A. Roberts, Rosario, Corliss, Koenen, & Austin, 2012).

To test our hypotheses, we took advantage of the unique overlap in NSHII and GUTS1 data. Respondents eligible for inclusion in the current analysis were as follows: Of the 2,144 children with SRS data from the nested NHSII study, 94 of these individuals are also GUTS1 participants who had data collected regarding their recalled childhood gender GNC. Of the 1,247 mothers with SRS data, 198 of them had children who are GUTS1 participants with data collected regarding their recalled childhood GNC. Of the 1,629 fathers with SRS data, 269 of them had children in GUTS1 with recalled childhood GNC data.

We analyzed child SRS score by their GNC category using cumulative logit models recommended for the analysis of ordinal response data (Lee, 1992). To evaluate our first hypothesis, that people with higher autistic traits have higher GNC, we examined data from the 94 children with both SRS scores from the nested NHSII study and GNC scores from GUTS1. Child SRS scores were compared to GNC score category (below median, above median but below top decile, and top decile) by calculating a median SRS score for each of these three GNC score categories. We used a cumulative logit model to determine how a 1 interquartile range (IQR) increase in SRS score statistically predicted the odds of being in a higher GNC score category. This model was adjusted for the sex and age of the child. In addition to an analysis of all 94 children, separate analyses were performed for males (n=47) and females (n=47), adjusted for age of the child.

In order to evaluate our second hypothesis, that parents with higher autistic traits have children with more GNC, we examined data from the 198 mothers and 269 fathers with SRS scores who also had a child with a GNC score. We analyzed mothers' and fathers' SRS scores and their children's GNC score category by calculating median SRS scores of mothers and fathers by GNC score category of their child. We used cumulative logit models to determine how a 1 IQR increase in the mother's SRS score or the father's SRS score predicted odds of their child being in a higher GNC score category. These models were adjusted for the sex and age of the child. In addition, analyses were performed stratified by child sex.

We also examined the relationship between SRS scores and GNC categories with the Kruskal-Wallis test. The Kruskal-Wallis test assigns ranks to SRS scores and compares ranks across GNC score categories. It does not assume a parametric distribution but sacrifices statistical power (Chan & Walmsley, 1997). Using a nonparametric Kruskal-Wallis test, child SRS scores were compared to the child's GNC across the three GNC score

categories. In addition, maternal SRS scores were compared to their child's GNC across the three GNC score categories using the Kruskal-Wallis test, and similarly, paternal SRS scores were compared to their child's GNC across the three GNC score categories using the Kruskal-Wallis test.

## Results

Characteristics of the index children are shown in Table 1. Data are stratified by whether the child participated in the nested SRS study within NHSII as an index case, identified by their mother as having ASD, or as a control participant.

In analyses of child SRS scores in relation to GNC, as the SRS score increases by 1 IQR, the odds ratio (OR) for being in a higher GNC score category (from below median, to above median but below top decile, to top decile) is 1.35 (95% CI=1.04, 1.76), indicating that higher SRS scores are associated with a higher GNC score category (p=0.03) (Table 2). For males, as the SRS score increases by 1 IQR, the OR for being in a higher GNC score category is 1.37 (95% CI=0.94, 1.99; p=0.10) and for females the OR is 1.30 (95% CI=0.87, 1.94; p=0.21) (Table 2).

In analyses of maternal SRS scores by child GNC, when a mother's SRS score is higher by 1 IQR, the OR for their child being in a higher GNC score category is 1.46 (95% CI=1.12, 1.90), indicating that mothers with higher SRS have children with higher GNC score category (p=0.005) (Table 2).

When a father's SRS score is higher by 1 IQR, the OR of their child being in a higher GNC score category is 1.06 (95% CI=0.82, 1.38), indicating no significant relationship between a father's SRS score and his child's GNC score category (p=0.66) (Table 2).

Kruskal-Wallis analysis demonstrates a significant association between the maternal SRS score and their child's GNC category (p=0.03). Kruskal-Wallis analyses does not demonstrate significant associations between the child's SRS score and the child's GNC category (p=0.18) nor between the paternal SRS score and their child's GNC category (p=0.40).

## Discussion

This is the first evaluation of a link between autistic traits in either children or their parents and childhood GNC in a national cohort. Our results suggest that higher autistic traits in children or their mothers are associated with higher degrees of GNC in the child. These results strengthen evidence of a relationship between ASD and gender development. Causality cannot be established using this research design, however these findings could be consistent with common hormonal (Berenbaum & Beltz, 2011; Hines, 2011; Knickmeyer et al., 2006; Voracek, 2010) or genetic causes, and may also support the assertion that the social relationship between parent and child is important for gender development (Fausto-Sterling, 2012). Specifically, findings may support maternal social responsiveness as a factor in child gender expression. Alternatively, the co-occurrence may reflect another

overarching characteristic, termed *neurodiversity* in some ASD literature, instead of an overlap of two distinct characteristics (Jaarsma & Welin, 2012; Kapp et al., 2013).

It is interesting that we did not find an association between paternal SRS score and child GNC. This may give more credence to the prenatal hormonal environment as a cause of co-occurring ASD and GNC, or suggest a higher level of influence of the mother-infant/child dyad compared to the father-infant/child dyad in gender development. This latter hypothesis is consistent with related data regarding other dimensions of child development. For example, a study of 112 two-parent families found mothers to be more involved than fathers in socialization of their children (Schoppe-Sullivan, Kotila, Jia, Lang, & Bower, 2013). However, a study of familiality of autism traits suggested higher SRS scores among fathers of children with ASD, but not mothers (De la Marche et al., 2012). Future research is warranted to understand the relation of maternal versus paternal autistic traits to the development of children's gender nonconforming expression.

It is important to note that both the cumulative logit analysis and the Kruskal-Wallis test assessing association between maternal SRS score and their child's GNC score category were statistically significant. However, when analyzing for association between child SRS score and the child's GNC score category, only the cumulative logit analysis and not the Kruskal-Wallis test was significant. This may be related to the more limited power of the non-parametric Kruskal-Wallis test and the smaller number of analyzed pairs of child SRS scores and child GNC scores (n=94) compared to the larger number of pairs of maternal SRS scores and their child's GNC scores (n=198). Alternatively, this may represent a stronger relationship between maternal autism traits and child GNC compared to a child's autism traits and their own GNC. Future research is warranted to examine these associations.

This study has several limitations. We found a significant association between child SRS score and GNC score category. However, our sample size was too small to capture significant associations when stratified by sex, although point estimates are similar in males, females, and the combined sample. Also, while the parent-infant dyad model of gender development was the basis for our initial hypotheses, we cannot make claims of causality using this study design, as we are unable to adjust for possible confounding factors including genetics and the hormonal milieu of pregnancy. Additionally, autistic traits and GNC are measured indirectly. Autistic traits in the children were measured by SRS reports as assessed by the mothers, whereas formal autism evaluations in a clinical setting would have provided a more rigorous assessment of autistic traits. The measurement of GNC was performed using a subjective questionnaire assessing recalled GNC in childhood and not an objective in-person gender conformity assessment. Variability in the acceptance of GNC across participants was not collected and therefore could not be controlled for in modeling. Finally, the NHSII and GUTS1 are not racially or ethnically diverse, and this limits the generalizability of our findings. Research with diverse samples assessing autistic traits of parents and children and gender nonconforming expression represents an important future endeavor.

The overlap of the NHSII and GUTS1 cohorts provided a unique opportunity to explore autistic traits in children and their parents and their associations to GNC in children. This

study should serve as a basis for further investigation into the importance of the parent-infant/child dyad on gender development and expression. Providers should be sensitive to the diversity of gender expression in children with autistic traits and in children of mothers with autistic traits. This sensitivity could help identify children who would benefit from services and support for both ASD and gender dysphoria.

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## ABBREVIATIONS

| | |
|---|---|
| **ASD** | autism spectrum disorder |
| **GNC** | gender nonconformity |
| **NHSII** | Nurses' Health Study II |
| **GUTS1** | Growing Up Today Study 1 |
| **SRS** | Social Responsiveness Scale |
| **IQR** | interquartile range |

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**Table 1**

Sex, Birth Year, Gender Non-conformity, and Social Responsiveness Scale Scores of Index Children by Case Status*

| | Cases (n=19) | Controls (n=75) |
|---|---|---|
| Sex | | |
| Males, n | 12 | 35 |
| Females, n | 7 | 40 |
| Year of Birth, Median | 1985 | 1985 |
| Childhood Gender Nonconformity | | |
| Below Median, n (%) | 6 (31.58) | 44 (58.56) |
| Above Median, Below Top Decile, n (%) | 9 (47.37) | 22 (29.33) |
| Top Decile, n (%) | 4 (21.05) | 9 (12.00) |
| Social Responsiveness Score‡, Mean (SD) | 103.16 (33.25) | 13.05 (12.38) |

* Cases are children identified by their mothers as having ASD as part of the nested study of autistic traits performed within NHSII. Controls are children not identified by their mothers as having ASD who participated in the same nested study.

‡ Social Responsiveness Scores increase as autistic traits increase

**Table 2**

Child, Mother, and Father Social Responsiveness Scale (SRS) Scores by Child's Gender Nonconformity

| | N | Gender Nonconformity of the Child | | | Odds of Higher Level of Gender Nonconformity Associated with 1 Interquartile range (IQR) Greater SRS Score‡ | *p value* |
|---|---|---|---|---|---|---|
| | | Below Median | Above Median, Below Top Decile | Top Decile | | |
| | | Median SRS Score (Interquartile range) | | | Odds Ratio (95% confidence interval) | |
| Child's SRS Score | 94 | 10 (20) | 16 (77) | 15 (97) | 1.35 (1.04, 1.76) | 0.03 |
| Males | 47 | 9 (17) | 23 (72) | 15 (97) | 1.37 (0.94, 1.99) | 0.10 |
| Females | 47 | 13 (24) | 11.5 (19) | 12.5 (119) | 1.30 (0.87, 1.94) | 0.21 |
| Mother's SRS Score | 198 | 13 (17) | 19 (20) | 18 (23) | 1.46 (1.12, 1.90) | 0.005 |
| Father's SRS Score | 269 | 17 (32) | 15 (23) | 19.5 (35) | 1.06 (0.82, 1.38) | 0.66 |

‡Models for Child's SRS Score, Mother's SRS Score, and Father's SRS Score adjusted for year of birth and sex of index child. Models stratified by sex adjusted for year of birth of index child

Ex. 6



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# Transgender and Gender Nonconforming Adolescent Care: Psychosocial and Medical Considerations

**Carly Guss, MD**[1], **Daniel Shumer, MD**[2], and **Sabra L. Katz-Wise, PhD**[1,3]

[1]Boston Children's Hospital, Division of Adolescent/Young Adult Medicine, Boston, MA

[2]Boston Children's Hospital, Division of Endocrinology, Boston, MA

[3]Harvard Medical School, Department of Pediatrics, Boston, MA

## Abstract

**Purpose of review**—Transgender individuals display incongruence between their assigned birth sex and their current gender identity, and may identify as male, female or elsewhere on the gender spectrum. Gender nonconformity describes an individual whose gender identity, role, or expression are not typical for individuals in a given assigned sex category. This update highlights recent literature pertaining to the psychosocial and medical care of transgender and gender nonconforming (TGN) adolescents with applications for the general practitioner.

**Recent findings**—The psychological risks and outcomes of TGN adolescents are being more widely recognized. Moreover, there is increasing evidence that social and medical gender transition reduces gender dysphoria, defined as distress that accompanies the incongruence between one's birth sex and identified gender. Unfortunately, lack of education about TGN adolescents in medical training persists.

**Summary**—Recent literature highlights increased health risks in TGN adolescents and improved outcomes following gender dysphoria treatment. It is important for clinicians to become familiar with the range of treatment options and referral resources available to TGN adolescents in order to provide optimal and welcoming care to all adolescents.

## Keywords

adolescent; gender identity; gender nonconforming; transgender

**Corresponding author:** Carly Guss, Boston Children's Hospital, Division of Adolescent/Young Adult Medicine, 300 Longwood Avenue, Boston, MA 02115, carly.guss@childrens.harvard.edu, 617-355-7181.

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**Resources**

http://www.lgbthealtheducation.org/
http://transhealth.ucsf.edu/
http://www.amsa.org/AMSA/Homepage/About/Committees/GenderandSexuality/TransgenderHealthCare.aspx
http://www.wpath.org/

## Introduction

Primary care providers may be the first medical professionals to whom a transgender or gender non-conforming (TGN) adolescent presents. This first impression may set the stage for a given adolescent's views of the medical field in general. Moreover, clinicians who care for adolescents have a responsibility to provide medical care that is non-judgmental and comprehensive (1). Research has increasingly been conducted with regards to the psychosocial needs of TGN adolescents following the establishment of treatment guidelines for transgender individuals by The Endocrine Society (2) and the World Professional Association for Transgender Health (WPATH) (3). Herein, the psychosocial and medical care of TGN adolescents is reviewed in order to provide guidance to general practitioners.

## Gender identity

It is important to become familiar with the terminology used in medical and TGN communities (Table 1 (3,4)). Transgender individuals display incongruence between their assigned birth sex and their current gender identity, defined as their internal identification as male, female or elsewhere on the gender spectrum (3). Gender nonconformity describes an individual whose gender identity, role, or expression are not typical for individuals in a given assigned sex category. Gender dysphoria is defined as distress that may accompany the incongruence between one's experienced or expressed gender and one's assigned sex. Gender identity and expression are not the same as sexual orientation (5). When discussing gender identity with patients, providers should inquire if patients have a preferred pronoun.

Gender identity typically develops in early childhood for both gender conforming and non-conforming youth, but can be a dynamic and evolving characteristic from childhood into adolescence and adulthood. For example, a pre-pubertal child who is gender nonconforming or who has apparent gender dysphoria may or may not identify as transgender later in life. The gender identity affirmed during puberty appears to predict the gender identity that will persist into adulthood (6). Estimates for the likelihood of gender dysphoria persisting from childhood into adulthood range from 2–27% depending on the study (6). Youth with persistent TGN identity into adulthood have greater gender dysphoria and are more likely to have experienced social transition, such as using a different name or changing their style of clothing to that which is stereotypically associated with another gender at some point during childhood (6).

## Primary care considerations for the TGN adolescent

As a primary care provider, it is important to use verbal and body language that demonstrates acceptance and openness to all patients, but especially to those who are TGN. The practitioner should inquire how the adolescent identifies their gender, and may open the conversation by using open-ended questions such as: "Many people struggle with gender. Is this an issue for you?" (7).

When performing a comprehensive medical and social history with a TGN adolescent there are particular issues that should be specifically addressed:

### Home

Gender nonconforming youth may experience conflict with family members who do not understand or accept their gender identification. It is important to assess for parental/family awareness of and support for the adolescent's current gender identification. Parental support is positively associated with condom use among transgender female youth (8) and with higher life satisfaction and fewer depressive symptoms among transgender adolescents (9,10). In situations where the TGN adolescent is not supported by family, the adolescent may be at risk for homelessness (10,11). It is important to ask TGN adolescents if they are concerned about homelessness following disclosure of their gender identity to their family.

### School

School is an important social environment for adolescents. A survey of New Zealand high school students found that students who self-identified as transgender were at increased risk of being bullied and in physical fights at school with more than half of them being afraid that someone would hurt or bother them at school (12). Similarly, a nationwide internet survey in the United States (US) found that TGN youth were at higher risk for bullying or harassment compared to their non-TGN peers (13). Adolescents who are gender nonconforming in their expression and behavior at early ages are at increased risk of bullying and verbal and physical abuse by adulthood (14). Moreover, the greater the gender non-conformity, the greater the victimization experienced at school (15).

An ally at school, such as a counselor or teacher, should be identified. Changing clothes for physical education classes or using the bathroom can be stressful or even dangerous for TGN adolescents (5). Bullying in a bathroom may occur as there is no adult supervision, which may result in TGN youth refraining from using the bathroom at school out of fear (5). A patient may not know if there are anti-harassment policies at their school, and this should be investigated.

### Substance Use

Although all adolescents should be asked about substance use, TGN youth may be at higher risk for us than non-TGN youth. One online survey found that TGN youth of any gender were more likely than non-TGN boys to have used alcohol, tobacco, marijuana, and other illicit substances in the past twelve months (13). Moreover, TGN youth who are bullied are at higher risk of substance use (13).

### Sexual Health

As with all adolescents, it is necessary to ask and counsel on matters related to sexual and reproductive health. As TGN patients may have significant dysphoria related to their genitals, it may be prudent to ask if they have preferred terms for their genitalia (7). Female to male (FTM) patients may have discomfort around pelvic exams or desire continuous oral contraceptives to suppress menses (16).

For those who are engaging in penile-vaginal sex, it is important to discuss contraception. Protection against sexually transmitted infections, including the use of condoms and/or dental dams, should be discussed with patients engaging in any type of sexual behavior. If

the patient is on cross-sex hormones, it is important to remind them that while a side effect may be infertility, such therapies should not be relied upon for contraception (7). For FTM patients who are on testosterone, they should be aware that testosterone is contraindicated in pregnancy and may have adverse effects on a developing fetus. Exogenous testosterone may increase sexual desire and clitoral pain (17). Male to female (MTF) patients on estrogen therapy may experience decreased sexual desire as a side effect of decreased testosterone concentrations (17,18). Decreased libido may also be a side effect of puberty suppressing medications (18).

### Mental Health

All adolescents should be evaluated for mental health disorders including depression, anxiety, and suicidality. The TGN adolescent may benefit from referral to mental health providers for a variety of reasons (3). Family counseling and psychotherapy may be necessary to address gender dysphoria, co-morbid mood disorders, and the effect of these conditions on the rest of the family. Assessment by a mental health professional is suggested prior to initiation of puberty blockers or cross-sex hormones to formally diagnose gender dysphoria and to support the TGN adolescent through the transition process (2,3). Additionally, mental health providers can act as liaisons for patients and families with medical and educational systems.

Recent research has focused on the mental health needs and risks of patients with gender dysphoria. Compared to matched non-TGN peers at a single community health center, TGN adolescents and young adults were at two to three times greater risk of depression, anxiety, and suicidal ideation (19). A survey of New Zealand high school students found that one in five students who identified as transgender had attempted suicide in the prior twelve months (12). These findings highlight the importance of assessing mood and suicidal ideation during visits with these patients. Providers should also ask about the timing of gender identity development, as gender nonconformity prior to age eleven is associated with an elevated risk of depressive symptoms (14).

Not surprisingly, following social and medical gender transition, there is reduced gender dysphoria and improved psychological functioning (20). This emphasizes the need to identify TGN adolescents early in order to refer them to appropriate counseling and specialists if they choose to undergo gender-affirmative treatments.

## Medical Management

Both The Endocrine Society and WPATH offer recommendations on the medical management of transgender adolescents (2,3). Medication regimens with dosing and administration route are summarized elsewhere (21). Although the medications used are commonly prescribed for other purposes, they are used off-label for treatment of gender dysphoria. Medical professionals have an ethical responsibility to help adolescents appropriately weigh the pros and cons of cross-sex hormonal therapies. (22). Clinicians may not feel comfortable managing such therapies themselves, but should be aware of potential side effects of hormonal interventions and any specific monitoring requirements. Providers

must also be aware of specialists to whom TGN adolescents can be referred for this important medical management.

### Early Puberty

If a patient presents with gender dysphoria in early puberty (sexual maturity rating 2), pubertal suppression with a GnRH agonist, such as leuprolide or histrelin, can be considered. This allows for further exploration of gender identity prior to the initiation of cross-sex hormones, which may have irreversible effects. Recommendations for monitoring TGN adolescents on pubertal suppressive medications can be found in Rosenthal's "Approach to the patient: transgender youth: endocrine considerations" (21).

### Late puberty

If the adolescent presents with gender dysphoria later in puberty (sexual maturity rating 4/5), GnRH agonists can be used to suppress the hypothalamic-pituitary-gonadal axis to potentially enable the use of lower doses of cross-sex hormones (21). According to the Endocrine Society guidelines, cross-sex hormones can be initiated around age 16 years (2). However, some institutions begin cross-sex hormones at age 14 years with a slow increase of hormones over 2–3 years (21). In addition to GnRH analogues, spironolactone can be used in MTF patients to reduce the effect of testosterone on its receptor.

Cross-sex hormones may be introduced in order to achieve feminizing or virilizing secondary sex characteristics so that a TGN patient's physical appearance will be more aligned with their gender identity. For MTF patients, 17-β-estradiol has numerous delivery routes. Side effects may include impaired insulin sensitivity and hyperprolactinemia (2). There is additional risk of thromboembolic events (18). For FTM patients, testosterone is most commonly given intramuscularly. Side effects include cystic acne, polycythemia, hypertension, adverse changes in lipid profile, possible insulin insensitivity, and risk of impaired fertility (2). Recommendations for monitoring TGN patients who are on cross-sex hormone therapies can be found in Rosenthal's "Approach to the patient: transgender youth: endocrine considerations" (21).

There is some data with regards to outcomes associated with cross-sex hormone treatment. A case report described the first patient treated with pubertal blockers followed by cross-sex hormone treatment in an Amsterdam clinic 22 years after initial treatment (23). This FTM individual received 4.9 years of a GnRH agonist followed by testosterone injections every 2–3 weeks. At age 35, he was found to have bone mineral density above the 50$^{th}$ percentile for females, normal serum values for lipids, hemoglobin A1C, glucose and insulin, and was living happily as a man without regrets regarding his gender transition. A more recent study of the short-term effects of cross-sex hormone therapy in FTM patients found that there were no deaths or severe adverse effects (17). Of 53 FTM patients, two had erythrocytosis and two had transient elevation of liver enzymes. Of the 53 MTF patients, three had transient liver enzyme increase. It is important to emphasize that body image dysphoria remits following cross-sex hormonal therapy and gender affirming surgery, while harassment decreases and patient quality of life and satisfaction with life improves (20).

### Surgical intervention

Gender affirming surgery (previously referred to as sex reassignment surgery) is an irreversible intervention and is considered the final phase of medical gender transition (15). The timing of the procedure remains controversial. Patients must have the cognitive ability to understand the risks and benefits of the procedure. They must also have adequate genital tissue for reconstruction (16). WPATH guidelines state that the patient should be of legal age for medical procedure consent and have lived continuously for 12 months in their identified gender (3). The Endocrine Society guidelines advise that surgical procedures should be done when a person is at least 18 years old (2). For FTM patients, one year of testosterone treatment is recommended prior to surgery (3). FTM patients may desire hysterectomy due to dysphoria associated with menses. If this procedure is performed, patients and primary medical providers must be told if the cervix remains, as pap smears for cervical cancer screening would still be indicated. Cross-sex hormone treatment continues after gender affirming surgery for continued feminization or masculinization and maintenance of bone health. Some transgender individuals decide to forgo gender affirming surgery either for personal or financial reasons, but may decide to continue on life-long cross-sex hormone treatment

## Barriers to Care

Several barriers to care exist for TGN patients. Lack of medical provider knowledge can make patients feel unwelcome and can hinder appropriate referral for mental health or hormonal interventions. A survey of medical schools in 2009–2010 found that the median number of hours of LGBT content was five hours with one-third of schools reporting no LGBT curriculum during the clinical years (24). Additionally, in a medical school class in Philadelphia, 74% of respondents had 2 hours or less of transgender health topics in medical school (25). However, the Philadelphia students who received an additional lecture on transgender health during their clerkship years had improved knowledge, attitudes and skills compared to students who had not received the lecture. Thus, the addition of even one medical school lecture may improve provider competency in caring for TGN patients.

Insurance coverage represents an additional barrier to care for TGN patients. In the US, cross-hormone or pubertal suppressive therapies are prescribed off-label and may be denied by insurance companies. Gender affirming surgery is infrequently covered by insurance and is a cost-prohibitive treatment for some. However, progress is being made in this arena. At the time of this publication, three US states have Medicaid programs that cover medical services for the treatment of gender dysphoria: California, Vermont, and Massachusetts (26).

In addition to cost, many TGN individuals have difficult or even discriminatory experiences with the medical system. A survey of transgender adolescents and adults from Ontario, Canada found that more than half of respondents reported a negative experience during care in an emergency department, and at least 1 in 5 had avoided the emergency room due to fears that their gender identity may negatively affect their care (27). The National Transgender Discrimination Survey, a national retrospective survey study of TGN adults in the US, found that half of respondents postponed seeking medical care when they were sick due to discrimination or financial concerns (28).

## Conclusion

Primary care providers are optimally positioned to welcome TGN adolescent patients to medical care and to create a medical home (28). This is especially important for a patient population that has historically been marginalized. While primary care clinicians may not feel equipped to prescribe hormonal therapies for this population, screening TGN adolescents, creating a safe environment for them in the medical system, and appropriately referring them to mental health and medical care is exceedingly important.

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**Key Points**

- Primary care providers are optimally positioned to welcome TGN adolescent patients to medical care and to create a medical home.
- TGN youth are at higher risk for bullying or harassment, substance use, and depression than their non-TGN peers.
- There are several published guidelines to help guide medical and surgical transition.
- Following social and medical gender transition, there is reduced gender dysphoria and improved psychological functioning.

**Table 1**

Terminology related to transgender and gender non-conforming youth(3,4).

| Term | Definition |
|---|---|
| Cross-sex hormones | Use of feminizing hormones in an individual assigned male at birth, or masculinizing hormones in an individual assigned female at birth |
| Female-to-Male (FTM) | Individuals assigned female at birth who identify on the masculine spectrum and may undergo gender affirming medical treatments to masculinize their body |
| Gender dysphoria | An individual's affective/cognitive discontent with the assigned sex. Refers to the distress that may accompany the incongruence between one's experienced or expressed gender and one's assigned sex. |
| Gender identity | An individual's internal identification as male, female or elsewhere on the gender spectrum |
| Gender non-conforming/gender atypical | An individual whose gender identity, role, or expressionis not typical of individuals with the same assigned sex in a given society and historical era |
| Genderqueer | A term which may be used by individuals whose gender identity and/or role does not conform to a binary understanding of gender as limited to the categories of male or female |
| Gender role | Personality, appearance, and behavior traits that society designates as masculine or feminine |
| Male-to-Female (MTF) | Individuals assigned male at birth who identify on the feminine spectrum and may undergo gender affirming medical treatments to feminize their body |
| Sex | Biological indicators of male and female, such as sex chromosomes, gonads, sex hormones, and internal/external genitalia. |
| Gender affirmation surgery (Sex reassignment surgery) | Surgery to change primary and/or secondary sex characteristics to affirm a person's gender identity. |
| Transgender | Adjective to describe a diverse group of individuals who cross defined categories of gender |
| Transsexual | Term to describe individuals who seek to change or have changed their primary and/or secondary sex characteristics through medical interventions (hormones and/or surgery) with a permanent change in gender role |
| Transition | Period of time when individuals change from the gender role associated with their sex assigned at birth to a different gender role. The nature and duration of transition are variable and individualized. |

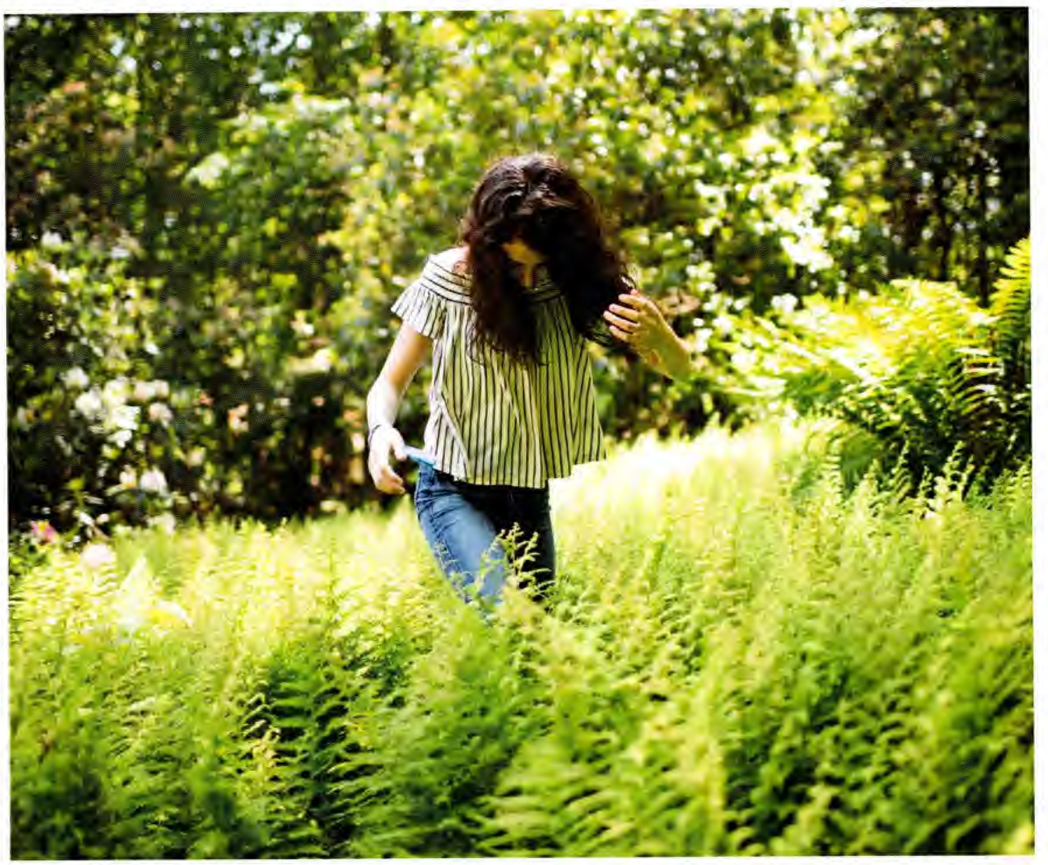
Ex. 7

SEPTEMBER 1, 2017 | 12 MIN READ

# When Sex and Gender Collide

Studies of transgender kids are revealing fascinating insights about gender in the brain

BY KRISTINA R. OLSON



Credit: *Lindsay Morris*

September 2017 Issue | Neuroscience

On arrival at a friend's house for dinner one night in the fall of 2008, I joined the evening's youngest guest, five-year-old Noah, who was playing on the couch. Little did I know he would single-handedly change the course of my career.

As a professor of developmental psychology, hanging out at the kids' table is not unusual for me. I study how children think about themselves and the people around them, and some of my keenest insights have come from conversations like this one. After some small talk, I saw Noah glance around the room, appear to notice that no one was looking and retrieve something

Skinner
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from inside his pocket. The reveal was slow but the result unmistakable: a beloved set of Polly Pocket dolls.

Over the next few years I got to know Noah well and learned more about his past (all names of children here are pseudonyms to protect their privacy). Noah's parents had first noticed that he was different from his brother in the preschool years. He preferred female playmates and toys more commonly associated with girls, but his parents were unfazed. As he got older, Noah grew out his previously short hair and replaced his fairly gender-neutral wardrobe with one that prominently featured Twinkle Toes—shoes that lit up in pink as he stepped. Unlike many similar kids, Noah's family, friends and school fully accepted him. They even encouraged him to meet other kids like himself, boys who flouted gender norms. Along with the other adults in Noah's life, I couldn't help but wonder: What did Noah's behavior mean? Was he gay? Could he just be a kid who paid less attention to gender norms than most? At the time I had no idea that these questions would soon guide my scientific research.

Life for Noah started to change when he hit third and fourth grade. Noah recently explained how at this time, it became increasingly apparent that although people accepted his preferences and befriended him nonetheless, the way he saw himself—as a girl—was at odds with the way others saw him. When people used his name and male pronouns, he realized that they thought of him as a boy. Noah remembers that this awareness made him increasingly unhappy—a feeling that had been rare just a few years earlier. According to his mom, previously cheerful and high-spirited Noah became sad and melancholy. This is when his family, after consulting with local therapists, reached a big decision that had been in the making for years. Noah came out as transgender, and accordingly Noah's friends, family and school community were asked to use a new name, Sarah, and to refer to Sarah as a girl.

Fourteen-year-old Sarah, photographed at home, knew from a young age that she was a girl rather than the boy she seemed to be at birth. Credit: Lindsay Morris

At this point I had been studying developmental psychology for a decade, mostly looking into how young children think about the social categories—race, gender, social class—around them. In my free time, I looked for research about kids such as Sarah. Not a single quantitative study had investigated young children who had "switched" gender. ("Sex" refers to the biological

categories male and female, whereas "gender" references one's identification with the social and cultural attributes and categories traditionally attached to each sex.) At that time nearly all adults who were transgender had transitioned much later in life, and almost no one had supported their early gender nonconformity (their desire to express preferences or behaviors that defy societal expectations for their sex). I wondered what we could learn about gender from such young pioneers as Sarah. What was the impact of transitioning on children's mental health and identity? What would this decision mean for their future?

## HOW WE LEARN GENDER

When most people hear about trans children, they are surprised. How could a three-year-old have such a clear sense of gender identity? People frequently compare early-identifying trans children with those who go through phases of believing they are cats or dinosaurs or who have imaginary friends. They use this comparison as evidence that no young child *knows* his or her identity or what is real or not real. Yet decades of work on gender development suggests these are precisely the ages at which nearly all kids are coming to understand their own and others' gender identities.


Source: "Parent Fights to Omit Gender on B.C. Child's Birth Certificate," by Maryse Zeidler, in CBC News. Published Online June 30, 2017. www.cbc.ca/news/canada/british-columbia/parent-fights-to-omit-gender-on-b-c-child-s-birth-certificate-1.4186221

In Western cultures (where most of this research has been done), within the first year of life infants begin to distinguish people by sex, seeing individuals as either male or female. By about 18 months toddlers begin to understand gendered words such as "girl" or "man" and associate those words with sex-matched faces. By 24 months children know of sex stereotypes (such as associating women with lipstick), and before their third birthday nearly all kids label themselves and others with gender labels that match their sex.

During the preschool years, large numbers of young people go through what gender researchers May Ling Halim of California State University, Long Beach, and Diane Ruble of New York University call the "pink frilly dress stage": most girls become obsessed with frilly princess dresses or similarly "gendered" clothing, whereas many boys prefer superhero gear or formal wear and actively avoid pink. Around this time children also often exhibit strong preferences for the company of same-sex friends, engage in activities

stereotypically associated with their sex and show a developing understanding that their sex is an enduring quality—believing that girls develop into women and boys into men.

Through the elementary school years, most children continue to associate themselves strongly with their sex group when asked both directly and indirectly. One experiment involves asking young participants to sort photographs of children on a computer screen into "boys" and "girls" while categorizing a set of words as either "me" words (like "me" and "myself") or "not me" words (like "they" and "them"). Researchers measure how quickly kids can make these categorizations when "boys" and "me" share one response key and "girls" and "not me" share another, compared with how quickly they can make the opposite pairings ("girls" with "me" and "boys" with "not me"). Past studies have found that an overwhelming majority of girls are faster at pairing "girls" with "me" and boys are faster at pairing "boys" with "me." Although scientists debate which aspects of development are innate or culturally constructed, or a combination of both, and not every child goes through the same gender pathway, most—including those children raised in families who vary in their parenting style, political beliefs, and racial and ethnic group membership—show the pattern we have described. And most parents, teachers and other adults never give it a second thought—except when kids start asserting that their gender is not what others expect it to be.

## EARLY DIFFERENCES

When I began the TransYouth Project in 2013, I wanted to understand whether, when and why young people such as Sarah do and do not behave like their peers in terms of their early gender development. The TransYouth Project is an ongoing study of hundreds of transgender and gender-nonconforming children. We focus on kids in the U.S. and Canada who are three to 12 years old when they begin the study, and we plan to follow them for 20 years.

What has been most surprising to me about our findings so far are the myriad ways in which trans kids' early gender development is remarkably similar to that of their peers. That is, children like Sarah look like other girls at every age but nothing like boys on measures of gender identity and preferences. Similarly, transgender boys (children who identify as boys but at birth were considered to be girls) perform like other boys on our tests. For example, one common observation in the preschool years is a strong hypergendered appearance—girls who *love* princess dresses; boys who avoid pink like it's the plague. We find the same thing in our youngest transgender children. The degree of their preferences for stereotypical clothes, as well as their tendency to prefer to befriend those of their self-identified gender and the degree to which they see themselves as members of their gender group, is statistically

indistinguishable from their peers' responses on the same measures throughout the childhood years.

Charlie prefers clothes and toys associated with girls but identifies as a boy He is pictured here at age 10. Credit: Lindsay Morris

Furthermore, when predicting their identities into the future, trans girls see themselves becoming women and trans boys feel that they will be men, just as other girls and boys do. Even when we present children with more indirect or implicit measures of gender identity—the measures that assess reaction times rather than children's more explicit words and actions—we have found that trans girls see themselves as girls and trans boys see themselves as boys, suggesting that these identities are held at lower levels of conscious awareness. All this research combines to show that transgender identities in even very young children are surprisingly solid and consistent across measures, contradicting popular beliefs that such feelings are fleeting or that children are simply pretending to be the opposite gender.

## THE ROOTS OF GENDER

But where does the feeling of gender come from in the first place? The science is still far from conclusive. Because of how early this sense of identity can emerge, researchers have been looking for genetic and neuroanatomical signs in transgender people. One approach scientists often use in studying genetics is to look at twins. A major difference between identical and fraternal twins is that the former share more of their genetic material than the latter. If researchers find more agreement in transgender identity among identical twins than in fraternal twins, they infer that genetics play some role. And in fact, this is exactly what early studies are finding (although identical twins may also share more aspects of their socialization and environment). For example,

in one 2012 review of the literature, Gunter Heylens of Ghent University in Belgium and his colleagues looked at 44 sets of same-sex twins in which at least one twin identified as transgender. They found that in nine of the 23 identical twin pairs, both siblings were transgender, whereas in no case among the 21 same-sex fraternal twin pairs were both twins transgender, suggesting transgender identity has some genetic underpinning. Despite these results, however, which particular genetic variations are involved is an open question.

Similarly, although some neuroscience studies have shown that brain structures of trans people resemble those of individuals with the same gender identity, rather than people with the same sex at birth, these findings have often involved small samples and have not yet been replicated. Further complicating interpretation of neuroscience results is the fact that brains change in response to experience, so even when differences appear, scientists do not know whether structural or functional brain differences *cause* the experience of a particular gender identity or *reflect* the experience of gender identity. Muddying the already murky waters, neuroscientists continue to debate whether even among people who are not transgender, there are reliable sex (or gender) differences in brains [see "Is There a 'Female' Brain?"]. Thus, whereas the topic is an active line of work in many research laboratories around the world, definitive conclusions about genetic and neural correlates of gender identity remain elusive.

Perhaps the most critical questions about transgender children, however, are about their well-being. Transgender adults and teens who did not go through the early social transition of kids such as Sarah and who were often rejected by peers and even their own families tend to have highly elevated rates of anxiety and depression. Estimates suggest that more than 40 percent of these largely unsupported trans teens and adults will attempt suicide. Many families like Sarah's report that these heartbreaking statistics are why they supported their children's early transitions.

My colleagues and I are finding—both in reports from parents and from kids themselves—that trans youth who make the social transition at a young age are doing remarkably well. They have depression rates comparable to their peers and only slightly elevated rates of anxiety. They also show very strong self-esteem. Whether these indicators of mental health stay strong as our cohort of trans children moves into the teen years remains to be seen, and certainly our all-volunteer sample is unlikely to be fully representative of all trans children alive today. Yet paired with work suggesting that interventions in adolescence (that involve not only social transitions but also hormonal therapy) are associated with improved mental health, these findings suggest that the high rates of depression, anxiety and suicide seen in earlier studies are not inevitable. Instead, as the world becomes more educated about transgender

people, as rejection and bullying decrease, and as these youth receive support and intervention at earlier ages, we are optimistic that mental health risks will decrease.

## "PINK BOYS" AND TOMBOYS

The first question I typically get when talking about transgender kids is something like, "Are you saying tomboys are actually transgender?" or "I used to be a boy who loved princess dresses. Are you suggesting I was transgender?" Of course, not all children who defy sex stereotypes as Sarah did are transgender. In fact, I would venture to say that most of them are not.

Sarah's decision to transition genders was made in elementary school. Sarah is shown with her parents here Credit: Lindsay Morris

One such kid is Charlie. On the surface Charlie seemed a lot like Sarah early in life. Both were assumed to be boys at birth, and both showed signs by the preschool years that they were different. As with Sarah, Charlie loved all things feminine. His mom recalls that by age two, Charlie loved pink sparkly clothing and would put a towel over his head pretending it was hair. Much like Sarah's family, Charlie's family introduced him to other boys who loved feminine stuff. And over the years some of these children, like Sarah, socially transitioned. But Charlie did not. I recently asked Charlie about his decision not to transition. He explained that his family (sometimes with the help of a therapist) spent a lot of time talking about social transitions and made it clear that they were onboard if that was what he wanted. Charlie said he considered this possibility in the back of his mind for several years but ultimately decided that although he unabashedly liked stereotypically "girl" things (in fact the very day I interviewed him, Charlie was wearing pink shorts, a purple T-shirt and a pink scarf to school) and even if he occasionally uses a girl's name at camp, at the end of the day Charlie feels that he is a boy. As his mom explained, Charlie said that what he really wanted was for the world to accept him as he is—to let him wear what he wanted to wear and do what he wanted to do. But he did not truly feel he was a girl.

My work with children such as Charlie is ongoing, but preliminary data from others suggest that distinctive developmental trajectories may differentiate Sarah and Charlie. For instance, the degree to which a child gravitates to toys and clothes associated with the opposite gender may distinguish kids who

ultimately identify as transgender from those who do not—on average, children like Sarah show even more gender nonconformity than children like Charlie. Other studies have suggested that the way kids talk about their gender identity—feeling you are a girl versus feeling that you wish the world was okay with your being a feminine boy (what Charlie's mom calls a "pink boy")—predicts the different paths of children like Sarah versus Charlie.

Researchers are also increasingly recognizing and studying people with nonbinary identities. Put simply, these are individuals who do not feel as if they are boys or girls, men or women, nor do they feel fully masculine or feminine. Instead many nonbinary people fall somewhere in the middle of a spectrum from masculine to feminine. To date, our research team has worked with several children who see themselves this way, but this group is not yet large enough from which to draw any strong conclusions.

What is undoubtedly true is that scientists have much to learn about children such as Sarah and Charlie. What does it mean to have a sense of yourself as a boy or a girl or something else? What makes a child more or less likely to identify that way? And how can we help all kids to be comfortable with themselves? Finding answers is especially difficult because gender is defined by culture, which constantly changes. In 1948, for instance, only 32 percent of adults believed women should wear slacks in public. Certainly feminine boys and masculine girls are not new; they are widely recognized in many indigenous cultures.

Today 14-year-old Sarah and 13-year-old Charlie are self-confident, smart and hardworking teens. Sarah plays piano, varsity field hockey and recently took up track. Charlie plays in a band and performs in theater. Both kids are popular and spend more of their time worrying about doing well in school and the complexities of adolescent social networks than about their gender. Both look to the future, excited about the possibilities that await them in college and beyond. Sarah says she wants to raise children with her future husband and aspires to make the world better for trans young people like herself. Charlie has dreams of moving to New York City to perform on Broadway. Both teens hope one day kids like them will be accepted for who they are regardless of the gender labels they use. In that hope, surely all of us can agree.



**MORE TO EXPLORE**

**Mental Health and Self-Worth in Socially Transitioned Transgender Youth.** Lily Durwood et al. in *Journal of the Academy of Child and Adolescent Psychology*, Vol. 56, No. 2, pages 116–123.e2; February 2017.

**The TransYouth Project:** http://depts.washington.edu/transyp

**FROM OUR ARCHIVES**

**Transgender Kids: What Does It Take to Help Them Thrive?** Francine Russo; *Scientific American Mind*, January 2016.

**KRISTINA R. OLSON** is a professor of psychology at Princeton University where she directs the Human Diversity Lab and the Trans Youth Project.

More by Kristina R. Olson


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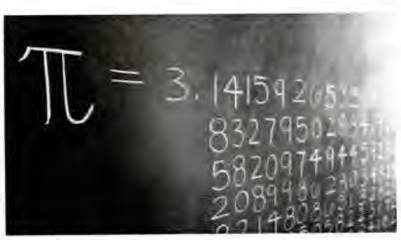


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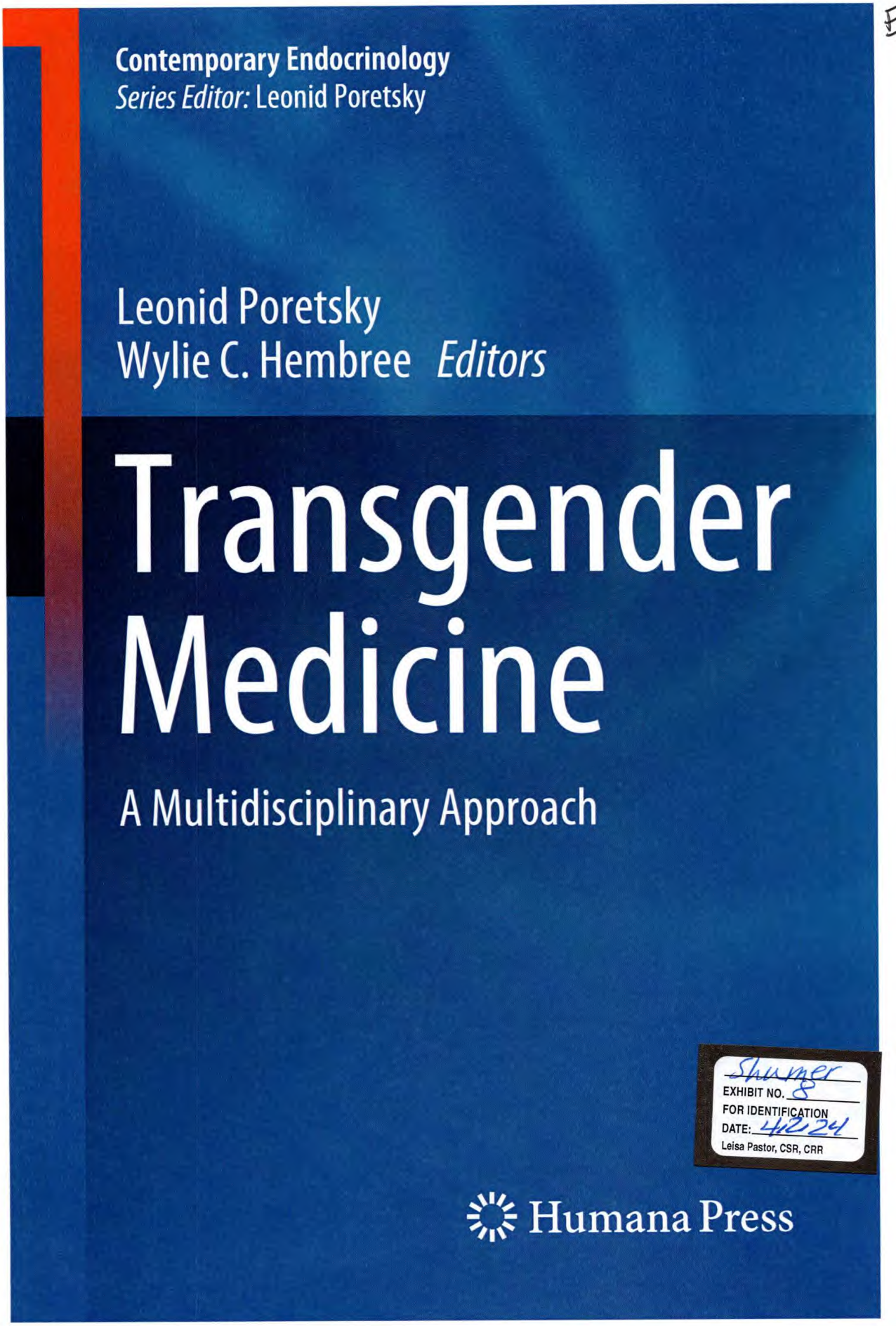


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# Series Editor Foreword

Transgender medicine, like transgender rights, is a new frontier. Endocrinologists, however, have been inhabiting this frontier for decades because of the patients' need for hormonal therapy. For this reason, I thought it would be appropriate to publish *Transgender Medicine: A Multidisciplinary Approach* in the *Contemporary Endocrinology* series.

Needless to say, comprehensive care of a transgender individual requires much more than hormonal therapy—primary care physicians, mental health professionals, pediatricians, plastic surgeons, specialists in reproductive health, and other medical specialists all have important roles to play. Further, because transgender patients' needs often extend beyond medicine, social workers, legal professionals, and spiritual counselors also may have to be involved. Hence, the interdisciplinary nature of this book.

I am thankful to an outstanding group of accomplished experts passionately devoted to this field who enthusiastically contributed their time and effort to this volume. I realize that there is no publication that can match our transgender patients' perseverance, determination, and courage in their pursuit of a fulfilling and happy life. I hope that this text will make a contribution to the development of the knowledge base sorely needed to help transgender individuals achieve their life goals.

New York, NY, USA — Leonid Poretsky, MD

# Preface

Medical and social care of transgender persons require complicated, multidisciplinary efforts with complex strategies and many unknowns. The biology of gender identity, gender dysphoria, and gender incongruence is still incompletely understood. The best medical practice options are often based upon retrospective or empirical studies rather than upon controlled, randomized, double-blind trials common to most other areas of medicine.

Even hormone therapy for transgender individuals is commonly based upon the strategies used for estrogen replacement in menopausal women or testosterone in hypogonadal men—treatment modalities whose goals differ from those of gender affirming hormone therapy in transgender individuals. As a result, treatment may lack consistent standards of care required to ensure an acceptable transition in hormone levels using the best available therapeutic options. To make things even more complex, in addition to hormone therapy, transgender persons have a multitude of coincident needs—medical, surgical, educational, social, and legal—all of which must be addressed.

In planning this volume, we attempted to address as many of these needs as possible. We have engaged a multidisciplinary group of experts and asked them to provide recommendations and advice based upon the best available evidence. Clearly, the important and compelling field of transgender medicine will continue to evolve and the next edition of this text (if and when it comes) may look very different. So, in the meantime, we hope that the reader will find this text a useful guide for safe and efficient care of transgender persons.

We are grateful to all contributors who embraced this project with immense enthusiasm and to our students who keep asking good questions and demanding evidence-based answers. Most importantly, we are indebted to those transgender persons from whom we continue to learn daily and with whom we are proud to share their accomplishments.

New York, NY, USA

Leonid Poretsky, MD
Wylie C. Hembree, MD

# Contents

# Contributors

**Ladan Ahmadi, MD** Department of Internal Medicine, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Brittany J. Allen, MD** Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

**Adrian Araya, MD** Department of Pediatrics, Mott Children's Hospital, Michigan Medicine, Ann Arbor, MI, USA

**Stephanie Baum, MD** Department of OBGYN and Reproductive Endocrinology and Infertility, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Boback M. Berookhim, MD** Department of Urology, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Kelly J. Chan, BA** Boston Medical Center and Boston University School of Medicine, Boston, MA, USA

**Deeangelys Colón, BA** Department of Endocrinology, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Dennis Dacarett-Galeano, BA** Icahn School of Medicine at Mount Sinai, New York, NY, USA

**Aaron Devor, Phd** University of Victoria, Victoria, BC, Canada

**Nancy Neveloff Dubler, AB, LL.B** Division of Medical Ethics, Department of Population Health, NYU School of Medicine, New York, NY, USA;
NYC Health + Hospitals, New York, USA

**Mark Fisher, MD** Department of Plastic and Reconstructive Surgery, Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, USA

**Sarah L. Fishman, MD, Phd** Division of Endocrinology, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Kelly McBride Folkers, MA** Division of Medical Ethics, Department of Population Health, NYU School of Medicine, New York, NY, USA

**Samantha Goldstein, DO** Department of Internal Medicine, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Zil Goldstein, MSN** Department of Medical Education, The Mount Sinai Health System, New York, NY, USA

**Oksana Hamidi, DO** Department of Endocrinology and Metabolism, UT Southwestern Medical Center, Dallas, TX, USA

**Wylie C. Hembree, MD** Division of Endocrinology, Columbia University College of Physicians and Surgeons, New York, NY, USA

**Jo Hirschmann, BCC, ACPE** Department of Spiritual Care and Education, Mount Sinai Beth Israel, New York, NY, USA

**Aron Janssen, MD** Department of Child and Adolescent Psychiatry, Hassenfeld Children's Hospital at NYU Langone, New York, NY, USA

**Noelle Marie Javier, MD** Department of Geriatrics and Palliative Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

**Susan Jelinek, BCC** Department of Pastoral Care, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

**Sean Lare, LCSW-C** Sean Lare Counseling and Consulting, Columbia, MD, USA

**Noah E. Lewis, Esq.** Transcend Legal, Jackson Heights, NY, USA

**Todd B. Nippoldt, MD** Department of Endocrinology, Diabetes and Nutrition, Mayo Clinic, Rochester, MN, USA

**Angelica Nocerino, MD** Department of Internal Medicine, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Maria Paliou, MD** Division of Endocrinology, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Adam D. Perry, MD** Department of Plastic and Reconstructive Surgery, Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, USA

**Richard R. Pleak, MD** Division of Child and Adolescent Psychiatry, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Glen Oaks, NY, USA

**Leonid Poretsky, MD** Friedman Transgender Health and Wellness Program, Division of Endocrinology, Lenox Hill Hospital, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, New York, NY, USA

**Tonia Poteat, Phd, MPH, PA-C** Department of Social Medicine, University of North Carolina, Chapel Hill, NC, USA

**Daniel Puneky, MD** Department of Internal Medicine, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Katherine Rachlin, Phd** Clinical Psychologist in Private Practice, New York, NY, USA

**Asa Radix, MD, MPH** Department of Medicine, Callen-Lorde Community Health Center, New York, NY, USA

**Tamar Reisman, MD** Division of Endocrinology, Diabetes and Bone Disease, Department of Internal Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

**Donna B. Riley, Phd, LCSW-R** School of Social Welfare, Stony Brook University, Stony Brook, NY, USA

**Joshua D. Safer, MD, FACP** Center for Transgender Medicine and Surgery, Mount Sinai Health System and Icahn School of Medicine, New York, NY, USA

**Jean Sawyer, Phd** Department of Communication Sciences and Disorders, Illinois State University (This is a university and not a hospital), Normal, IL, USA

**Warren M. Seigel, MD, MBA, FAAP, FSAHM** Division of Adolescent Medicine, Bioethics Consultation Service, Department of Pediatrics, NYC Health + Hospitals/Coney Island, Brooklyn, NY, USA

**Shervin Shadianloo, MD** Division of Child and Adolescent Psychiatry, Zucker Hillside Hospital, Glen Oaks, NY, USA

**Daniel Evan Shumer, MD, MPH** Department of Pediatrics, Mott Children's Hospital, Michigan Medicine, Ann Arbor, MI, USA

**Tomer Singer, MD** Department of OBGYN and Reproductive Endocrinology and Infertility, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Mark Smith, MD** Department of Plastic and Reconstructive Surgery, Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, USA

**Leon Telis, MD** Department of Urology, Lenox Hill Hospital, Northwell Health, New York, NY, USA

**Emilee Walker-Cornetta, BCC** Department of Pastoral Care and Education, New York-Presbyterian Hospital, Columbia University Irving Medical Center, New York, NY, USA

# Chapter 9
# Endocrine Care of Transgender Children and Adolescents


**Daniel Evan Shumer and Adrian Araya**

## Introduction

An estimated 0.7% of youth ages 13–17 in the United States identify as transgender according to a 2017 report, the largest percentage of any measured age group [1]. Dozens of US comprehensive clinical care programs [2] and likely, hundreds of individual providers across the country and around the world are now providing hormonal care for transgender youth. Current treatment approaches have their roots in the so-called "Dutch Protocol", consisting of confirmation of a diagnosis of gender dysphoria by a mental health professional, pubertal suppression at Tanner stage 2, and treatment with gender-affirming hormones in later adolescence [3]. This treatment strategy has subsequently been codified by the World Professional Association for Transgender Health (WPATH) Standards of Care for the Health of Transsexual, Transgender and Gender-Nonconforming People (version 7, 2012) [4] and the Endocrine Society Clinical Practice Guideline for Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons (2017) [5]. Other seminal resources outlining current best practices include the UCSF Guidelines for Primary and Gender-Affirming Care of Transgender and Gender Nonbinary People, section titled "Health considerations for gender nonconforming children and transgender adolescents" [6] and Rosenthal's *Approach to the patient: transgender youth: endocrine considerations* [7]. In this chapter, we aim to review the current standards and provide practical guidance for the clinical care of transgender youth.

D. E. Shumer (✉) · A. Araya
Department of Pediatrics, Mott Children's Hospital, Michigan Medicine, Ann Arbor, MI, USA
e-mail: dshumer@med.umich.edu


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## Terminology

A brief review of terminology related to transgender youth serves as an introduction to subsequent sections. Note that this lexicon influx, and some terms which were previously in common use have been replaced and may be considered offensive by some. Below are some currently used terms and their definitions [5, 7–9].

*Gender identity*: an internal sense of oneself as a boy or girl, man or woman, somewhere along a gender spectrum, or as having no gender (agender). This is not the same as *sexual orientation*, defined below.
*Assigned sex (sex assigned at birth or natal sex)*: the sex assignment made upon birth, typically male or female, based on the appearance of the external genitalia and/or based on information on the infant's chromosomal or hormonal sex. Persons born with a disorder of sex development, in which the classification as male or female may be less clear, may identify with the term *intersex*.
*Transgender*: an umbrella term describing individuals who identify with a gender that is different from gender assigned at birth; may or may not connote gender dysphoria or desire to seek an intervention.
*Transgender girl/woman*: a transgender person who identifies as a girl or a woman.
*Transgender boy/man*: a transgender person who identifies as a boy or man.
*Cisgender*: a person whose assigned sex is congruent with gender identity; a person who is not transgender.
*Agender*: a person whose gender identity is not aligned with any gender.
*Gender expression*: ways in which a person may express gender identity through appearance, clothing, and behavior.
*Gender attribution*: process by which others make an assessment of an individual's gender based on the person's expression.
*Sexual orientation*: a person's feelings of romantic interest or sexual attraction directed toward members of one or more sex or gender (*gay, lesbian, bisexual, straight*) or no such attractions (*asexual*).
*Gender dysphoria*: conflict between one's gender identity and assigned sex which results in distress. Gender dysphoria is further defined by the American Psychiatric Association's Diagnostic and Statistical Manual (DSM-5), and in previous versions was referred to as *gender identity disorder*. The DSM separates gender dysphoria in children from gender dysphoria in adolescents and adults. Furthermore, in children, two of the following six criteria must be present to meet the DSM clinical criteria [10]:

1. A strong desire to be of the other gender or an insistence that one is the other gender.
2. A strong preference for wearing clothes typical of the opposite gender.
3. A strong preference for cross-gender roles in make-believe play or fantasy play.
4. A strong preference for the toys, games, or activities stereotypically used or engaged in by the other gender.

5. A strong preference for playmates of the other gender.
6. A strong rejection of toys, games, and activities typical of one's assigned gender.
7. A strong dislike of one's sexual anatomy.
8. A strong desire for the physical sex characteristics that match one's experienced gender.

Likewise, in adolescents and adults, two of the following six criteria must be present to meet criteria:

1. A marked incongruence between one's experienced/expressed gender and primary and/or secondary sex characteristics.
2. A strong desire to be rid of one's primary and/or secondary sex characteristics.
3. A strong desire for the primary and/or secondary sex characteristics of the other gender.
4. A strong desire to be of the other gender.
5. A strong desire to be treated as the other gender.
6. A strong conviction that one has the typical feelings and reactions of the other gender.

*Transition*: the hormonal, surgical, or social interventions taken to live as one's affirmed gender; specific terms *hormonal transition*, *surgical transition*, and *social transition* refer to those individual processes within one's transition.
*Hormone blockers*, or "*blockers*": term commonly used by patients and families referring to medications that delay the progression of puberty such as gonadotropin-releasing hormone (GnRH) agonists.
*Gender-affirming hormone treatment*: use of medications that will provide secondary sexual characteristics consistent with gender identity, specifically testosterone, or estrogen treatments.
*Gender-affirming surgeries*: surgical interventions providing physical characteristics congruent with gender identity. Patients and families may refer to chest surgeries "top surgery" and genital surgeries as "bottom surgery." Gender-affirmation surgery is preferred over the term *gender-reassignment surgery*.

## Sex Differentiation and Puberty

Prior to discussion of the treatment of gender dysphoria in youth, a foundation in normal growth and development is required, beginning with sex differentiation. In fetal life, the undifferentiated gonad, under direction from the sex chromosomes, becomes differentiated as a testis or an ovary. During the first trimester, the testis or ovary is stimulated by human chorionic gonadotropin from the placenta. Later in fetal life, the hypothalamic–pituitary–gonadal axis develops with gonads receiving stimulation from, and providing feedback to, the central nervous system. The differential production of testosterone, anti-Mullerian hormone, and estrogen in the

fetus accounts for the development of male and female internal and external reproductive organs, known as the primary sex characteristics. After birth, gonads continue to produce differential levels of these hormones during a so-called "mini-puberty of infancy", but quickly thereafter become quiescent. Therefore, prepubertal male and female children share a similar hormonal milieu [11]. This fact has significant application for prepubertal transgender youth. Transgender youth who desire to make a social transition is not encumbered by incongruent secondary sex characteristics, the changes that occur during puberty. For example, young transgender girls do not have a low voice, an Adam's apple, facial hair, or masculine facial features; young transgender boys do not have breasts or a feminine body habitus; and therefore gender expression alone (their dress, hairstyle, and behavior) can allow for successful attribution from others as their desired gender. Transgender youth, therefore, require no medical intervention.

Secondary sex characteristics are the group of sex-specific changes that occur during puberty. These changes begin as the GnRH pulse generator matures within the hypothalamus. When secreted in a pulsatile fashion, GnRH acts upon the anterior pituitary causing the pulsatile release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which, in turn, provides stimulation to the gonads. This process occurs in parallel with adrenarche, during which adrenal androgen production increases, manifested by the development of pubic hair and apocrine body odor [12, 13].

In males, LH stimulates testicular Leydig cells to produce testosterone and FSH stimulates maturation of germ cells and testicular enlargement. Development of male secondary sex characteristics is driven by the production of testosterone and conversion to dihydrotestosterone at end organs. Pubertal development in boys typically occurs as early as 9 years of age to as late as 15 years of age with mean age of 11–12 years. Physical manifestation of central puberty presents first with testicular enlargement and scrotal reddening and thinning. These changes are the hallmark of Tanner stage 2. Peak height velocity is achieved, on average, by age 14 [14]. Importantly, while an adolescent male may have fully developed genitalia (Tanner stage 5), continued production of testosterone in young adulthood further acts to contour the facial skeleton. This has relevance when considering whether to use GnRH agonists in transgender girls or young women presenting later to care in order to limit further facial and skeletal masculinization.

In females, puberty is driven by estrogen production. LH stimulates ovarian theca cells to produce androgens which are aromatized to estrogen in granulosa cells. FSH is responsible for follicular recruitment [13]. Pubertal development typically occurs as early as 8 years of age to as late as 14 years of age with mean age of 10–11 years. Physical manifestation of central puberty presents first with the development of glandular breast tissue characterized by elevation of breast and papilla, the hallmark of Tanner stage 2 [15]. Peak height velocity is achieved in Tanner stage 2–3 prior to menses, with menses occurring 2–2.5 years after Tanner stage 2 begins [16].

## Historical Perspectives

The current hormonal management of transgender youth evolved from strategies first described by Delemarre-van de Waaal and Cohen-Kettenis at the Amsterdam Gender Clinic in 2006, and was subsequently referred to as the "Dutch Protocol" [3]. This protocol was derived from several important observations: (i) prior to the onset of puberty, no hormonal intervention is necessary; (ii) after the onset of puberty, the development of secondary sex characteristics can exacerbate a young person's gender dysphoria and also cause permanent changes to the young person's body, incongruent with their gender identity; (iii) the onset of puberty occurs at an age when providers were not comfortable starting medications which would cause irreversible effects; (iv) older adolescents and young adults can make appropriately informed decisions about gender-affirming hormone treatments and gender-affirming surgeries. The specific timeline outlined by Delemarre-van de Waal and Cohen-Kettenis dictated that youth with consistent cross-gender identity could begin GnRH agonist at Tanner stage 2 or 3 *and* at an age older than 12 [3]. The supposition was made that prepubertal youth may or may not have persisting gender dysphoria and that persistent dysphoria could not be predicted. By waiting until Tanner stage 2 or 3, the young person would experience some pubertal development, and the exacerbation of dysphoria would be diagnostic of persisting dysphoria into later adolescence and adulthood [17, 18]. Age 12 was chosen as an age when adolescents were able to make medical decisions with their caretakers [19]. Pubertal suppression could also be applied to adolescents presenting after age 12 and in later stages of pubertal development with a goal to halt further progression of puberty. Gender-affirming hormone treatments could be introduced at age 16, an age chosen because at this age Dutch adolescents were considered adults in the context of medical decision making. Pubertal development aligned with gender identity was achieved by gradually increasing doses of testosterone or estrogen every 6 months until adult hormonal levels were achieved. GnRH agonist treatment was continued at least until adult hormonal levels were reached and preferably until gonadectomy. Gender-affirming surgeries were deferred until age 18 in the initial descriptions of the protocol.

The Dutch Protocol provided the framework for subsequent guidelines [4–7] and current clinical practices. The authors noted that hormonal treatment for transgender youth is a controversial topic, but argued that nonintervention is not a neutral option; postponing medical intervention until adulthood may portend negative mental health outcomes for transgender youth [20]. Long-term outcomes data related to the first cohort of patients treated under the Dutch Protocol demonstrated positive mental health outcomes in young adulthood, in contrast to the large mental health disparities faced by untreated transgender persons [21]. That said, the evolving landscape of gender identity has led to the evolution of contemporary care. For example, the Dutch Protocol relied heavily on age cutoffs for medical decision making, largely based on Dutch law at the time. These cutoffs are being reconsidered by many clinicians in favor of other factors including appropriate pubertal

timing, stability of gender identity, patient maturity, ability to understand risks and benefits, and family readiness. Second, gonadectomy was an assumed goal of the initial patients described by the Dutch Protocol. Clinicians today acknowledge that not all transgender youth who are appropriate candidates for hormonal intervention will desire or will be able to afford gonadectomy or other gender-affirming surgeries [22].

## Contemporary Management

The WPATH Standards of Care (SOC) [4] and the Endocrine Society's guidelines [5] currently serve as the basis for clinical care of transgender youth. As mentioned before, guidance published by UCSF [6] and also independently by Rosenthal [7] compliment these guidelines and provide logistical detail. These resources and our clinical experience guide future sections of the chapter.

## The Clinical Care Team

The WPATH SOC and Endocrine Society both strongly recommend that medical providers work with mental health professionals with expertise in the diagnosis of gender dysphoria prior to medical intervention. This recommendation highlights the importance of confirming the diagnosis of gender dysphoria prior to embarking on a medical intervention. In addition, because transgender children and adolescents have increased the risk of suicidal ideation, suicide attempt, depression, and anxiety, evaluation by a mental health professional can be helpful in the diagnosis of comorbid conditions and can help coordinate complimentary mental health treatment as appropriate [23, 24].

The logistics of how mental health professionals work with medical providers is variable and often influenced by local resources. Large hospital-based gender clinics may employ social workers or psychologists to perform independent gender and psychosocial assessments for patients presenting for care [25]. Other providers may form informal networks with community based mental health providers, and ask for letters of support from these providers prior to initiation of hormonal interventions. Additionally, psychiatrists may work within gender teams or as consultants to evaluate and treat patients with unmet psychiatric needs such as depression and anxiety.

Hormone prescribers may have received initial medical training in any number of specialties including, but not limited to, general pediatrics, pediatric endocrinology, adolescent medicine, family medicine, or gynecology. The most important requirement of the hormone provider is that they have an interest in working with this vulnerable patient population—the medical aspects of care can be learned.

Other team members may include: legal consultants (to assist with gender marker changes on legal documents, or to advocate for insurance coverage); nursing (to provide education related to medication administration, for example to teach self-administration of testosterone); plastic surgery; and speech and language pathologists (to evaluate and treat voice dysphoria). Individual providers not working as part of a formal gender team may familiarize themselves with local transgender-friendly resources in these fields. In addition, all staff members working with hormone providers should be trained on topics of gender identity, including the use of preferred names and pronouns. This includes, but is not limited to, scheduling staff, receptionists, medical assistants, phlebotomists, and radiology technicians. Leaders of gender teams may need to think critically about the cultural competency of the health system in which they work and advocate at administrative levels in order to improve care for patients.

## Hormonal Interventions

Hormonal interventions used in the treatment of adolescents with gender dysphoria include medications that suppress natal hormone production or action (pubertal suppression), and medications which promote the development of secondary sex characteristics of the affirmed gender (gender-affirming hormone treatment). Factors involved in deciding which interventions are appropriate for the individual patient include the current pubertal stage of the patient, the patient's stated goals of treatment, the maturity level of the patient and their ability to understand risks and benefits of intervention, and the consent and support of parents or guardians (Fig. 9.1). Unfortunately, insurance coverage and affordability of interventions may also dictate what interventions are available to individual patients.

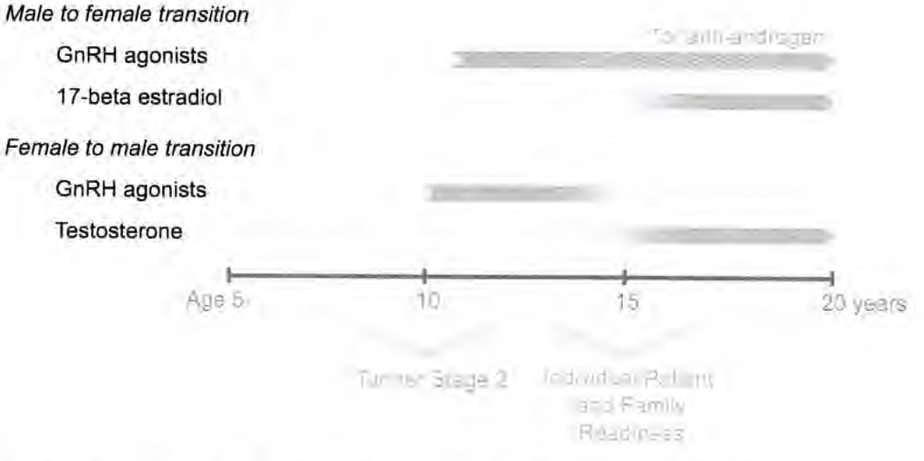


**Fig. 9.1** General timeline for medical interventions in transgender youth/adolescents

## Pubertal Hormone Suppression/Inhibition

### GnRH agonists

Both the WPATH SOC and Endocrine Society suggest children with gender dysphoria are eligible for treatment with GnRH agonist medications starting at Tanner stage 2, regardless of age [4, 5]. The goals of suppression include (i) prevention of development of unwanted secondary sex characteristics, (ii) mitigation of the accompanying dysphoria associated with puberty, and (iii) the ability to delay decisions around gender-affirming hormone treatment.

GnRH agonist medications, initially used in pediatrics for the treatment of central precocious puberty, provide continual stimulation of the GnRH receptor. When stimulated continuously, as opposed to in pulsatile fashion, gonadotrophs in the anterior pituitary are inhibited from releasing LH and FSH. Treatments with GnRH agonists, therefore, inhibit pituitary stimulation of the gonads with a goal of suppressing production of the sex hormones, testosterone, and estrogen [26].

GnRH agonists are formulated as intramuscular injections (such as leuprolide acetate 1-month or 3-month preparations) and subcutaneous implants (histrelin, implanted annually). Factors such as preference for more frequent injections versus less frequent surgical implantation procedures and cost/insurance coverage may influence the choice of formulation [7, 23, 27]. In situations where GnRH agonist treatment is desired but not available or affordable, treatment with medroxyprogesterone acetate, which works both to inhibit the hypothalamic–pituitary–gonadal axis and to inhibit gonadal steroidogenesis, has been described. This treatment has been shown to reduce but not completely inhibit sex hormone production in transgender adolescents [28, 29].

The classic scenario when GnRH agonist treatment is prescribed is the young adolescent patient, male or female, presenting at Tanner stage 2. The efficacy of GnRH agonist in this situation is well documented—the young person will be spared the development of unwanted secondary sex characteristics; if the decision is made to proceed with gender-affirming hormone therapy in later adolescence, the adolescent will theoretically only develop secondary sex characteristics of the affirmed gender. This treatment strategy has the potential to avoid dysphoria associated with development of sex characteristics incongruent with gender identity and may obviate need for masculinizing chest surgery (top surgery) in transgender boys and the need for voice training, chondrolaryngoplasty (tracheal shave), facial feminization, and facial and body hair removal for transgender girls [30]. That said, the majority of patients presenting to care may not present at Tanner stage 2. In our clinical practice, about two-thirds of adolescent patients present to care at a more advanced pubertal stage. In these cases, the decision regarding whether to consider GnRH agonist treatment is more complex.

The following factors should be considered when discussing GnRH agonist use for the transgender adolescent presenting at a pubertal stage more advanced than Tanner stage 2: (i) Is more pubertal development expected? (ii) What are the goals

of treatment? (iii) Is the patient currently a candidate for gender-affirming hormone treatment? (iv) Is the patient male or female? Brief case examples will highlight how answers to these questions can assist in medical decision making.

*A transgender girl presents at Tanner stage 4.* Despite the fact that she started male puberty 2 years prior, she has very little facial or body hair, her facial bone structure appears still quite gender neutral. She is a candidate for estrogen therapy and she and her family are considering starting estrogen in 1 year. In this case, there is more masculinization of the facial structure expected, as is often true of adolescent transgender girls in mid-puberty. A significant goal of treatment, in this case, would be to limit masculine facial structure and facial hair development. Regardless of whether this patient decides to start estrogen now or in the future, GnRH agonist treatment could be considered. Concurrent use of GnRH agonist plus estrogen in transgender young women can also be beneficial. This "block-and-replace" strategy can be advantageous—the estrogen dose required to both suppress testosterone production and promote feminization when given as monotherapy may be significantly higher than when used concurrently with GnRH agonist [6].

*A transgender girl presents at Tanner stage 5 to discuss initiation of gender-affirming hormonal treatment.* On clinical exam, the patient has developed adult facial and body hair and is already engaged in the removal of this hair using electrolysis. She has a masculine appearing facial bone structure and is planning to request facial feminization surgery in the future. She is also contemplating vaginoplasty with gonadectomy in the future. This patient does not have expected further masculinizing pubertal development—she is fully masculinized. Treatment with GnRH agonist will not prevent further pubertal development. She may still benefit from concurrent use of GnRH agonist with estrogen in order to limit the required estrogen dose. However, the cost–benefit analysis in this situation is different than the previously described scenario, especially if there is a financial burden to the patient for initiation of GnRH agonist.

*A transgender boy presents at Tanner stage 3 breast development.* He is not yet a candidate for testosterone but is concerned about the prospect of further breast development. In this situation, GnRH agonist could be useful in limiting further breast development and the associated dysphoria accompanying this development. While breast development may not entirely regress on treatment, future mastectomy (top surgery) may require a less invasive incision if breasts do not develop past their current stage (i.e., a periareolar incision may be used rather than an inframammary incision).

*A transgender boy presents at Tanner stage 5 breast development.* He is not currently interested in starting testosterone; however, he is experiencing significant distress associated with his menses. In this scenario, GnRH agonist would inhibit the menstrual cycle, however, other interventions, such as progesterone-only contraceptive medications may have the same effect, and is further described below. The cost–benefit analysis may favor this approach over GnRH agonist treatment. GnRH agonist treatment could be reconsidered if other strategies are not successful [6].

*A transgender boy presents at Tanner stage 3–4 breast development for discussion of gender-affirming hormone treatment.* In this scenario, testosterone therapy is initiated prior to the completion of female puberty. Unlike estrogen monotherapy, testosterone monotherapy is more effective at suppressing further development of female secondary sex characteristics and the additional benefit of concurrent use of GnRH agonist is likely minimal.

In addition to GnRH agonist medications, several other medications should be included in the discussion of pubertal hormone suppression in transgender adolescents.

As previously mentioned, medroxyprogesterone acetate has been described for suppression of puberty when GnRH agonist treatment is not available. Medroxyprogesterone acetate can be given as a daily oral preparation (up to 40 mg/day) or as an intramuscular preparation (150 mg every 3 months) for males or females [7]. Commercially available progesterone-only contraceptive pills, when taken daily, can promote amenorrhea in transgender boys with menses-based dysphoria (for example, norethindrone 5–15 mg daily).

Spironolactone, initially developed as a potassium-sparing diuretic, additionally works to inhibit the synthesis and action of testosterone. Spironolactone (100–300 mg daily) is commonly prescribed to transgender girls and women who have already developed secondary hair (facial hair, body hair) as a means to slow hair growth and limit further hair development.

Prior to initiation of pubertal suppression, a baseline medical evaluation is recommended including height, weight, and blood pressure measurements and Tanner staging. Baseline laboratory evaluation of LH, FSH, estradiol, and testosterone can be used in confirming central puberty and for comparing to posttreatment assessments [5]. Rosenthal suggests assessment of LH, FSH, estradiol (in females), testosterone (in males) at baseline and every 3 months, assessments of calcium, phosphorus, alkaline phosphatase, and 25-hydroxyvitamin D at baseline and annually, and bone age and bone densitometry at baseline and annually in transgender youth treated with pubertal suppression [7]. Assessment of bone metabolism markers has been suggested due to the concerns regarding delaying bone density accrual, further described later in the chapter. Bone age evaluation can help the prescriber understand the individual patient's timing and tempo of growth and development, specifically with regards to height accrual.

## Gender-Affirming Hormonal Interventions

Gender-affirming hormones, specifically testosterone and estradiol, are used to promote development of secondary sex characteristics of the affirmed gender. Specifically, testosterone is used in transgender boys to promote the development of facial and body hair, voice deepening, masculinization of facial structures and of fat and muscle distribution. Testosterone monotherapy, as mentioned above, also typically causes amenorrhea during use. Estradiol is used in transgender girls to

promote breast development and development of a feminine body habitus. Unlike GnRH agonists, which have been described as *reversible* interventions, many of the changes which occur from use of gender-affirming hormones are more permanent. Therefore, careful assessment and thorough discussion of risks, benefits, and expectations of treatment are critical.

The timing of initiation of gender-affirming hormone therapy is a complex decision based on individual, family, social, and societal factors. As discussed, in the initial "Dutch Protocol" the age 16 was used. Many transgender adolescents, however, are deemed to have clear gender dysphoria and are requesting these interventions with parental support at much younger ages. From a social perspective, it may be challenging for a transgender child living in all contexts as their affirmed gender to wait until age 16 to start puberty—an age significantly older than what is typical for their peers. Furthermore, for a child who started GnRH agonist treatment at Tanner stage 2, perhaps as young as 8 or 9 years old, restricting the use of gender-affirming hormone therapy until age 16 would artificially delay their pubertal development, including growth spurt and bone density accrual, by over a half-decade. Given these concerns, many providers treat transgender youth with testosterone or estrogen at ages younger than 16 years. Rosenthal notes the use of age 14 in his review [7]. The Endocrine Society, in its 2017 revised Clinical Practice Guideline, comments: "we recognize that there may be compelling reasons to initiate sex hormone treatment prior to age 16 years, although there is minimal published experience treating prior to age 13.5–14 years of age [5]." As the evolution on age continues, providers seem to be acknowledging that individual readiness factors, rather than age cutoffs, are important when considering the use of gender-affirming hormones.

Testosterone is most commonly prescribed as testosterone cypionate or enanthate and given as an intramuscular (IM) or subcutaneous (SC) injection. For treatment of youth receiving GnRH agonist treatment concurrently, the goal of treatment is to mimic normal male puberty. This can be achieved by prescribing gradually escalating doses over time, such as starting with 12.5 mg/week (or 25 mg/2 weeks) and gradually increasing to 50–100 mg/week (or 100–200 mg/2 weeks) SC based on clinical progress [7]. For older adolescents, especially when not prescribed GnRH agonist concurrently, prescribers can more rapidly increase dosing or start at the lower end of the final dose range. The adult maintenance dose should provide enough testosterone for masculinization, should suppress menses, should limit excessive androgen effects such as acne vulgaris, and should provide for a measured testosterone level in the normal adult male range. In our clinical practice, most commonly prescribed adult dose of testosterone used to achieve these goals is 50 mg SC weekly. Testosterone has classically been prescribed as an intramuscular injection, however, subcutaneous administration has become a popular alternative delivery method as it is easier for self-administration and has been shown to be effective [31]. When prescribing testosterone for home administration, it should e noted that injectable testosterone is suspended in oil and is too thick to draw up through small caliber needles. In our practice, we prescribe a 1 cc or 3 cc syringe, a 21 gage removable needle for drawing up the testosterone, and a 25 gage

5/8 inch removable needle for injecting into the subcutaneous tissue. A demonstration injection is performed in our office using saline.

Other preparations of testosterone include transdermal gel and patch products. Transdermal gels and patches can be prescribed with a similar graduated dosing strategy to the adult dose of 50–100 mg daily for gel, 4 mg for the patch. Disadvantages of the gel, especially in the pediatric population, is the care needed to ensure that the gel does not come in contact with family members, and the need for daily administration. Testosterone patches provide for less flexibility with dosing increments and may irritate the skin. Both gels and patches are more expensive than injectable products. We suggest reserving use of gels and patches for cases of needle phobia or per patient preference after the full adult dose of injectable testosterone has been achieved.

Feminizing hormonal treatment for transgender girls is achieved with 17-beta-estradiol. Pharmaceutical products containing other conjugated or synthetic estrogens are not preferred due to unfavorable risk profiles [32]. 17-beta-estradiol can be prescribed as oral tablets, transdermal patches, or injectable products, with oral or transdermal administration most common in pediatric practice. Similarly to testosterone dosing, estrogen dosing varies with the clinical situation. For transgender girls concurrently treated with GnRH agonist, estrogen dosing can start low and proceed gradually to mimic normal female puberty. For example, oral 17-beta-estradiol can be initiated at 0.25 mg PO daily and advance to 2 mg oral daily based on clinical progress. Transdermal dosing could start with a portion of a 25 mcg patch (for example, 12.5 mcg by cutting the patch in half), and progressing to 100 mcg patch over time based on clinical progress. When treating an adolescent not concurrently prescribed GnRH, estrogen the dose required to suppress testosterone production and promote feminization is higher. For example, starting doses could be 2 mg oral or 100 mcg transdermal with increases to 6 mg or 300 mg, respectively. Goals of treatment, in this case, are to promote desired feminine development while suppressing testosterone; a concrete goal may be to keep measured testosterone level under 100 ng/dL.

Ongoing monitoring is required for patients prescribed gender-affirming hormone therapy. Assessments should focus on the clinical effects of the intervention, and how these effects align with the patient's goals of treatment. Ongoing review of mental health concerns, other medical concerns, general well-being, and social impacts of transition should also be discussed. For patients treated with 17-beta-estradiol, signs and symptoms of insulin resistance and hyperprolactinemia should be reviewed. Patients treated with testosterone may be at risk for hyperlipidemia, insulin resistance, and polycythemia, which guides recommendations for interval laboratory evaluation. In our experience, cystic acne is the most commonly encountered unwanted side effect of testosterone treatment, which can respond to reductions in testosterone dosing or standard acne interventions. Given these concerns, Rosenthal suggests baseline and quarterly assessments of height, weight, blood pressure, Tanner staging, LH, FSH, testosterone and/or estrogen, complete blood counts, renal function, liver function, fasting lipids, glucose, and hemoglobin A1c for at least the first year of treatment. Potassium should be included if a patient

is treated with spironolactone. If a patient had been previously treated with GnRH agonists, assessments of calcium, phosphorus, alkaline phosphatase, 25-hydroxyvitamin D, bone densitometry, and bone age may also be helpful [7]. When appropriate adult dosing is determined and remains unchanged, monitoring frequency can be reduced, eventually to annual assessments as clinically appropriate.

## Special Considerations for Youth

*Bone density*

Puberty is a time of relatively rapid skeletal maturation and accrual of bone mass. When puberty is suppressed at Tanner stage 2, there is a concern for a relative decrease in bone mineral density compared to untreated peers. There are data to suggest that current protocols do result in a decrease in apparent bone mineral density *z*-score, but with improvement toward normal after initiation of gender-affirming hormone therapy [33]. However, another study demonstrated a decline in bone mineral density *z*-score during GnRH agonist treatment without full catchup by age 22 [34]. Ultimately, clinicians are advised to discuss the risk of lower bone density at the onset of treatment, screen calcium, and vitamin D intake and treat deficiencies in an attempt to mitigate this potential risk.

*Stature*

There are a few little data regarding the final impact of pubertal suppression and gender-affirming hormone therapy on stature. While stature may not be a concern for all patients, it is not uncommon in our experience that transgender boys may desire a taller stature than their expected female height and transgender girls may be concerned about excessive height. Bone age assessments can be helpful in determining growth potential. Height growth can be expected until growth plates fuse. Hormonal interventions including use of GnRH agonists, estrogen, and testosterone have the potential to affect the duration of growth plate patency. The action of growth hormone, adrenal androgens, and sex hormones on the growth plate likely all contribute to height growth; estrogens are also known to promote growth plate closure. Broadly speaking, we recommend that decisions regarding timing of hormonal interventions be based primarily on patient readiness, however, significant concerns around stature could be influenced by the timing of therapy and dosing. For example, a longer course of pubertal delay followed by slower escalation in testosterone dosing may allow for more time for growth in a transgender boy. A more rapid escalation in estrogen dosing may marginally reduce unwanted height growth in a transgender girl. Trials examining these strategies are lacking.

*Fertility*

One of the most challenging aspects of providing patient-centered care to transgender youth and their families is engaging in discussions regarding fertility. Transgender youth, especially those presenting prior to or around the onset of puberty, are seldom concerned about the impact of medical interventions on fertility, and often even less interested in discussing this topic. This ambivalence is likely age appropriate, shared by their cisgender peers, and may not predict their future feelings. For example, a study of transmen indicates the majority desire to have children [35].

Development of mature sperm and oocytes occurs during puberty. Therefore, progressing through natural puberty is a requirement for fertility. When discussing fertility with a patient and family presenting at Tanner stage 2, it should be noted that patients with central precocious puberty treated with GnRH agonists have normal reproductive function after discontinuation of GnRH agonist and progression through natal puberty [36]. However, patients considering GnRH agonist therapy for gender dysphoria may not decide to allow their natal puberty to progress in later adolescence, choosing instead to bridge to gender-affirming hormone treatment. If that decision is made, there will never be maturation of sperm or eggs and no opportunity for gamete preservation.

It should be noted that trans men who maintain a uterus and ovaries have achieved pregnancy by cessation of testosterone and achieve pregnancy by ovarian stimulation [21].

Patients presenting after puberty should be advised that future fertility could be compromised by prolonged use of gender-affirming hormones. While there are examples of preserved fertility after hormonal transition, fertility options can be expanded by use of gamete cryopreservation [37]. In our practice, preservation of sperm is more likely to be desired than oocytes, likely due to cost and logistics.

*Consent*

Because adolescents are unable to independently consent for medical care, decisions on hormonal transition are shared among patients, parents, and medical providers. The best outcomes are achieved when all parties are in agreement with the medical plan [38]. Adolescents and their parents should be counseled on risks and benefits of treatment prior to proceeding [39]. In our experience, disagreements regarding the timing of transition can often be resolved by reviewing the goals of treatment, the potential risks of nontreatment, and encouraging professional family counseling when indicated.

In our experience, older adolescents who meet criteria for hormonal transition have often done independent research on transition, may be connected with other transitioning youth on social media, and are eager to start testosterone or estrogen, while their parents may not be ready to provide consent. In these situations, we recommend meeting the parents where they are: affirming the fact that this is an important family decision, celebrating the love and support they are demonstrating by bringing their child in for assessment, providing education around gender

identity and the rationale for current standards of care, and reviewing risks and benefits of treatment and of nontreatment. While consent for hormonal transition may not occur at an initial visit, we have seen this approach successful in shifting parental attitudes in favor of consenting to hormonal transition over time when it is clinically indicated.

More challenging situations arise when there are disagreements between two parents or guardians of a child or adolescent, especially when parents are separated and perhaps engaged in joint custody or a custody dispute. In these situations we have found it helpful to engage with legal and/or ethical experts within our health system for guidance on how to proceed on an individual basis.

## Summary

Several consensus guidelines outline best practices for the medical management of transgender youth. These guidelines describe the use of pubertal suppression and gender-affirming hormones to reduce gender dysphoria. As more transgender youth are now presenting to medical attention than in previous generations, medical providers caring for youth in any capacity should expect to see transgender youth in their practice and be knowledgeable about the basics of gender-affirming care. Providers interested in prescribing gender-affirming hormonal interventions should familiarize themselves with current standards and guidelines and develop a strategy for the provision of multidisciplinary care including mental health support and knowledge of community resources.

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**SHORT REPORTS**

Ex. 9

# Evaluation of Asperger Syndrome in Youth Presenting to a Gender Dysphoria Clinic

Daniel E. Shumer, MD, MPH,[1,2] Sari L. Reisner, ScD,[3] Laura Edwards-Leeper, PhD,[4] and Amy Tishelman, PhD[1,5,6,7]


## Abstract

***Purpose:*** There is evolving evidence that children and adolescents with gender dysphoria have higher-than-expected rates of autism spectrum disorder (ASD), yet clinical data on ASD among youth with gender dysphoria remain limited, particularly in North America. This report aims to fill this gap.
***Methods:*** We conducted a retrospective review of patient chart data from 39 consecutive youth ages 8 to 20 years (mean age 15.8 years, natal male: $n=22$, natal female: $n=17$) presenting for evaluation at a multidisciplinary gender clinic in a large U.S. pediatric hospital from 2007 to 2011 to evaluate the prevalence of ASD in this patient population.
***Results:*** Overall, 23.1% of patients (9/39) presenting with gender dysphoria had possible, likely, or very likely Asperger syndrome as measured by the Asperger Syndrome Diagnostic Scale (ASDS).
***Conclusion:*** These findings are consistent with growing evidence supporting increased prevalence of ASD in gender dysphoric children. To guide provision of optimal clinical care and therapeutic intervention, routine assessment of ASD is recommended in youth presenting for gender dysphoria.

**Keywords:** Asperger syndrome, autism spectrum disorder, gender dysphoria, transgender, lesbian, gay, bisexual, and transgender (LGBT) youth.

## Introduction

There is evolving evidence that children and adolescents with gender dysphoria have higher than expected rates of autism spectrum disorder (ASD).[1–3] Gender dysphoria denotes a persistent incongruence between one's biologic sex and current gender identity causing clinically significant distress and impairment.[4] The association of gender dysphoria with ASD, with cases often categorized as Asperger syndrome (AS), was initially reported in a series of case reports.[5–7] The association was measured more formally in a Dutch study which reported a 7.8% prevalence of ASD in patients presenting for evaluation at a gender dysphoria clinic,[8] a rate much higher than expected based on the prevalence of ASD in the general population. In addition, children with ASD seen at a large U.S. hospital-based neuropsychology clinic were 7.59 times more likely than non-referred children to have gender variance as measured by the parent-reported Child Behavior Checklist.[9]

However, clinical data on ASD among youth with gender dysphoria remain limited, particularly in North America where no published studies, to our knowledge, have formally evaluated the prevalence of children and adolescents with ASD presenting with gender dysphoria. The current study aimed to fill this gap by providing a descriptive analysis of ASD data from youth patients referred to a multidisciplinary gender clinic in a large U.S. pediatric hospital.

## Methods

We reviewed clinical data from 39 consecutive patients ages 8 to 20 years (mean age 15.8 years, natal male: $n=22$, natal female: $n=17$) presenting for evaluation at a multidisciplinary gender clinic from 2007 to 2011 based in a large U.S. pediatric hospital. All study activities were approved by the Boston Children's Hospital Institutional Review Board, and patient data were protected by use of sound research methods and use of de-identified data.

[1]Department of Medicine, Division of Pediatric Endocrinology, [3]Department of Pediatrics, [5]Department of Psychology, [6]Department of Psychiatry, and [7]Department of Urology, Boston Children's Hospital, Boston, Massachusetts.
[2]Department of Pediatrics and Communicable Diseases, Division of Pediatric Endocrinology, University of Michigan, Ann Arbor, Michigan.
[4]Pacific University School of Professional Psychology, Hillsboro, Oregon.

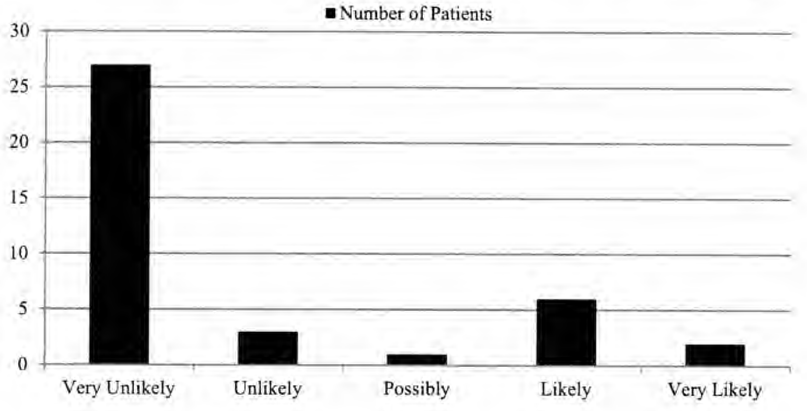


**FIG. 1.** Probability of Asperger Syndrome as determined by the Asperger Syndrome Diagnostic Scale.

As part of an initial evaluation, a single clinic psychologist performed a battery of psychological assessments, routinely administering the Asperger Syndrome Diagnostic Scale (ASDS)[10] in an attempt to identify children who may have co-occurring gender dysphoria and ASD. The ASDS was designed for use in children ages 5-to-18 years and was validated in a sample of 115 children with the diagnosis of AS.[11] It is a parent-completed, pen-and-paper measure containing 50 statements that are rated as observed or not observed. The statements describe AS-typical behaviors within 5 subscales: language (9 items), social (13 items), maladaptive (11 items), cognitive (10 items), and sensorimotor (7 items). For example, an item within the social subscale, "Avoids or limits eye contact," provides the parent with choices "Observed" or "Not observed." The total number of observed items within the subscale yields the subscale's raw score with corresponding percentiles within each subscale. Adding together these subscale raw scores yields a total raw score that is converted into an Asperger Syndrome Quotient (ASQ). The ASQ translates to a "Probability of Asperger Syndrome." Probabilities are expressed as "Very likely" (ASQ >110), "Likely" (ASQ 90–110), "Possibly" (ASQ 80–89), "Unlikely" (ASQ 70–79), and "Very unlikely" (ASQ ≤69).[10] The ASDS was chosen as a clinical tool for our program because it could be administered and scored quickly, serving as a screening tool for ASD.

Data were extracted manually from individual patient charts by one member of the study team and entered into Excel. Descriptive statistics (frequencies, means, standard deviations) were conducted.

## Results

Overall, 9 of the 39 participants (23.1%) had an ASQ above 80, corresponding to a "Probability of Asperger Syndrome" of "Possibly" ($n=1$), "Likely" ($n=6$), or "Very likely" ($n=2$) (Figure 1). Of these nine participants scoring highly on the ASDS, 5 were assigned a male sex at birth and 4 were assigned a female sex at birth. Average age at evaluation of the high ASQ group was 16.2 years (range 12.0–18.8). The prevalence of ASDS among natal males (22.7%, $n=5/22$) and natal females (23.5%, $n=4/17$) in our patient population was not significantly different (Fisher's exact test $P=0.95$).

Prior to presenting to the gender clinic, one patient had a long-standing diagnosis of autism, one had a long-standing diagnosis of Asperger syndrome, and two had been recently given a diagnosis of Asperger syndrome by a referring psychologist. Subscale analysis of the 9 patients with high ASQ scores demonstrates highest scores in the cognitive subscale, followed by the maladaptive subscale (Figure 2). Review of documentation would suggest that the identified patients represent a range of autism severity with more representation in the high-functioning end of the clinical spectrum. None of the four patients already diagnosed with an ASD had been diagnosed with other mental health disorders.

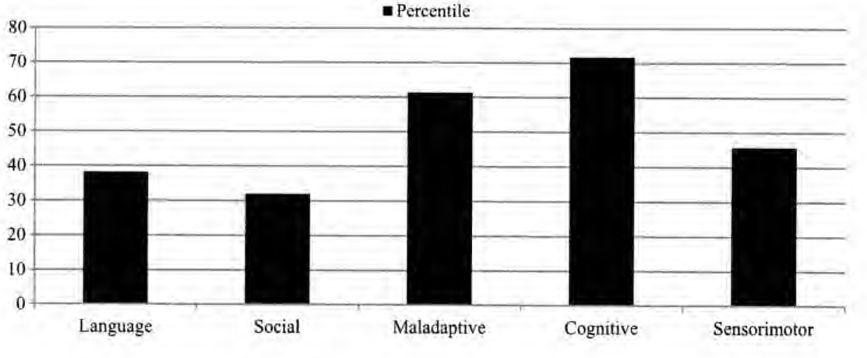


**FIG. 2.** Mean Subscale Percentile* Scores of the 9 Patients with ASQ Scores >80. *Percentile refers to the percentile score described on the Asperger Syndrome Diagnostic Scale scoring form.

TABLE 1. DESCRIPTION OF 9 PATIENTS WITH ASQ >80

| *Age (years)* | *Sex assigned at birth* | *ASQ* | *ASQ Interpretation^* | *ASD Diagnosis* | *Other mental health diagnoses* |
|---|---|---|---|---|---|
| 17.8 | F | 116 | Very likely | Asperger syndrome* | — |
| 15.6 | M | 111 | Very likely | Asperger syndrome | — |
| 12.0 | M | 101 | Likely | Autism | — |
| 12.5 | M | 107 | Likely | — | Learning Disorder, ADHD, Bipolar Disorder |
| 14.8 | M | 103 | Likely | — | ADHD, Selective Mutism, PTSD |
| 18.1 | F | 97 | Likely | Asperger syndrome* | — |
| 18.8 | F | 97 | Likely | — | Anxiety Disorder |
| 18.7 | M | 92 | Likely | — | — |
| 17.1 | F | 82 | Possibly | — | Dysthymic Disorder, Social Phobia |

*Diagnosed by referring psychologist during assessment for gender dysphoria.
^Likelihood of diagnosis of Asperger syndrome based on ASQ.
ASQ, Asperger syndrome quotient; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; PTSD, post-traumatic stress disorder.

Of the five patients not previously diagnosed with an ASD, four had been diagnosed with other mental health disorders (Table 1). Comparison of rates of co-morbid mental health disorders between patients with high versus low ASQ scores was not performed; previous description of this clinic's patient population demonstrated a rate of significant psychiatric history at presentation at 44.3%.[12]

## Discussion

This is the first report of formal screening for ASD in patients presenting to a North American child and adolescent gender clinic. Our finding, that 23% of patients presenting with gender dysphoria had possible, likely, or very likely Asperger syndrome as measured by the ASDS, is consistent with growing evidence of increased prevalence of ASD in gender dysphoric children.[1–3,8] This number is higher than the previously published co-occurrence rate of 7.8% in the Dutch pediatric gender clinic, however the numbers should not be compared directly since we used a screening tool while the Dutch group used a diagnostic test, the Diagnostic Interview for Social and Communication Disorders—10th Revision.[8] It is interesting to note the near equal rate of positively screened males and females. This finding conflicts with the typical male prevalence of ASD.

The evaluation and treatment of children and adolescents with gender dysphoria is guided by professional guidelines or standards of care.[13,14] These guidelines suggest evaluation by a mental health professional who not only explores the diagnosis of gender dysphoria, but who also assesses for co-occurring mental health conditions. Anxiety, depression, and suicidality are common in children and adolescents presenting to gender clinics.[15,16] The psychological evaluation performed is not standardized, with different clinics performing diverse batteries of psychological testing.[12,17,18] Our data support inclusion of ASD screening as part of any comprehensive gender assessment, especially as diagnosis of ASD has implications for management of gender dysphoria. For example, a patient with ASD and gender dysphoria may require specialized psychosocial interventions, focused on navigating unique social challenges encountered during hormonal and social transition from the natal sex to the affirmed gender. Youth have the right to appropriate assessment, diagnosis, and treatment of both ASD and gender dysphoria to ensure optimal clinical care.

A limitation of this study is that we report results of a screening test that is not validated as an ASD diagnostic tool in the absence of other confirmatory information. It is important to restate that the ASDS was not validated in a general population-based sample, but rather in a sample of 115 children with the diagnosis of AS. This limits our ability to know how closely we are measuring true ASD. For example, some items on the ASDS may be naturally observed in non-ASD gender dysphoric youth. Specifically, an item on the cognitive subscale "appears to be aware that he or she is different from others," and an item on the maladaptive subscale "does not change behavior to match the environment," might capture expected observations in a gender dysphoric child. Thus, scrupulous attention to symptomology during ASD diagnostic evaluation of gender non-conforming youth is essential to minimize any risk of misclassifying gender dysphoric youth with high functioning ASD due to symptom overlap (e.g., feeling different from peers, social isolation, etc.). Importantly, certain symptoms may be associated with both diagnoses, but stem from vastly different origins. Another consideration is the potential for presentation bias. First, children with ASD may be more likely to express their gender dysphoria than children without ASD. ASD may minimize awareness of social stigma, the same stigma that might cause non-ASD children to repress gender dysphoria. Second, children with co-occurring ASD and gender dysphoria may be more likely to be referred to a specialty center than other children with gender dysphoria, who may be managed locally. Therefore, our findings describe rates of positively screened ASD in children presenting for medical assessment and management of gender dysphoria to a specialty referral center, as opposed to the general co-occurrence of ASD and gender dysphoria. The true relationship of ASD and gender dysphoria requires a population-based study design. Finally, this is a retrospective chart review and results should be further examined using prospective research methods.

## Conclusion

Our findings are consistent with growing evidence supporting increased prevalence of ASD in gender dysphoric

children. Future research is needed to validate measures of ASD for use with a gender dysphoric patient population. In addition, longitudinal follow-up studies of co-occurring gender dysphoria and ASD will allow for better quantification of the problem and improved understanding of etiological factors. Differences in androgen exposures on the developing fetal brain have been suggested as a potential contributor in gender development[19–22] as well as in the development of ASD.[23,24] Genetic factors have also been implicated in both ASD[25] and gender dysphoria.[26] In addition, it has been proposed that the social rigidity typical of ASD contributes to inflexibility of gender and contributes to increased prevalence of ASD in gender dysphoric children.[8] Gaining scientific and clinical insight into children and adolescents with co-occurring gender dysphoria and ASD could advance understanding of development of both gender and autism, as well as guide diagnostic practices, clinical care, and therapeutic intervention. To guide provision of optimal clinical care and therapeutic intervention, we recommend routine assessment of ASD in youth presenting for gender dysphoria.

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### Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
*Daniel E. Shumer, MD, MPH*
*University of Michigan Health System*
*Division of Pediatric Endocrinology*
*Medical Professional Building, Room D1205*
*1500 E. Medical Center Drive*
*Ann Arbor, MI 48109-0718*

*E-mail:* dshumer@med.umich.edu

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# Mental health of transgender youth in care at an adolescent urban community health center: A matched retrospective cohort study

**Sari L. Reisner, ScD, MA**[1,2], **Ralph Vetters, MD, MPH**[3], **M Leclerc, MPH**[4], **Shayne Zaslow, MA, MS**[2], **Sarah Wolfrum, MPH**[2], **Daniel Shumer, MD**[5], and **Matthew J. Mimiaga, ScD, MPH**[1,2,6]

[1]Department of Epidemiology, Harvard School of Public Health, Boston, MA

[2]The Fenway Institute, Fenway Health, Boston, MA

[3]Sidney Borum Jr. Health Center, Boston, MA

[4]Boston University School of Public Health, Boston, MA

[5]Division of Endocrinology, Boston Children's Hospital, Boston, MA

[6]Department of Psychiatry, Harvard Medical School, Boston, MA

## Abstract

**Purpose**—Transgender youth represent a vulnerable population at risk for negative mental health outcomes including depression, anxiety, self-harm, and suicidality. Limited data exists to compare the mental health of transgender adolescents and emerging adults to cisgender youth accessing community-based clinical services; the current study aimed to fill this gap.



Address Correspondence To: Sari Reisner, ScD, MA, The Fenway Institute at Fenway Health, 1340 Boylston Street, 8th floor, Boston, MA 02215. sreisner@fenwayhealth.org. Phone: 617-927-6017.

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**Contributors' Statement**

**Sari L. Reisner:** Dr. Reisner conceptualized and designed the study, conducted all statistical analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

**Ralph Vetters:** Dr. Vetters conceptualized and designed the study, critically reviewed the manuscript, and approved the final manuscript as submitted.

**M Leclerc:** Co-Author Leclerc conducted manual chart review and data extraction from patient charts, conducted quality assurance activities to ensure integrity of the data, assisted with literature review for the manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted.

**Shayne Zaslow:** Co-Author Zaslow wrote the initial query to extract data from patient charts, designed the data collection instruments and database, assisted with data collection and quality assurance, reviewed and revised the manuscript, and approved the final manuscript as submitted.

**Sarah Wolfrum:** Co-Author Wolfrum assembled the matched cohort of patients for chart review, supervised data collection, conducted data quality reviews, reviewed and revised the manuscript, and approved the final manuscript as submitted.

**Daniel Shumer:** Dr. Shumer critically reviewed the manuscript, and approved the final manuscript as submitted.

**Matthew J. Mimiaga:** Dr. Mimiaga critically reviewed the manuscript, and approved the final manuscript as submitted.



Shumer
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Leisa Pastor, CSR, CRR

**Methods**—A retrospective cohort study of electronic health record (EHR) data from 180 transgender patients age 12–29 years seen between 2002–2011 at a Boston-based community health center was performed. The 106 female-to-male (FTM) and 74 male-to-female (MTF) patients were matched on gender identity, age, visit date, and race/ethnicity to cisgender controls. Mental health outcomes were extracted and analyzed using conditional logistic regression models. Logistic regression models compared FTM to MTF youth on mental health outcomes.

**Results**—The sample (n=360) had a mean age of 19.6 (SD=3.0); 43% white, 33% racial/ethnic minority, and 24% race/ethnicity unknown. Compared to cisgender matched controls, transgender youth had a two- to three-fold increased risk of depression, anxiety disorder, suicidal ideation, suicide attempt, self-harm without lethal intent, and both inpatient and outpatient mental health treatment (all $p<0.05$). No statistically significant differences in mental health outcomes were observed comparing FTM and MTF patients, adjusting for age, race/ethnicity, and hormone use.

**Conclusions**—Transgender youth were found to have a disparity in negative mental health outcomes compared to cisgender youth, with equally high burden in FTM and MTF patients. Identifying gender identity differences in clinical settings and providing appropriate services and supports are important steps in addressing this disparity.

**Keywords**

mental health; transgender; gender minority; adolescent; health disparity

## Introduction

*Transgender* youth have an assigned sex at birth that is different from their current gender identity[1]. Gender identity refers to a person's internal felt sense of self[2]. Transgender adolescents and emerging adults represent an underserved and under-researched population with specific medical and mental health needs[3,4]. U.S. population-level surveys do not routinely include survey items to identify transgender youth respondents; therefore, there is a lack of national epidemiologic data to document and monitor health disparities by gender identity[1], including among youth[5]. Despite the dearth of quality comparative national-level data on the mental health of transgender versus cisgender (non-transgender) youth, local and regional studies suggest transgender adolescents and emerging adults are a subpopulation of youth burdened by adverse health indicators, particularly in the mental health domain including depression, anxiety, suicidality, and self-harm behaviors[6–11].

Clinical settings and electronic health records (EHR) have been identified as important and under-utilized sources of information about sexual minority (lesbian/gay/bisexual) and gender minority (transgender) health[12,13]. Clinical settings and EHR are particularly valuable for transgender health in light of the dearth of comparative data that exist to understand the health and wellbeing of transgender relative to cisgender patients. Only a small handful of studies using transgender youth patient data have been conducted in clinical settings in the U.S., and most of these have occurred in multidisciplinary gender clinics[7,14,15]. Spack and colleagues conducted a chart review study to explore characteristics of 97 children and adolescents age < 21 years (mean age=14.8; SD=3.4) with Gender Identity Disorder (GID) seen consecutively between 1998 and 2009 at a multidisciplinary

gender clinic at Boston Children's Hospital in Massachusetts. Overall, 44% (n=43) of patients presented for medical care with significant psychiatric histories, including diagnoses of depression (58%), general anxiety disorder (16%), a history of self-mutilation (21%), and/or one or more suicide attempts (9%)[15]. Another study conducted at Children's Hospital, Los Angeles in California examined associations between quality of life measures and psychosocial factors among 66 youth age 12 to 24 with GID who received care between 2011 and 2012. Perceived burden–the extent to which transgender identity interferes with life activities or causes distress–was positively correlated with greater depression and negatively associated with self-reported life satisfaction[7].

These clinical studies offer valuable information about transgender youth accessing services at multidisciplinary gender clinics at U.S. pediatric medical centers. However, there are limitations. Youth in these studies all received a psychiatric GID diagnosis per the Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)[16]. Given the 2013changes to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) which changed diagnoses from to Gender Dysphoria, research is needed that a) does not use GID as a sole inclusion criteria, and b) refrains from conceptualizing gender identity variation as psychopathology[17]. Patients presenting to specialized multidisciplinary gender clinics may not represent the larger population of transgender patients, including those who do not meet diagnostic criteria for Gender Dysphoria. The youth in these studies tend to be from higher socioeconomic status families that have health insurance, present for medical care with their parents/families—meaning their guardians are engaged in some way —and are largely white (non-latino/hispanic)/caucasian[14,15]. In addition, U.S. studies of transgender youth in clinical settings have not included a cisgender comparison group which is essential to examine mental health disparities[18].

There are no published studies that utilize EHR data to examine the mental health of diverse transgender youth with varied socioeconomic and racial/ethnic backgrounds presenting to U.S. community-based primary care youth clinic settings. Community-based health clinics are a unique point of entry to care for youth, especially for people of low socioeconomic and racial/ethnic minority backgrounds[19]. In 2008, children and youth made up 33% of all patients seen in over 1100 Federally-Qualified Community Health Centers (FQCHC), and they were more likely to be uninsured, poor, or from a racial/ethnic minority background than those seen in private practice settings[19,20]. Examining gender differences among transgender youth who access community-based primary care youth clinic settings is also important to understand whether and how healthcare utilization and service needs differ for FTM and MTF youth patients.

This study is designed to compare the mental health of transgender and cisgender youth in a community-based setting. To achieve this goal, this study: (1) examines mental health indicators among diverse transgender youth engaged in care at an urban pediatric and young adult community-based health center; (2) tests whether transgender youth patients bear increased mental health burden compared to matched cisgender patients; (3) explores differences in psychiatric diagnoses between FTM spectrum and MTF spectrum youth patient populations.

## Patients and Methods

### Study Design, Participants, and Procedures

A retrospective observational cohort study of electronic health record (EHR) data was conducted at the Sidney Borum, Jr. Health Center, an urban community-based health center serving youth in Boston, Massachusetts that is part of Fenway Health. Transgender patients age 12–29 years seen for one or more medical and/or behavioral health care visits between 2002–2011 were included in this study. Transgender patients (n=180) were identified by an EHR code "transgender" based on self-reported transgender identity on patient registration forms, behavioral health assessment forms, or direct communication with medical or behavioral health professionals during clinical visits. Direct patient communication of transgender identity to a physician or behavioral health professional was documented in narrative notes on the clinical visit and/or listed as a diagnosis of Gender Identity Disorder[16] in the patient's diagnostic history. All study activities were reviewed and approved by the organization's Institutional Review Board.

### Description of Clinical Context

During the period covered by data collection from the Sidney Borum, Jr. Health Center clinical site, annual visits by unduplicated patients varied between 2,000 to 3,000 patients per year at the clinic. Clinicians providing care for transgender youth at the site included MDs, nurse practitioners, LICSWs and MSWs all working collaboratively as a team. This team met regularly once to twice a month to review cases and assess medical and behavioral health protocol applicability before supporting hormones for gender transition and writing prescriptions for hormones and other adjunct medications. Transgender care for youth under age 18 years required family participation, broadly defined, and the consent of the youth's guardians, including state-appointed guardians in some situations. Youth age 18 years and older could consent to care supporting gender transition for themselves. Health insurance or the ability to pay for services was required for transition-focused transgender care at the clinic. However, with the implementation of Massachusetts state health insurance reform starting in 2006, many barriers to access to care for transgender youth were removed.

### Matched Sampling

Matched sampling was utilized to reduce bias, increase precision, and control for confounding in this observational study[21]. Transgender youth were categorized as being on the female-to-male (FTM) spectrum (assigned a female sex at birth and identify as man, male, transgender, FTM, trans man, trans masculine) or on the male-to-female (MTF) spectrum (assigned a male sex at birth and identify as woman, female, transgender, MTF, trans woman, trans feminine). The 106 FTM and 74 MTF patients were matched to cisgender patient controls on: (1) visit date: an office visit +/− 3 months of the office visit where the transgender patient received a transgender "flag" in their patient chart or the office visit where this was first reported; (3) gender identity; (3) age; and (4) race/ethnicity. If a patient's ethnicity was latino/hispanic and their race was listed as something other than latino/hispanic, the patient was categorized as multiracial and matched to other multiracial individuals. Six transgender patients (3.3% of the transgender patient sample) were partially

matched on age and gender identity only, not on race/ethnicity, due to the few number and homogeneity of younger age patients.

A Structured Query Language (SQL) query pulled the matching criteria for each transgender patient, and a second query was done to find a match for each patient. When multiple patients matched, a randomly generated number was assigned to each possible control, and the matching cisgender patient with the highest randomly generated number was assigned as the control. Once a control was selected they were removed from the pool of available matches.

For transgender patients that did not have an exact match on all matching criteria, the matching criteria were ranked (as numbered previously) and adjusted in a systematic way in order to obtain a match for the patient. When no match was found, the criterion that patients must match on race/ethnicity was removed. If still no matches were found then the age of matches was expanded to be +/− one year of the case patient. These revisions to the matching criteria were sufficient to find matches for all of the transgender patients in the cohort.

A Microsoft Access database was created with separate forms and tables corresponding to each category of the data extraction measures. SQL queries extracted demographic and some medical information from the EHR, which was then exported into the Access database. Data about patients' mental health history were obtained by individual manualized chart review.

## Measures

Demographic data were extracted from patient registration and behavioral intake forms, as well as clinical visit physician narratives. Demographics extracted included age (continuous in years calculated by subtracting date of first appointment from date of birth), race/ethnicity (white, black, latino/hispanic, other race/ethnicity, multiracial, missing/unknown), gender identity (non-gender minority female, non-gender minority male, FTM, MTF), and cross-sex hormone use (yes/no).

Depression and anxiety disorders were recorded only for patients with physician-endorsed diagnoses listed in the EHR per DSM-IV-TR criteria[16]. Patient self-report of lifetime suicidality (suicidal ideation and suicide attempt captured separately), self-harm without lethal intent (non-suicidal self-injury; NSSI; e.g., cutting, burning, other self-harm behaviors), outpatient mental health care (e.g., psychotherapy), and inpatient mental health care (e.g., inpatient psychiatric hospitalization, substance abuse treatment) were recorded in data abstraction from physician clinical visit narratives.

## Data Analysis

SAS version 9.3 statistical software was used for data analysis. Statistical significance was pre-determined at the alpha 0.05-level. Univariable, descriptive statistics (frequencies, means, standard deviations (SD)) were estimated. Bivariate statistics compared transgender and cisgender youth. T-test statistics were estimated for continuous variables (with appropriate tests for normality) and $\chi^2$ test statistics were used for binary and categorical variables. Conditional logistic regression models for matched pairs data[22] compared

transgender and matched cisgender youth to examine between-group differences in mental health. To examine within-group differences, logistic regression models restricted to transgender youth were fit to compare FTM and MTF patients, regressing each mental health outcome on gender identity (FTM vs MTF) (unadjusted), then adjusting for age and race/ethnicity, and finally adjusting for age, race/ethnicity, and cross-sex hormone use. Risk Ratios (RR) and 95% Confidence Intervals (95% CI) were estimated rather than Odds Ratios (OR) because the prevalence of outcomes was > 10%[23].

## Results

### Demographics

The overall sample had a mean age of 19.6 (SD=3.0), 42.5% were white, 33.3% were racial/ethnic minority, and 24.2% were race/ethnicity unknown. As expected due to matching by age and race/ethnicity, no significant differences were found by age and race/ethnicity comparing transgender and cisgender youth (Table 1). The majority (61.7%; n=111) of transgender youth were being treated with cross-sex hormones.

### Between-Group Differences: Comparing Transgender and Cisgender Youth

Compared to cisgender matched controls, transgender youth had an elevated probability of having DSM-IV-TR diagnosed depression (50.6% vs 20.6%; RR=3.95; 95% CI=2.60, 5.99) and anxiety (26.7% vs 10.0%; RR=3.27; 95% CI=1.80, 5.95) (Table 2). Transgender youth also disproportionately endorsed suicide ideation (31.1% vs 11.1%; RR=3.61; 95% CI=2.17, 6.03), suicide attempt (17.2% vs 6.1%; RR=3.20; 95% CI=1.53, 6.70), and self-harm without lethal intent (16.7% vs 4.4%; RR=4.30; 95% CI=1.95, 9.51) relative to matched controls. A significantly greater proportion of transgender youth compared to matched cisgender controls accessed inpatient mental health care (22.8% vs 11.1%; RR=2.36; 95% CI=1.33, 4.20) and outpatient mental health care (45.6% vs 16.1%; RR=4.36; 95% CI=2.69, 7.05) services.

### Within-Group Differences: Comparing FTM and MTF Transgender Youth

FTM and MTF transgender youth were compared on mental health indicators. No statistically significant differences in mental health indicators were found comparing FTM and MTF adolescent and emerging adult patients, including after adjustment for age, race/ethnicity, and hormone use (Table 3).

## Discussion

The current study fills a key gap in the existing mental health research literature on transgender adolescents and emerging adults. First, in a transgender patient population not defined solely by GID and presenting at a community-based youth clinic, this study found high prevalence of depression, anxiety, suicide ideation, suicide attempt, self-harm without lethal intent, and lifetime inpatient mental health care utilization, corroborating research in other clinical settings[7,14,15,24] and in convenience sample studies[6,9,10,25,26]. Second, this study's ability to compare mental health in transgender and cisgender patients in a community-based setting provides a unique addition to the literature. Findings demonstrate

that a significantly higher proportion of transgender adolescent and emerging adult patients were burdened by mental health concerns than cisgender youth. Third, no statistically significant differences in mental health were found between FTM and MTF transgender youth patients. This suggests equally high burden of mental health disorders in FTM and MTF adolescent and emerging adult patients. Findings point to the need for gender-affirming mental health services and interventions to support transgender youth. Community-based clinics should be prepared to provide mental health services or referrals for transgender patients.

Study findings should be interpreted alongside several limitations. First, nearly half of transgender patients were accessing outpatient mental health services, and transgender patients were more likely to access mental health services than cisgender youth. Therefore, transgender youth may be more likely to have had a DSM-IV-TR-based depression and/or anxiety diagnosis in their EHR which could inflate prevalence estimates (i.e., issues of measurement equivalence). Second, as a retrospective chart review this study is subject to common limitations of this research design (e.g., incomplete documentation, information that is unrecorded, variance in the quality of information recorded by medical professionals)[27]. Third, several transgender patients were partially matched to cisgender patients on age and gender identity only which may have introduced some bias in study findings. Fourth, youth in this study were seeking care at an urban community-based health center; thus, findings may not generalize to other clinic settings and geographic locations. Lastly, the elevated mental health burden among transgender youth is hypothesized to result from experiences of social stress such as family rejection, bullying, violence, victimization, and discrimination which occur due to disadvantaged social status [28,29]. These potential confounding variables were not captured in our chart review. Future research is needed to contextualize the mental health concerns of transgender adolescent and emerging adult patients in community-based clinic settings, including prospective assessment of social stressors and mental health symptoms and diagnoses over time. Such longitudinal investigations will also allow for specific consideration of developmental processes that may accompany mental health outcomes in different developmental periods, which the current study was not able to examine due to the age-matched design.

A strength of this study is that the sample was not restricted to youth with a GID diagnosis. As reflected in recent changes to the 2013 Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5)[30] which removed GID as a diagnosis and replaced it with Gender Dysphoria, being transgender is no longer conceptualized as a disorder. Over the past 10 years there has been a move away from pathologizing transgender people in mental health and clinical settings[31]. It generally accepted that wide spectrum of non-pathological diverse gender identities and gender expressions exist[31–33]. Thus, this study offers unique comparative data that directly compare the health and wellbeing of transgender and cisgender youth using a non-pathological perspective of gender variation.

Reducing health disparities[34]—through addressing inequities—is a core aim of Healthy People 2020[35]. Collecting gender-inclusive measures in patient settings is recommended for health services research and surveillance efforts to monitor health disparities and improve clinical practice[12,13]. A two-step approach is recommended where assigned sex at birth and

current gender identity are both assessed, either routinely at patient registration and/or during clinical care. Clinical assessment of patient reported outcomes (PROs)[36,37] can be implemented as part of routine clinical care visits for transgender youth to collect data that will inform clinical practice and future intervention development to reduce mental health disparities.

## Conclusion

The current study is one of the first studies in the U.S. to document mental health disparities by transgender status in youth using patient data and a controlled design to compare transgender and cisgender adolescents and emerging adults. Based on these findings, and consistent with prior clinical recommendations[38–40], it is recommended that primary care providers include gender identity as part of a basic patient history. Training programs and continuing education programs for primary care providers and mental health providers should include gender identity education. Providers should familiarize themselves with community resources for transgender youth. Patients with a transgender identity or history should be recognized as having higher risk for mental health concerns and should be carefully screened and evaluated. Patients identified with co-occurring transgender identity and mental health concerns should be seen by a mental health provider who is qualified to provide evidence-based care with sensitivity to the diversity of gender identity and expression.

The Sidney Borum, Jr. Health Center, the clinic site where this study took place, while devoting a good part of its resources to the care of transgender youth, is still a primary care clinic for adolescents and emerging adults. Therefore, this study shows that expanded care for transgender youth can be provided in the context of overall pediatric care: integration of behavioral health, psychiatry, and pediatric primary care – a medical home approach – can more than adequately support the medical and behavioral health needs of transgender youth and provide a locus of care for reduction of psychiatric outcomes described by the study. Including questions about gender as well as sexuality in standardized annual health reviews in pediatric practices in combination with recognized adolescent depression screenings can identify transgender youth at high-risk for self-harm and other mental health outcomes. The practice of care at this clinic creates a framework within which risk behaviors can potentially be addressed and may serve as a model for other youth-oriented clinics so that transgender youth feel safe, accepted, and receive the gender affirming care they need and deserve.

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## Abbreviations

| | |
|---|---|
| **GM** | gender minority |
| **MTF** | male-to-female |

**FTM** female-to-male

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## Highlights

- Transgender youth represent a vulnerable population at-risk for negative mental health outcomes including depression, anxiety, self-harm, and suicidality.
- Limited mental health data are available in this patient population from community-based clinic settings, including comparative data that examine disparities in mental health outcomes.
- Transgender patients have disparately negative mental health outcomes compared to their non-transgender counterparts, with equally high burden for FTM and MTF youth.
- Clinicians serving transgender youth should screen for mental health concerns.
- Collecting gender-inclusive measures in electronic health records is recommended, including assigned sex at birth and current gender identity at patient registration.

**Implications and Contributions**

Transgender youth were found to have a disparity in negative mental health outcomes compared to cisgender youth, with equally high burden in FTM and MTF youth. Identifying gender identity differences in clinical settings and providing appropriate services and supports are important steps in addressing this disparity.

**Table 1**

Sociodemographics: Comparing Transgender Youth and Cisgender (Non-Transgender) Controls (n=360).

| | Transgender n=180 50.0% | | Cisgender n=180 50.0% | | Bivariate Statistics | |
|---|---|---|---|---|---|---|
| | Mean | (SD) | Mean | (SD) | t-test (df) | *p*-value |
| Age | | | | | −0.78 (358) | 0.435 |
| Continuous in Years | 19.7 | 3.1 | 19.5 | 3.0 | | |
| | n | % | n | % | $\chi^2$ (df) | *p*-value |
| Race/Ethnicity | | | | | 7.18 (5) | 0.208 |
| 1 White | 87 | 48.3 | 66 | 36.7 | | |
| 2 Black/African American | 17 | 9.4 | 23 | 12.8 | | |
| 3 Latino/Hispanic | 19 | 10.6 | 23 | 12.8 | | |
| 4 Other Race/Ethnicity | 12 | 6.7 | 10 | 5.6 | | |
| 5 Multiracial | 9 | 5.0 | 7 | 3.9 | | |
| 6 Unknown Race/Ethnicity | 36 | 20.0 | 51 | 28.3 | | |
| Race/Ethnicity | | | | | 5.77 (2) | 0.056 |
| Racial/Ethnic Minority | 57 | 31.7 | 63 | 35.0 | | |
| White (Non-Hispanic) | 87 | 48.3 | 66 | 36.7 | | |
| Unknown Race/Ethnicity | 36 | 20.0 | 51 | 28.3 | | |

**Table 2**

Between-Group Differences Documenting Mental Health Disparities: Transgender Compared to Matched Cisgender (Non-Transgender) Youth Patients (n=360).[+]

| | Transgender n=180 | | Cisgender n=180 | | Transgender Versus Cisgender | | Total Sample n=360 | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | RR (95% CI) | *p*-value | n | % |
| Depression (DSM-IV-TR Diagnosis) | 91 | 50.6 | 37 | 20.6 | **3.95 (2.60, 5.99)** | **<0.0001** | 128 | 35.6 |
| Anxiety (DSM-IV-TR Diagnosis) | 48 | 26.7 | 18 | 10.0 | **3.27 (1.80, 5.95)** | **0.0001** | 66 | 18.3 |
| Suicide Ideation | 56 | 31.1 | 20 | 11.1 | **3.61 (2.17, 6.03)** | **<0.0001** | 76 | 21.1 |
| Suicide Attempt | 31 | 17.2 | 11 | 6.1 | **3.20 (1.53, 6.70)** | **0.002** | 42 | 11.7 |
| Self-Harm Without Lethal Intent | 30 | 16.7 | 8 | 4.4 | **4.30 (1.95, 9.51)** | **0.0003** | 38 | 10.6 |
| Inpatient Mental Health Services | 41 | 22.8 | 20 | 11.1 | **2.36 (1.33, 4.20)** | **0.004** | 61 | 16.9 |
| Outpatient Mental Health Services | 82 | 45.6 | 29 | 16.1 | **4.36 (2.69, 7.05)** | **<0.0001** | 111 | 30.8 |

[+] Participants were matched on age, race/ethnicity, and visit date.

**Table 3**

Within-Group Differences: Comparing FTM and MTF Transgender Youth Patients (n=180).

| | FTM (n=106) | | MTF (n=74) | | FTM Versus MTF Transgender[+] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Bivariate | | Age- and Race- Adjusted | | Age-, Race-, and Hormone- Adjusted | |
| | n | % | n | % | RR (95% CI) | *p*-value | RR (95% CI) | *p*-value | RR (95% CI) | *p*-value |
| Depression (DSM-IV-TR Diagnosis) | 58 | 54.7 | 33 | 44.6 | 1.50 (0.83, 2.73) | 0.182 | 1.17 (0.54, 2.51) | 0.697 | 1.64 (0.86, 3.09) | 0.131 |
| Anxiety (DSM-IV-TR Diagnosis) | 28 | 26.4 | 20 | 27.0 | 0.97 (0.50, 1.90) | 0.927 | 0.47 (0.19, 1.17) | 0.105 | 0.77 (0.37, 1.61) | 0.490 |
| Suicide Ideation | 32 | 30.2 | 24 | 32.4 | 0.90 (0.48, 1.71) | 0.750 | 1.09 (0.47, 2.53) | 0.834 | 0.99 (0.50, 1.96) | 0.979 |
| Suicide Attempt | 16 | 15.1 | 15 | 20.3 | 0.70 (0.32, 1.52) | 0.367 | 0.50 (0.18, 1.41) | 0.188 | 0.86 (0.38, 1.95) | 0.713 |
| Self-Harm Without Lethal Intent | 21 | 19.8 | 9 | 12.2 | 1.78 (0.77, 4.15) | 0.179 | 1.68 (0.69, 4.10) | 0.256 | 1.75 (0.71, 4.30) | 0.222 |
| Inpatient Mental Health Services | 23 | 21.7 | 18 | 24.3 | 0.86 (0.43, 1.74) | 0.680 | 0.99 (0.39, 2.49) | 0.982 | 0.96 (0.46, 2.03) | 0.922 |
| Outpatient Mental Health Services | 50 | 47.2 | 32 | 43.2 | 1.17 (0.65, 2.13) | 0.603 | 1.18 (0.54, 2.61) | 0.676 | 1.43 (0.75, 2.71) | 0.277 |

[+] Age, race/ethnicity, and cross-sex hormone use were not statistically significant in any of the fitted models.

FTM = Female-to-male. MTF = Male-to-female. RR = Risk Ratio. 95% CI = 95% Confidence Interval.

Ex. 11

**In the Matter Of:**

*K.C., ET AL*

*-v-*

*INDIVIDUAL MEMBERS OF MEDICAL LICENSING BOARD OF INDIANA, ET AL*

---

**Daniel Shumer, M.D.**

*May 16, 2023*

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UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF INDIANA
INDIANAPOLIS DIVISION

K.C., et al.,

Plaintiffs,

-v-

THE INDIVIDUAL MEMBERS OF THE MEDICAL LICENSING BOARD OF INDIANA, in their official capacities, et al.,

Defendants.

CASE NO. 1:23-cv-00595-JPH-KMB

The deposition upon oral examination of DANIEL SHUMER, M.D., a witness produced by means of videoconference and sworn before me, Melody M. Goodrich, CM, Notary Public in and for the County of St. Joseph, State of Indiana, taken on behalf of the Defendants, with the witness being located in Ann Arbor, Michigan, and all other participants appearing via videoconference, on Tuesday, May 16, 2023, at 9:02 a.m., pursuant to the Federal Rules of Civil Procedure.

STEWART RICHARDSON & ASSOCIATES
Registered Professional Reporters
(800)869-0873



APPEARANCES
(All participants via Zoom videoconference)

FOR THE PLAINTIFFS:

Harper S. Seldin, Esq.
Chase Strangio, Esq.
AMERICAN CIVIL LIBERTIES UNION FOUNDATION
125 Broad Street
New York, NY 10041
hseldin@aclu.org
cstrangio@aclu.org
and
Kenneth J. Falk, Esq.
Gavin M. Rose, Esq.
ACLU of INDIANA
1031 East Washington Street
Indianapolis, IN 46202
kfalk@aclu-in.org
grose@aclu-in.org

FOR THE DEFENDANTS:

Thomas M. Fisher, Esq.
Razi Lane, Esq.
OFFICE OF THE ATTORNEY GENERAL
302 West Washington Street
IGCS Fifth Floor
Indianapolis, IN 46204-2770
tom.fisher@atg.in.gov
razi.lane@atg.in.gov

ALSO PRESENT: Shawn Weyerbacher
Brad Davis
John Vastag



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**a female body, but I identify now as a boy."**
**And I'm asking apart from that person's communication of their understanding of their gender identity, how would you know what their gender identity is?**
MR. SELDIN: Object to form.
A Well, I think a lot of it does have to do with that person's understanding of their gender identity, and, you know, this is -- this is what mental health professionals are trained to do, to work with folks, partner with patients and families, to help understand these really challenging concepts.
There is no test -- there's no blood test. There's no X-ray. But the work of highly-qualified mental health professionals helps to inform the rest of the medical team whether someone's -- whether a person that we're seeing may benefit from intervention for gender dysphoria.
**Q What is the error rate for determining if someone is transgender?**
MR. SELDIN: Object to form.
A Well, I've never really thought of it that way. You know, I think that what's more clinically

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relevant is, you know, the -- the treatment of gender dysphoria -- right? -- so that if someone has -- has a diagnosis of gender dysphoria and may benefit from treatment, then the likelihood that that person's gender identity persists across time is extremely high.
**Q Okay. But is there an error rate of diagnosing gender dysphoria?**
MR. SELDIN: Object to form.
A In my clinical experience, the patients that I've seen with a diagnosis of gender dysphoria, you know, has had -- have had -- for example, I haven't had a patient that I've treated with gender dysphoria that then comes back several years later and says, "Guess what? My gender identity is -- I was completely wrong. I'm cisgender."
So I have not had that experience, no.
**Q Well, but you're in this case testifying as an expert, right? You're not just testifying based on your clinical experience. That was my understanding at least. Is that correct?**
MR. SELDIN: Object to form.
A Yes, I'm testifying as an expert.
**Q So according to the literature and all that**

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**you're aware of, what is the error rate for diagnosing gender dysphoria?**
MR. SELDIN: Object to form.
A I certainly have -- there's certainly people out there that have -- that describe that at one point they thought they were transgender, received treatment, and now identify as cisgender. The rate of that happening seems to be less than 1 percent.
**Q What's your basis for that?**
A So I think that there's -- the statement that the rate of someone that is treated for gender dysphoria all of a sudden identifying as cisgender being extremely low comes from lots of difference sources. Right?
So there's -- there's some, for example, longitudinal studies of treatment of gender dysphoria. There are retrospective studies. There's, you know, the -- I think the -- the way to quantify what you're asking is challenging -- right? -- because you can't capture everyone that has ever identified as transgender and then compared them later on.
What you can come closer to doing, that is people that have received medical care. Right?

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And so in review of cases of people with gender dysphoria that have received medical care, numbers of people that identify as cisgender subsequently, is low.
**Q Well, I guess I'm wondering, to have -- if you are designing a study to capture the error rate of diagnosing gender dysphoria, what would be necessary to have a reliable result? How would you structure that study?**
MR. SELDIN: Object to form.
A So let me back up for a second because, you know, the diagnosis of gender dysphoria -- there's a specific definition of gender dysphoria. Right?
So if a person has gender dysphoria at any one point in time, then that person is meeting specific criteria. Right? So that a person with gender dysphoria, for example, has a gender identity that's different from the sex assigned at birth. They meet 206 of the other criteria. It's affecting them clinically in some -- it's affecting them negatively in other aspects of their life. So if a person has gender dysphoria, by definition they meet that definition. They meet those criteria.
So at that point in time, there's no error

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rate. That person has gender dysphoria. So you're asking a different question.

**Q Well, no. I think let's go there. So is it impossible to misdiagnose gender dysphoria in your view?**

MR. SELDIN: Object to form.

A Just like all diagnoses in the DSM, the diagnosis is based on a clinical interview. So, for example, if a -- if you ask a patient a question and they give you a false answer, then you may diagnose them with gender dysphoria because they are not being truthful. But if a person is not able to participate in the interview, then you would have a harder time diagnosing gender dysphoria.

You know, I'm speaking as a pediatric endocrinologist that doesn't make a diagnosis of gender dysphoria, of course, but -- but just like the diagnosis of depression or schizophrenia or anxiety, all of these have clinical criteria, and so someone is diagnosed based on meeting those criteria.

**Q Well, I guess when using a diagnostic tool in trying to determine whether it's a useful diagnostic tool, as a scientist is it important**

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**to know what the error rate of that tool is?**

MR. SELDIN: Object to form.

A Well, gender dysphoria is defined as the -- someone meets the diagnosis of gender dysphoria only if they have the criteria outlined in the DSM. So I'm not -- I guess I'm not understanding your question.

**Q I guess I'm wondering how you know that that test gets it right every time.**

MR. SELDIN: Object to form.

A Okay. I think I understand. So I think maybe -- maybe implicit in your question is, well, what do we do with the diagnosis of gender dysphoria. Right? So if someone meets criteria for gender dysphoria, what does that mean and what does that imply for the future?

If a person meets criteria for gender dysphoria, for example, and they -- well, okay. Let me back up for a second.

We use gender -- the diagnosis of gender dysphoria to make medical decisions. Right? So a person that does not meet the diagnosis of gender dysphoria wouldn't require intervention. A person that does meet the criteria for gender dysphoria, I would want to know how does that

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affect them. Is that something that is making it harder for them to accomplish tasks, like going to school or getting a job or leading a happy, healthy, productive life?

As a whole, we understand that people with gender dysphoria may benefit from medical interventions -- right? -- such as gender-affirming hormones, for example. So if -- if we then take data from the use of gender-affirming hormones to treat gender dysphoria and we see improvement or positive impact on the gender dysphoria, then that helps to validate that the criteria used to diagnose gender dysphoria is helpful.

**Q So you don't really know until you treat the child whether your diagnosis was correct?**

MR. SELDIN: Object to form.

A No, that's not what I said.

I said that because of the body of evidence in the -- regarding the positive effects of treatment of gender dysphoria, we understand that -- that the use of that diagnosis can be helpful in making management decisions.

**Q Can be helpful. Are there times when it's not helpful?**

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MR. SELDIN: Object to form.

A I don't -- I don't really think I can -- I'm not really sure I understand.

**Q Well, you said "can be helpful." And I'm wondering -- okay. I'm still going back to my question. Is it always helpful? Is it an unassailable diagnostic tool?**

MR. SELDIN: Object to form.

A Well, I certainly think that in treating transgender adolescents, the -- whether or not they meet criteria for gender dysphoria is an extremely helpful thing to know.

**Q Is it unassailable?**

MR. SELDIN: Object to form.

A Can you define that.

**Q Is it always 100 percent right?**

MR. SELDIN: Object to form.

A I don't think anything is 100 percent right in any aspect of medicine, but I think that the confidence that I have in, for example, the assessment that -- members of the multidisciplinary team that I work with I find to be extremely helpful in having really challenging conversations with patients and families about what the medical options might be.

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**Q So not always 100 percent right. What is the error rate?**

MR. SELDIN: Object to form.

A I think this is too abstract to answer in that way. So I think, you know, if you could, be more specific in, you know, a specific situation.

**Q Doctor, all I'm asking is if you know if there is an error rate for diagnosing gender dysphoria.**

MR. SELDIN: Object to form. Asked and answered.

A I don't have more -- a more precise answer or number than I've already shared with you.

**Q And what number is that?**

MR. SELDIN: Object to form.

A I don't know what the error rate of diagnosis of gender dysphoria is. What I do know is that patients that have received -- that receive a diagnosis of gender dysphoria and are treated with gender-affirming care, I believe the error rate or the rate of people that later on in the future say, "Turns out I'm cisgender and I" -- "Turns out I'm cisgender," is less than 1 percent.

MR. SELDIN: Tom -- Mr. Fisher, is now a good time for a little mid-morning break or --

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MR. FISHER: Yeah. Sure. That's fine. Let's go ahead and take a break.

(Recess taken from 9:57 a.m. to 10:02 a.m.)

BY MR. FISHER:

**Q Doctor, I think you said that there are some who are transgender that do not experience gender dysphoria; is that accurate?**

A I would agree.

**Q So let's start with the preadolescents. About how many preadolescents do you think -- or is there evidence showing that are transgender but not gender dysphoric?**

MR. SELDIN: Object to form.

A I don't think I can give you a number. I think what I would say is that gender identity exploration is a normal function of childhood so that -- you know, in childhood we're always putting on different hats and exploring the world around us and how we interact with that world.

So, you know, I think that the -- for example, if a -- if a -- someone assigned male at birth is experimenting with wearing different types of clothes or different types of play, that doesn't necessarily mean that they have a difference in gender identity, for example. They

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may -- if a child has a difference in gender identity, they may or may not have any distress associated with that.

But the percentage of young people who are experiencing different degrees of gender identity difference I don't know.

**Q Let's just move into the range of adolescents. Maybe let's take somebody -- the range of kids from beginning of Tanner Stage 2 up through, I guess, 15. Is that a useful range -- age range in your mind?**

MR. SELDIN: Object to form.

A Sure.

**Q And I'm wondering, within that age range, do you have a -- is there any data that shows or do you have an estimate of percentage of transgenders who do not experience gender dysphoria?**

MR. SELDIN: Object to form.

A So there are some efforts to understand the number of people that identify as transgender, for example, in the United States today, and that number seems to be somewhere below 1 percent and above .5 percent.

**Q Okay.**

A And then the number of people then presenting to

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clinical care for gender dysphoria is much lower than that -- than that figure.

**Q What are your sources for those numbers?**

A Let me think. So I think that there's been -- there's a national survey in 2015 that was aiming to understand the prevalence of gender identity difference in the U.S. I think that there's some -- some -- an effort to quantify the percentage --

**Q Doctor, I'm sorry. You're breaking up. We're having a hard time getting you.**

A Sorry. Is that better?

**Q Yes. I don't know where the problem was, but you were starting to talk about what -- I was asking what studies supported the numbers you were mentioning, and so if we could just start there.**

A Yeah. So the things that come to my mind are, I think, a 2015 national transgender survey. I believe there's been some work done in Minnesota, if I'm not mistaken, trying to quantify the percentage of young people that are identifying as transgender, and so that's where I'm pulling that number, somewhere between .5 and 1 percent, from.

**Q Okay. That 2015 survey, who was surveyed?**

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A Now I'm just trying to remember if that was the one that came up -- that did offer that percentage. But there is a 2015 survey of -- I think it's called the National Transgender Survey, I think, published by the Williams Institute, which was surveying people from across the U.S. and territories to learn more about the health and well-being of transgender Americans.

**Q How was that survey conducted?**

A If I recall, there was a recruitment strategy to try to identify a diverse sampling of transgender people from all 50 states and different territories, recruiting from medical clinics, from snowball sampling, from online advertisements, to try to identify more people from different parts of the country.

**Q And how was it conducted?**

A Surveys.

**Q No. But, I mean, was it mail? Telephone? What was it?**

A If I'm not mistaken, I think majority online, but there may have been some mail. I'm not a hundred percent on that.

**Q Are you familiar with any criticisms of that survey?**

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MR. SELDIN: Object to form.

A Not specifically.

**Q Okay. When, Doctor, in your understanding was the first teen gender clinic opened in the United States?**

MR. SELDIN: Object to form.

A I want to say in the early 2000s.

**Q How many teens were diagnosed with gender dysphoria from 2000 to 2010?**

MR. SELDIN: Object to form.

A I don't know the answer to that.

**Q How about the decade -- or 12 years, 2011 to 2023?**

MR. SELDIN: Object to form.

A I don't know the number of people diagnosed.

**Q Do you have any sense of the volume dynamic of that diagnosis over that time?**

MR. SELDIN: Object to form.

A Sorry. Can you --

**Q I'm wondering if you have a sense of whether the volume of teen diagnosis with gender dysphoria has increased in those two decades.**

MR. SELDIN: Object to form.

A There has been more -- more adolescents diagnosed with gender dysphoria in the last decade, specifically as there's been more access to health intervention.

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**Q Sorry.**

A I can just barely see the top of your head.

**Q Oh, I'm so sorry.**

**Is that better?**

A Yes. Thank you.

**Q Okay. I can't see myself so I didn't really know.**

A You're like (indicating).

MR. SELDIN: Don't deprive us of the view of that sharp tie, Mr. Fisher.

BY MR. FISHER:

**Q I'm sorry. Were you finished with your answer, Doctor?**

A I think so, yes.

**Q Okay. So back to paragraph 28 of your declaration, if you could pull that up. I'm sorry. This is 4, right? Exhibit 4.**

**And you'll recall earlier I read the first clause of that first sentence, and now I'm going to switch focus to the second clause, which says, "Attempts to force transgender people to align their gender identity with their birth sex (sometimes descried as 'conversion therapy') have**

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**been found to be both harmful and ineffective."**

**Do you see that?**

A I do. And I do believe that word was supposed to be "described." So sorry about that typo.

**Q No. That's okay. I was actually going to try to fix it for you, but then I couldn't figure out if it was "decried" or "described." So I thought I'd let you do it. Okay. Thank you.**

**And then you cite -- later in the paragraph you cite Turban 2020a, right? Do I have that -- right. Turban 2020a. Campbell 2002, but I think that may be actually 2022.**

A Okay.

**Q And in Fish, you say 2022, but might actually be 2020. I got those dates just, I think, from your bibliography.**

**Anyway, you're with me, though? Yes?**

A I'm with you.

**Q Okay. So let's start with that Turban study.**

**So this is Exhibit 5. Let's go ahead and pull that up.**

(Shumer Exhibit 5 marked.)

MR. FISHER: I'm sorry. Turban -- JAMA Psychiatry, Association Between Recalled Exposure. There we go.

**what's going on there?**
MR. SELDIN: Object to form.
A Yes. So I would say the -- I think how -- how our -- our social worker describes it as a biopsychosocial assessment --
**Q Biopsychosocial. Okay.**
A -- which is, I think, just a fancy way of saying learning everything there is to learn about this patient's understanding of gender identity and also getting a good sense of other aspects of their life.
**Q How long does that process take, that assessment?**
A So the first visit with the social worker is typically scheduled for three hours, and then based on that visit, you know, the social worker could determine what further visits are required for the assessment.
**Q But it could be only one three-hour visit, and then it gets forwarded to you for a medical visit?**
MR. SELDIN: Object to form.
A You know, it really depends. I think sometimes patients coming in already have had an assessment with a treating therapist that they've known for several years and are coming with, for example, a



letter from the summary of the biopsychosocial assessment that's already been done by someone that's known them for a long time. So in that situation, oftentimes the one visit with our social workers is all that's required to sort of confirm. But in other situations, subsequent visits are necessary.
**Q Do you have a sense for sort of an average?**
MR. SELDIN: Object to form.
A An average number of visits with the social worker?
**Q Yes. Before the medical visit.**
A It's very individualized but, you know, somewhere between one and two visits on average.
**Q Over how many weeks or months would those one or two visits likely occur?**
A I think it's really variable. Right? So, like, for example, at an initial assessment visit with the social worker, the recommendation might be, you know, I want you to, you know, continue to evaluate and explore your gender identity working with, you know a local mental health provider for the next year. And then we'll set up a return visit.
Or it could be, you know, we haven't been



able to go through everything that I wanted to go through to understand you so let's set up another visit at my next available appointment.
So, you know, I think it -- it's -- you know, I think that it's -- when we're dealing with individual people here in medicine, it's not so much, like, you know, triage, done; assessment, bing; visit, check mark. You know, every single person requires a lot of individual thought, you know. How can I -- what is this person telling me about themselves? How can I help them? And, you know, what are some barriers to care? What -- what is unique about this person that allows us -- that requires time for considering X, Y, or Z?
So when you say "protocol" -- right -- I can say that we have some sort of a protocol which involves those sort of four phases that you outlined. But beyond that, the protocol breaks down when you're talking about individual people and their specific needs.
**Q Do you track data for how long people -- how long your patients take from triage through the medical visit?**
MR. SELDIN: Object to form.



A I don't formally track data, but, you know, I work there and can give estimates about how long things take.
**Q So how long do you think it takes on average from triage to medical visit?**
A Probably four to eight months.
**Q Is it -- if there's going to be maybe -- strike that.**
**Do you ever prescribe either pubertal suppressants or hormones at the first medical visit?**
MR. SELDIN: Objection to form.
A Yes.
**Q Okay. So the monitoring, tell me about how frequently you are monitoring your patients where there's a medical intervention.**
A Typically I see patients every three months.
**Q Through their 18th birthday, or for how long?**
A Every three months over the first year of treatment for sure. And then, like I say, because everyone is so individual and different, you create a plan as to what follow-up looks like moving forward.
So I have some patients that are, you know -- that are doing so well that our visits

**here, these individuals, the -- which I understand that to be describing the cohort included in the study -- "These individuals of whom an even higher percentage than the general population were pursuing higher education seemed different from the transgender youth in community samples with high rates of mental health disorders, suicidality, and self-harming behavior and poor access to health services."**

**Does that give you any concern about how representative this study is of the general -- when it talks about, you know, comparing this cohort to the general population?**

MR. SELDIN: Object to form.

A So I want to back up for a second and say that they're not saying that in choosing these patients to participate in the study initially they were pursuing higher education at higher rates. Of course, they were young adolescents. Right?

So what they're saying is the patients that participated, that they're describing in this study, are going on to have -- what I think they're implying -- is successful lives and saying that this seems different than the youth



in community samples with higher rates of these other problems.

This is something that I get to witness every day too, where, you know, oftentimes patients are coming to see me with, you know, parents feeling sort of hopeless, that there's no -- there's no future in sight and, you know, as I'm -- as we talked about graduating these kids to adult care, I do have that privilege of watching them, you know, successfully adulting in ways that we didn't -- that maybe their parents couldn't envision a few years before.

So I think that's what these authors are describing, that sort of phenomenon of, wow, our patients are doing well, they're going to college, they're getting jobs. Kind of in contrast to sort of what we're seeing from -- because I think that sentence is sort of read out of context there.

**Q Oh. Well, let's look at page 703. Right above "References."**

A Okay.

**Q "Third, despite absence of pretreatment differences on measured indicators, a selection bias could exist between adolescents of the**



**original cohort that participated in this study compared with nonparticipants."**

**Does that give you any concern about the results -- the reliability of the results here?**

MR. SELDIN: Object to form.

A Yeah. You know, this is just an aspect of research. It gets messy because we -- you know, because we can't do a randomized control trial, then we need to be creative about how we can attack these specific issues.

So if we are saying, okay, how do patients do over the long term with this type of care, how are we going to conduct that study? Okay. We need to conduct that study at a place where people go to receive this care. Right? So you couldn't do this study without doing it in a medical center that is doing the care.

So inherent in that is the idea that, well, not everyone's getting care. Right? If you're -- if you're farther away from Amsterdam, maybe you're less likely to get the care. If you don't have a car or maybe, you know, have -- have parents that won't bring you to the clinic, maybe you won't get the care.

So those -- you know, so then the next



question is, okay, so if we did this same treatment to kids that weren't able to get the care for one reason or another, would we have the same results? And so that in the -- in the limitation section here, that's what the author is asking the reader to think about. Right? Would we have the same results if we did this study on all the people that didn't have a car back in 1990 to get to these clinics or didn't know -- their doctor didn't know about the clinic existing?

And I think that that's left for the sort of contemplation of the reader -- or the common sense or the clinical sense of the reader to say, again, how generalizable is this to me, to my practice?

So to me, you know, these patients are not exactly the same as the patients that I'm seeing in the office. They live in Michigan, not Amsterdam. It's 2023, not 1990s. So those are differences.

Are those differences relevant? Maybe. Does this paper still help to inform me that gender-affirming care might be helpful? I think it does.

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I think that this is -- you know, this is just the nature of research. The limitation is that the study wasn't done on your patient. Right? And you have to extrapolate from what's learned from others on to your own patient.

**Q Is it your understanding that all of the participants in this study were getting psychiatric support?**

MR. SELDIN: Object to form.

A Yes.

**Q Does the study purport to control for psychiatric support in any way?**

MR. SELDIN: Object to form.

A So the study's describing this particular clinic experience with their care, which they, I think, do a very nice job of describing what that care is. It involves psychological support, gender-affirming care.

And so, you know, again I would go back to the same thing I just said. If I'm working in a place where there is not available psychiatric support or psychological support for patients, then I might say, hmmm, how am I going to use this study? Is it generalizable to me?

It looks like these patients, they improved

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with this package of psychological support, gender-affirming care, you know, seemingly supportive environment, and their outcomes were good.

Unfortunately, you know, me as a hypothetical person, unfortunately in my situation, I have something that maybe is similar to their psychologic support. They have a therapist, but it's not exactly the same as, you know, what they're describing they did in terms of psychological support. So is this paper generalizable to me?

And so, again, I think that goes back to -- back to the clinical sense and common sense of the reader, that without providing that psychological support, would the treatment that I'm proposing -- or without providing psychological support exactly how it's outlined in Amsterdam, does my -- would my treatment result in similarly favorable outcomes?

And so, you know, I think that, for the most part, providers of gender-affirming care today, the takeaway here is that, yeah, you don't give gender-affirming care, such as GnRH agonist or gender-affirming hormones, in a vacuum; that, you

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know, you don't give people medication and say, "Well, hope things go all right. Let's never talk about this again"; that you're also advocating for the patient to be well-supported, supported in their family, supported in their school, to connect with mental health professionals, to help them -- help their journey.

So how I would, I guess, answer your question is it seems like, you know, we have some pretty compelling data to say that this model of care works. So how -- how can I best replicate that using the resources that I have?

**Q And is it proper to infer causation from the medical interventions on this -- based on this paper?**

MR. SELDIN: Object to form.

A Well, I think that there's some compelling reasons to -- and for some causation -- right -- that there's -- you know, I think that the authors do a nice job of describing, you know, the intervention that they received, that there's -- you know, that there's an outcome that is quite different from what's expected based on the general population. So then if you're

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thinking about, okay, what's the causation? All right? So it could be -- it could be this package of care. Right? That could be one of the causes.

Now, in order to dispel that theory, I need to think about, well, what are other potential -- potential causes for these people doing so well compared to the general trans population. Could it be that their situation is somehow very different from those in the general population? Is there some -- you know, is this -- do I buy that there's a significant, you know, selection bias?

You know, I think that you can't -- you can't ever, you know, assume 100 percent causation in this type of study design, but I think the authors do a pretty nice job of, you know -- of explaining why causation should be considered.

And, you know, when I read this study, the conclusion that I reach is that, gosh, it seems like these patients are doing quite well after this intervention. It's nice that they were also involved in this really seemingly well-run clinic. And so, you know, while I -- while I

endeavor to provide high-quality care, let me learn from their experience in applying that to my own patients.

**Q So did the authors use the -- what I think is sometimes referred to as the UGDS, the Utrecht Gender Dysphoria Scale?**

MR. SELDIN: Object to form.

A I believe so.

**Q Are you aware whether they switched the version, male to female and female to male, after the transition interventions?**

MR. SELDIN: Object to form.

A You know, I have heard sort of this question raised about this paper before, and I'm not exactly sure that -- I don't want to make a mistake in answering it. If there's a part in the paper that is relevant to pull up, maybe I can review it with you in more detail.

**Q Yeah. I'm really just asking about awareness. You've heard it. I've heard it. I don't know that there's anything in the paper that tells us exactly. I just wondered if you were aware of that or had any information about that.**

MR. SELDIN: Object to form.

A Yeah. I'm not sure that I have -- that I'm the



most eloquent in sort of feeding back your -- you know, opponents' talking points about this particular problem. But I think it has something to do with, you know, after transition, that, you know -- so, first of all, what is the Utrecht Gender Dysphoria Scale? It's a pretty simple tool that is, you know, basically asking questions like this is how I feel about my chest, about my face, about my body.

And my understanding is that there's sort of a version that is designed to be asked to people assigned male at birth, people assigned female at birth. And so I think that the -- you know, I think in the beginning -- right? -- the Utrecht was presumably asked of people using the -- the version that's according to their assigned sex. And then subsequently, after transition, the question is, well, which version of this do we use? Right? You know, I think -- I think inherent in that is, like, well, what's the difference? What are we talking about here?

And I think that the point here is that there's an effort to try to quantify something that's hard to quantify, which is sort of what you've been asking me about, right? How do we



quantify gender dysphoria? That's hard.

So I think the Utrecht Gender Dysphoria Scale is an effort to try to quantify gender dysphoria. It's not perfect. I don't think that many people use it. But, you know, I think that people are picking out this question about which form was used to -- on which patient at which time.

To me, I think that sort of misses the point, that, you know, the primary outcome of this is -- is, you know -- is well-being which, you know, they describe how they measure it in lots of different ways.

So, yeah, I think that it's an interesting question. How -- what version of the Utrecth Gender Dysphoria Scale would you use before and after transition? You know, I don't really have the answer. But I guess that's to say I'm aware that this question exists, but I'm not sure that it's -- that it's something that makes me feel feelings one way or another in general about the study as a whole.

**Q Okay. So I think I want to go back to Exhibit 14, which might -- it's hard -- there it is. The date is in the lower right. There it**



**is. So this says 2010.**

**I guess I'm -- my understanding is that this is the original de Vries, and then what's at Exhibit 15 is the follow-up study.**

**Do you have enough familiarity with this to tell me if you think that's true?**

MR. SELDIN: Object to form.

A So this is the 2010, right?

**Q Yeah.**

A Yeah. So what was your question?

**Q I'm wondering if this is -- if I'm right in saying that this is the study that -- you know, the de Vries -- first de Vries study published with this cohort, and then the 2014 that we just looked at is a follow-up of this same group with fewer participants.**

A Yeah. You know what? I'm not -- I'm not sure.

**Q Okay. But are you familiar with this paper generally?**

A Yes.

**Q And, again, what is this paper telling us?**

A So this is more of trying to assess shorter term measurables at different time points. And I think -- if you just give me a second, I can take a quicker look here.

Ex. 12



or experience with postinfection syndromes. The relationship of long Covid to ME/CFS has been brought into focus by the CDC, the NIH, the WHO, and Anthony Fauci, the chief medical advisor to President Joe Biden and director of the National Institute of Allergy and Infectious Diseases. Going forward, research may yield complementary insights into the causation and clinical management of both conditions. The CDC has developed guidelines and resources on the clinical management of ME/CFS that may also be applicable to patients with long Covid.

An audio interview with Dr. Phillips is available at NEJM.org

Fourth, to respond holistically to the complex clinical needs of these patients, more than 30 U.S. hospitals and health systems — including some of the most prestigious centers in the country — have already opened multispecialty long Covid clinics. This integrative patient care model should continue to be expanded.

Fifth, the ultimate success of the research-and-development and clinical management agendas in ameliorating the impending catastrophe is critically dependent on health care providers' believing and providing supportive care to their patients. These beleaguered patients deserve to be afforded legitimacy, clinical scrutiny, and empathy.

Addressing this postinfection condition effectively is bound to be an extended and complex endeavor for the health care system and society as well as for affected patients themselves. But taken together, these five interrelated efforts may go a long way toward mitigating the mounting human toll of long Covid.

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From the COVID Collaborative, Washington, DC (S.P.); and the Harvard T.H. Chan School of Public Health, Boston (M.A.W.).

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# Criminalization of Gender-Affirming Care — Interfering with Essential Treatment for Transgender Children and Adolescents

Simona Martin, B.S., Elizabeth S. Sandberg, M.D., and Daniel E. Shumer, M.D., M.P.H.

On April 6, 2021, the Arkansas state legislature overrode a veto by the governor to pass legislation making it illegal for medical professionals to provide gender-affirming treatment to patients with gender dysphoria who are younger than 18 or to refer them to other clinicians for such treatment. Several other states have similar legislation pending. As physicians and a physician-in-training who provide gender-affirming care, we are deeply concerned that these political actions threaten the health and well-being of transgender children and adolescents. We have found that such young people are courageous and resilient, yet profoundly vulnerable. Moreover, they already have higher-than-average risk for suicidality and are disproportionately likely to experience violence.

Gender identity — the deeply felt internal sense of oneself as male, female, or somewhere else on the gender spectrum — may or may not align with the sex one was assigned at birth. When it does not align, the umbrella term "transgender" is often used to denote this incongruence. Although not all transgender young people feel distress related to their gender identity, when distress is present and persistent, a mental health professional with experience in gender-identity evaluations may diagnose gender dysphoria.

Gender dysphoria can be treated with both nonmedical and medical interventions. The former may include therapy, coming out to loved ones, or using a chosen name or pronouns and dressing or grooming in a way that

matches one's gender identity (making a social transition); the latter may include hormonal or (when age appropriate) surgical treatments to bring the person's physical characteristics more closely in line with their gender identity or to prevent developmental changes that don't align with this identity. Decisions regarding the appropriate treatment for each individual patient are made by the patient, the parents, and the health care team and are guided by evidence-based standards put forth by organizations such as the Endocrine Society, the World Professional Association for Transgender Health, and the American Academy of Pediatrics. Each person has their own gender journey, and there is no one-size-fits-all approach to this kind of care.

Pediatric gender clinics originated in the 1980s in Amsterdam. Dutch physicians recognized that transgender children tended to face mental health challenges during adolescence, as secondary sex characteristics developed, and that early intervention could be lifesaving. They also appreciated the value of delaying decisions that could have a permanent effect on a child. To resolve these conflicts, they created a protocol under which puberty would be paused using medications at Tanner stage 2 (the period during which signs of central puberty are first detected, most often between 8 and 15 years of age) if gender dysphoria had persisted, thereby forestalling the development of unwanted and potentially permanent secondary sex characteristics with a reversible intervention. Gonadotropin-releasing hormone (GnRH) analogues, or "puberty blockers," have been used by pediatric endocrinologists for more than 30 years for the treatment of precocious puberty. These agents have well-known efficacy and side-effect profiles, and their effects are reversible. In later adolescence, treatment with gender-affirming hormones could be initiated if gender identity remained incongruent with the sex assigned at birth.

The Dutch-developed treatment model was shown to result in long-term improvements in the well-being of adolescents with gender dysphoria[1] and was the basis for current guidelines formalizing the treatment of gender dysphoria. These guidelines recommend using GnRH analogues at Tanner stage 2 and prescribing hormone therapy later in adolescence if the patient, the parents, and the medical team all agree with this approach. Today, prescribing these therapies is coupled with education on the safe use of such medications and with close surveillance for potential risks associated with therapy — for instance, monitoring for changes in bone health in children taking GnRH agonists, for risk factors for blood clotting with estrogen therapy, and for polycythemia with testosterone therapy. With proper monitoring and education, the risks associated with these therapies can be mitigated, and the benefits are substantial: use of hormone therapy is associated with improved quality of life, reduced rates of depression, and decreased anxiety among transgender people.[2]

A recent survey of U.S. high school students conducted by the Centers for Disease Control and Prevention found that 1.8% of students identify as transgender. More than one third of transgender adolescents surveyed had attempted suicide in the previous 12 months.[3] As clinicians caring for this population, we are alarmed by this statistic, but we see it as a call to action. We know that mental health disparities between transgender and cisgender children are not inevitable and that with support from their families and communities and access to evidence-based mental health and medical interventions, transgender children and adolescents can survive and thrive.

A multidisciplinary approach to treating transgender young people has been shown to alleviate gender dysphoria when treatment occurs in a supportive environment that attends to the patient's mental, social, and physical needs. Young people who receive such gender-affirming care report improvements in their overall well-being, and their level of well-being is generally in line with that of their cisgender peers — and sometimes it's higher.[4] Having access to gender-affirming care in childhood and adolescence can have profoundly important mental health benefits: one study found that transgender adults who had had access to puberty suppression during adolescence had lower odds of suicidal ideation than those who wanted such treatment but hadn't received it.[5]

Under the new Arkansas law, known as the Save Adolescents from Experimentation (SAFE) Act, physicians who provide gender-affirming therapy for transgender people younger than 18 will be subject to loss of licensure and could be sued. The law's name implies that following evidence-based guidelines while working closely with patients and families

is a form of experimentation. The law references inaccurate information about the care of gender-diverse young people, stating that genital surgeries are being recommended for people younger than 18. In reality, guidelines indicate that genital surgeries should be delayed until the person reaches the age of legal adulthood in their country, which in the United States is 18 years. The law also states that there are no long-term data on the use of puberty-blocking drugs for the treatment of gender dysphoria, when multiple studies have revealed long-term positive outcomes for transgender people who have undergone puberty suppression.[4,5]

The content of the Arkansas law, and that of similar bills that have been proposed in other states, is not based on data, medical literature, or correct information about the process of treating transgender adolescents. We believe these bills threaten the health, well-being, and survival of transgender children and young adults. By penalizing physicians for practicing evidence-based medicine, the legislation nullifies their expertise and interferes with therapeutic relationships among physicians, patients, and families. It strips power from patients and families who are already marginalized. And although the stated purpose of the legislation is to protect adolescents, we believe that criminalizing what has been shown to be lifesaving treatment will do the opposite — and that the consequences could well be tragic.

Disclosure forms provided by the authors are available at NEJM.org.

From the University of Michigan Medical School (S.M.) and the Division of Pediatric Endocrinology, Diabetes, and Metabolism, Department of Pediatrics, Michigan Medicine, University of Michigan (E.S.S., D.E.S.) — both in Ann Arbor.

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# When Low Tech Wins

Jacqueline Baras Shreibati, M.D.

By April 2020, I was ready for video. The Covid-19 crisis required that patients, clinicians, and staff stay home, so clinics throughout the United States had ramped up their telehealth practices. The federally qualified health center (FQHC) where I work had spent several weeks thoughtfully developing the workflows for video visits. I was eager to virtually see my patients — mostly elderly, Spanish-speaking, and low income — and be a part of the digital health revolution.

When I started, I thought there was a hierarchy in which in-person care was the gold standard, video visits were second, and telephone visits the method of last resort. But a year later, I don't think of them as best, good, and OK. I see them as different — each ideal for different contexts. And just as people with visual impairment may have heightened use of their other senses, my year of telephone care during the Covid-19 pandemic has cultivated my capacity to connect with patients solely through sound.

It took only a day or two to discover the benefits of video communication. A patient called in to say he had run out of one of his pills. He lived alone and didn't understand the labels on his pill bottles. Had he missed doses of his blood thinner or his vitamins? During our video visit, he showed me the empty bottle, and I was able to promptly identify the needed medication and arrange for home delivery.

But video was not the norm for my telehealth practice in 2020, for a simple reason: more often than not, it was problematic for my patients. Many of them had basic phone plans with restricted data for video calls. Others struggled to set up the video platform on their phone or to find a private space to speak openly with

REVIEW

Ex. 13

# Transgenderism and reproduction



Guy T'Sjoen[a,b], Eva Van Caenegem[a], and Katrien Wierckx[a]

**Purpose of review**
The development of new reproductive medicine techniques creates opportunities for preserving fertility in transgender persons. Before, losing fertility was accepted as the price to pay for transitioning.

**Recent findings**
The desire for children is present in many trans persons, as in the general population. Ethical concerns are sometimes raised against the preservation of fertility; however, the only unique aspect of this group is the gender transition of one of the parents. All other elements such as same sex parenthood, use of donor gametes, social stigma, etc., can be found in other groups of parents. Not all reproductive options for all trans persons are equal because not only the gametes are of importance, but also the sex of the (future) partner. In trans women, the best option to preserve gametes is cryopreservation of sperm by preference initiated before starting hormonal therapy. In trans men, donor sperm is most often used, but in theory, there are three options available to preserve fertility: oocyte banking, embryo banking and banking of ovarian tissue.

**Summary**
Fertility is possible for both trans men and women, but it requires timely cryopreservation of gametes or stopping cross-sex hormones and possible fertility treatments which are costly and may be unpleasant. Centers should elucidate their policy and inform trans persons on the possibilities and limitations.

**Keywords**
fertility, reproduction, transgender

## INTRODUCTION

Gender dysphoria is a condition that often needs treatment through state-of-the-art hormonal and surgical interventions [1–3]. Because of the effects of treatment on fertility, it seems like transitioning to the desired sex and reproduction is incompatible. It is often assumed that losing reproductive function is the price to pay for transition. Even if reproductive need and rights of men and women have been recognized for more than 50 years [4], there are still many medical experts, even those involved in transgender care, who remain skeptical regarding discussing possible procreation after sex reassignment. It has taken up to 2001 before the World Professional Association for Transgender Health's (WPATH's) Standards of Care contained a paragraph describing the need to discuss reproductive issues with transgender individuals before starting hormonal treatment [5]. Yet, the majority of female-to-male and male-to-female transsexual persons (trans men and trans women, respectively) are of reproductive age at the moment of transition [6] and have relationships following transition [7,8]. Consequently, as any other couple, they might desire to have children. The development of medically assisted reproduction as a field of medicine shows that the wish to have a genetically related child is recognized in general.

Also among fertility specialists, there is a discussion whether transsexual persons should be assisted in their need for parenthood in post-transition relationships [9,10]. Initially, this debate was held in relation to donor inseminations of female partners of trans men. In fact, the question underlying is if transsexual persons can be 'good' parents, without negative influence on the gender identity and the sexual orientation of the child [11,12]. This discussion reminds of the debate held many years ago regarding homosexual couples [13]. However, it is now clear that the debate no longer is whether transsexual persons need to be assisted, but how [14]. The general well-being of transsexual persons after sex reassignment surgery therapy has

[a]Department of Endocrinology and [b]Center for Sexology and Gender problems, Ghent University Hospital, Ghent, Belgium

Correspondence to Guy T'Sjoen, MD, PhD, Department of Endocrinology, 9K12 I.E., Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium. Tel: +32 9 332 21 37; e-mail: guy.tsjoen@ugent.be

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## KEY POINTS

- The World Professional Association for Transgender Health Standards of Care and the Clinical practice guidelines of the Endocrine Society clearly state that transsexual persons should be encouraged to consider fertility issues before starting cross-sex hormonal treatment.
- The majority of transsexual men and women are of reproductive age at the moment of transition and have relationships following transition.
- Reproductive options for all trans persons are not equal because not only the gametes are of importance, but also the sex of the (future) partner.
- In trans men, use of donor sperm is most common, but in theory, there are three options available to preserve fertility: oocyte banking, embryo banking and banking of ovarian tissue
- In trans women, sperm cryopreservation is advised before starting hormonal therapy.

been documented [15,16] and many have normal relationships with children from earlier relationships or with their current partners.

A large group of trans men and women state that fertility is important and that loss of fertility as a result of sex reassignment surgery is problematic. A questionnaire in 101 trans women [12] showed that 77% opinioned that the possibility to freeze sperm had to be discussed and offered by the professionals treating them. A small majority (51%) would have cryopreserved sperm, or at least seriously considered this, if it had been proposed. A minority of the respondents was concerned about the possible risk of genetic transfer of transsexuality to their children, or found the idea to preserve sperm in conflict with their female gender identity. Many trans women regretted not being able to become pregnant themselves. In a small study performed in collaboration by two centers, only a minority of trans women chose sperm cryopreservation when systematically counseled [17▪].

A similar questionnaire in 50 trans men showed that more than half had a desire to have children, and 37.5% would have cryopreserved oocytes if it had been possible [18▪▪].

## TRANSSEXUAL PERSONS AND THEIR CHILDREN

Before we discuss the practical problems that occur when wanting to fulfill the wish for a child after sexual reassignment surgery, it is worthwhile to review the literature concerning relations of trans persons and their children from earlier relationships. Not many studies have been performed; but from all available information, it seems that transsexuality of one of the parents does not have a negative influence on the psychosexual or gender identity development of the children. Children will suffer from a (problematic) divorce, but this is not different from the consequences of divorce for other reasons [19–22]. Green [19,20] described 34 children who kept contact with their transsexual parent after transition and did not find any clinical significant cross-gender behavior in these children. Even if some children were harassed at school or outside because of having a trans parent, this always remained mild and transient and could be resolved. All children had a reasonable insight in the process of sex reassignment of the parent and the treatment. Even if children may encounter difficulties as a consequence of the family setting, that does not automatically mean that reproduction in this family setting is ethically unacceptable [23]. One should look at the situations that are similar in some aspects. The only unique aspect of this group is the gender transition of one of the parents. All other elements, such as same sex parenthood, use of donor gametes, and social stigma, can also be found in other groups of parents.

White and Ettner [21] conducted a study evaluating the experiences of therapists who worked with trans persons with children and found that children could better adjust to the sex reassignment of the parent if they were younger and if there were less familial conflicts. In 2007, these authors confirmed their results in a group of 55 children [22]. In fact, these results are similar to those of studies with children of lesbian couples [24,25]. Also in these children, it was shown that psychosexual and gender identity development are not different than those of other children. The only problems that sometimes occur are related to the nonacceptance of the homosexuality of the parents and experience of discrimination. Because of all these reasons, Green [19,20] pleas against interrupting contact between the trans parent and his or her children. Unfortunately, this is not the point of view of many courts of justice in the Western world. In many countries, transsexuality will be seen as a sufficient reason to interrupt contact between trans persons and their children. Often this occurs on the initiative of the ex-partner, and this is harmful for the children.

## REPRODUCTIVE OPTIONS FOR TRANS WOMEN

Even if a majority of trans women will have relationships with men after transition, there are quite a

number in which this is not the case. This illustrates the known fact that sexual orientation and gender identity are different entities [26,27]. We can deduct that not all reproductive options for all trans persons are equal because not only the gametes are of importance, but also the sex of the (future) partner.

In trans women, feminizing hormonal therapy will lead to hypospermatogenesis and eventually azoospermia [28,29]. The azoospermia will become irreversible after some time. Moreover, surgical reassignment with removal of testicles will of course lead to irreversible sterility. The best option to preserve gametes is cryopreservation of sperm, by preference initiated before starting hormonal therapy. These sperm samples may be used at a later point in time to inseminate a female partner if quality is good, or can be used for in-vitro fertilization (IVF) or intracytoplasmatic sperm injection (ICSI) if quality is poorer. In theory, even a testicular biopsy can be preserved, as little spermatocytes are needed to attain fertilization and pregnancy through ICSI. In case, there is a (future) male partner, the situation is the same as for male homosexual couples, and there will be a need for treatment through an oocyte donor and surrogate mother. The possibility to become pregnant herself is to date impossible for trans women. Uterus transplantation is technically feasible [30,31], but the real chances of success in a human are unknown. Recently, one pregnancy after uterus transplantation has been attained in Turkey, however terminated in the 8th week. As immunosuppressive therapy is necessary that could be contra-indicated during a pregnancy [32], there remain medical concerns regarding uterus transplantations [33▪].

## REPRODUCTIVE OPTIONS FOR TRANS MEN

Trans men can have female partners and within these families a desire for children may become prominent. Currently, most of these couples will ask to be treated with the help of donor sperm insemination. In theory, this treatment does not differ in any way with the treatment of another heterosexual couple with untreatable male infertility. As stated earlier, there is some discussion in the literature concerning the ethical aspects of this treatment [11,12], but experience learns that children growing up in these families are not different in their psychosexual development as compared to other children. Unlike in many European countries, hysterectomy and oophorectomy are not necessary for legal sex reassignment in the USA. As a result, some trans men do not choose this surgery and are still able to give birth themselves. Thomas Beatie, a trans man from the USA, decided to become pregnant himself because of fertility problems of his wife. After interruption of testosterone treatment, he conceived thrice with donor sperm and gestated three pregnancies [34]. To date, no specific guidelines are available on this particular matter.

Regarding the possibility of using own gametes, there are clear differences between trans men and women. Masculinizing hormonal therapy in trans men will lead to irreversible amenorrhea; androgen therapy does not deplete primordial follicles nor affects the developmental capacity of the follicles [35]. Discussion is ongoing whether this leads to a situation that is physiologically comparable to polycystic ovarian syndrome (PCOS), but in any case, histologically hyperplasia of ovarian cortex and stroma is found [36–38]. Of course, ovariectomy will lead to irreversible ovarian failure. For preservation of reproductive possibilities, in theory, there are three options available: oocyte banking, embryo banking and banking of ovarian tissue. These options are in fact the same for women who underwent radiation or chemotherapy for malignancies [39] or more recently in women opting for 'social freezing' [40]. Cryopreservation of oocytes requires hormonal stimulation and oocyte retrieval, as for IVF treatment, after which the oocytes are vitrified. With oocyte vitrification, there are improving results on viability, fertilization and pregnancy [41]. The question remains whether trans men wish to undergo the process of hormonal stimulation and oocyte retrieval, but it could be an option if transition has just started. Freezing of embryos is also a theoretical possibility; but for this procedure, spermatocytes have to be available, coming from a male partner or from a sperm donor. Preservation of embryos is a routine activity in all fertility centers and has high efficiency. Most trans men, if they want to take steps toward preservation of fertility, will choose to cryopreserve ovarian tissue at the time of hysterectomy and ovariectomy, as this will not require an additional surgical procedure. Ovarian tissue can be cryopreserved successfully, but so far, there are no pregnancies described after thawing and in-vitro maturation (IVM) of this tissue. Only after autotransplantation, pregnancies have been described, but this is not an option for transsexual men [42]. The results of IVM of follicles and oocytes derived from ovarian tissue fragments, followed by IVF in humans are still unsatisfactory. Freezing of oocytes, embryos or ovarian tissue from transsexual men encompasses they will need a surrogate mother if they have a male partner or a sperm donor if they

have a female partner. In an ethical discussion, questions may arise if in a trans man with a female partner, oocytes should be frozen. Would this social indication be worth the medical investment? Such requests should be carefully discussed with the initiators and the fertility center should announce its policy.

Even if for the time being, there are several limitations in the possibility of a trans person with a desire for children in current or future relationships, it is possible that in the future different options will become available. Research to create gametes through stem cell technique is ongoing, in which from somatic cells like skin or muscle tissue through nuclear transplantation embryonal stem cells can be derived, which then are stimulated *in vitro* to differentiate to spermatocytes or oocytes. Even if for the moment, there have not been successful results in humans, research in animals is advanced [43,44]. It is evident that these techniques of reproduction would mean a revolution, for all men and women who do not possess gametes and who now require oocyte or sperm donation, and par excellence also for homosexual and transsexual persons.

## CONCLUSION

In conclusion, we can state that not all theoretical reproductive options for trans persons are as available, because a lot depends on the partner they have or will have in future relationships. It also has to be underlined that not all forms of medical-related reproduction are available everywhere, that these treatments are often expensive and not free from risks. Future use of cryopreserved gametes cannot be guaranteed and will depend on the quality of the gametes, success rate of the proposed technique, the center's policy and local legislature. However, professionals who assist trans persons have to consider the desire for children as a serious and important aspect of transition, and discuss all possible options. If it is decided that gametes will be cryopreserved, all future possibilities and limitations have to be discussed in full detail.

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### Conflicts of interest

*There are no conflicts of interest*

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