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ORIGINAL ARTICLE

Consensus Parameter: Research Methodologies to Evaluate Neurodevelopmental Effects of Pubertal Suppression in Transgender Youth

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Abstract

Purpose: Pubertal suppression is standard of care for early pubertal transgender youth to prevent the development of undesired and distressing secondary sex characteristics incongruent with gender identity. Preliminary evidence suggests pubertal suppression improves mental health functioning. Given the widespread changes in brain and cognition that occur during puberty, a critical question is whether this treatment impacts neurodevelopment.

Methods: A Delphi consensus procedure engaged 24 international experts in neurodevelopment, gender development, puberty/adolescence, neuroendocrinology, and statistics/psychometrics to identify priority research methodologies to address the empirical question: is pubertal suppression treatment associated with real-world neurocognitive sequelae? Recommended study approaches reaching 80% consensus were included in the consensus parameter.

Results: The Delphi procedure identified 160 initial expert recommendations, 44 of which ultimately achieved consensus. Consensus study design elements include the following: a minimum of three measurement time points, pubertal staging at baseline, statistical modeling of sex in analyses, use of analytic approaches that account for heterogeneity, and use of multiple comparison groups to minimize the limitations of any one group. Consensus study comparison groups include untreated transgender youth matched on pubertal stage, cisgender (i.e., gender congruent) youth matched on pubertal stage, and an independent sample from a large-scale youth development database. The consensus domains for assessment includes: mental health, executive function/cognitive control, and social awareness/functioning.

Conclusion: An international interdisciplinary team of experts achieved consensus around primary methods and domains for assessing neurodevelopmental effects (i.e., benefits and/or difficulties) of pubertal suppression treatment in transgender youth.

Keywords: expert consensus; Delphi; puberty blockers; GnRHa; transgender; adolescents

Introduction

Standards of care established by the World Professional Association for Transgender Health[1] and the Endocrine Society[2] recommend pubertal suppression for gender dysphoric transgender youth during early puberty (i.e., Tanner stages 2–3).[3,4] Pubertal suppression is achieved through administration of gonadotropin-releasing hormone agonists (GnRHa). When administered in early puberty, GnRHa suppress endogenous sex hormone production and prevent the development of undesired and irreversible secondary sex characteristics, thereby minimizing distress associated with pubertal development incongruent with gender identity.[5] For youth who later decide to initiate estrogen/testosterone (gender-affirming hormones [GAH]) treatment to induce development of the desired secondary sex characteristics, pubertal suppression may minimize the need for more invasive, surgical interventions (e.g., facial and chest surgery). For youth who decide not to pursue GAH treatment, discontinuing GnRHa will reactivate the hypothalamic-pituitary-gonadal axis and endogenous puberty will resume.[6]

Three longitudinal studies have examined psychosocial outcomes in GnRHa-treated transgender youth; two (conducted by the same research group) followed a single cohort over time, immediately before initiating GAH ($N=70$)[7] and later in early adulthood after surgery for gender affirmation ($N=55$).[8] The third study compared groups of GnRHa-treated ($n=35$) and untreated ($n=36$) youth longitudinally.[9] Findings across these studies include significant reductions in depressive symptoms and improvement in overall psychosocial functioning in GnRHa-treated transgender youth. A fourth cross-sectional study compared adolescents diagnosed with gender dysphoria (GD), who were treated with GnRHa and close to starting GAH treatment ($n=178$), adolescents newly referred for GD evaluation ($n=272$), and cisgender adolescents recruited from the general population ($n=651$) on self-reported internalizing/externalizing problems, self-harm/suicidality, and peer relationships.[10] Before medical treatment, clinic-referred adolescents reported more internalizing problems and self-harm/suicidality and poorer peer relationships compared to age-equivalent peers. GnRHa-treated transgender adolescents had fewer emotional and behavioral problems than clinic-referred, untreated adolescents and had comparable or better psychosocial functioning than same-age

cisgender peers. In addition to studies of youth, the 2015 U.S. Transgender Survey included questions about past gender-affirming medical treatment, including pubertal suppression. These questions were asked retrospectively and linked to reported current and lifetime mental health.[11] Individuals who received pubertal suppression treatment ($n = 89$), when compared to those who wanted pubertal suppression, but did not receive it ($n = 3405$), had lower odds of endorsing lifetime suicidal ideation on the survey. Given these five studies and the presumed reversibility of GnRHa treatment, pubertal suppression is increasingly offered to early pubertal transgender youth. It is important to note that there has been only one longitudinal report of adult outcomes,[8] and questions remain regarding the potential for both positive *and* disruptive effects of pubertal suppression on neurodevelopment.[12–14]

The pubertal and adolescent period is associated with profound neurodevelopment, including trajectories of increasing capacities for abstraction and logical thinking,[15] integrative thinking (e.g., consideration of multiple perspectives),[16,17] and social thinking and competence.[18,19] During this period, there is a developmental shift toward greater exploration and novelty seeking,[20,21] salience of peer perspectives and interactions,[22] and accelerated development of passions/interests and identities.[23] These developments lay the groundwork for adult functioning.[18,24] At the level of the brain, several primary neurodevelopmental processes unfold during adolescence, including myelin development[25] and changes in neural connectivity[26]; synaptic pruning[27] and gray matter maturation[28,29]; changes in functional connectivity[30]; and maturation of the prefrontal cortex[31] and the "social brain" network.[19] Adolescent neurodevelopmental processes underlie mental health risks, resilience, and outcomes.[32,33]

Considerable research has addressed the effects of puberty-related hormones on neurodevelopment, including hormone manipulation studies in nonhuman animals and observational studies in humans. Animal studies demonstrate pubertal hormones exert broad neuronal influence, including effects on neurogenesis, differentiation, apoptosis, dendritic branching, spine density, and regional gray and white matter volumes.[30,34] Androgen and estrogen receptors are found in high density within the hypothalamus and amygdala, and are also present in the hippocampus, midbrain, cerebellum, and cerebral cortex of the rodent and monkey.[35–37] This widespread receptor distribution in rodents may explain the diverse effects of pubertal hormones on both reproductive and nonreproductive behaviors, including anxiety, scent-marking, and food guarding.[34] In human studies, pubertal progression has been linked to developmental changes in reward,[38] social,[39] and emotional processing[40] as well as cognitive/emotional control.[41] However, consensus regarding pubertal impacts at the neural level—such as puberty-associated changes observed in magnetic resonance imaging (MRI) measures—has been more difficult to achieve.[42] Distinct puberty-related neurodevelopmental trajectories have been differentiated by sex.[43]

The combination of animal neurobehavioral research and human behavior studies supports the notion that puberty may be a *sensitive* period for brain organization:[44–46] that is, a limited phase when developing neural connections are uniquely shaped by hormonal and experiential factors, with potentially lifelong consequences for cognitive and emotional health. Studies have linked early life adversity to early puberty onset[47] and early puberty onset to poorer mental health.[48] There is also some evidence to suggest that delayed puberty onset predicts slightly poorer adult functional outcomes.[49] Taken as a whole, the existing knowledge about puberty and the brain raises the possibility that suppressing sex hormone production during this period could alter neurodevelopment in complex ways—not all of which may be beneficial.

Two small studies have assessed impacts of pubertal suppression on neural and cognitive functioning in peripubertal transgender youth. Staphorsius et al. compared brain and behavioral responses of GnRHa-treated (8 transgender girls [birth-assigned male] and 12 transgender boys [birth-assigned female]) and untreated transgender youth (10 of each sex) during an executive function task.[50] No group differences were found in task load-related brain activation; GnRHa-treated transgender girls demonstrated poorer performance compared with untreated transgender boys and cisgender controls. Schneider et al. evaluated a single pubertal transgender girl undergoing GnRHa with MRI scans of white matter and cognitive assessments at baseline (before GnRHa initiation) and at 22 and 28 months of pubertal suppression treatment.[51] During follow-up, white matter fractional anisotropy (i.e., a measure of axonal diameter, fiber coherence, and myelination) did not increase in the manner otherwise expected during puberty. By 22 months of pubertal suppression treatment, working memory scores dropped by more than half a standard deviation.

Larger-scale, longitudinal studies are required to understand possible neurodevelopmental impacts of pubertal suppression over time in transgender youth. Suppressing puberty may reduce dysphoria and diminish risks for poor mental health in this population, thereby exerting neuroprotective effects. If pubertal suppression disrupts aspects of neurodevelopment, it is possible these effects are temporary, with youth "catching up" developmentally after transitioning to GAH treatment or discontinuing GnRHa. However, pubertal suppression may prevent key aspects of development during a sensitive period of brain organization. Neurodevelopmental impacts might emerge over time, akin to the "late effects" cognitive findings associated with certain oncology treatments.[52] In sum, GnRHa treatment might produce a myriad of *varied* impacts, both positive and disruptive.

The goal of this study was to develop a framework in which these questions could be asked, and ultimately answered. We identify priority research methodologies that can be used to address the empirical question of how pubertal suppression in transgender youth may affect neurodevelopment and real-world functioning. Given the complexity of neural development during the pubertal period and the novelty of developmental research with transgender youth, this study employed a Delphi consensus method to leverage international expertise in neurodevelopment, gender development, puberty/adolescence, neuroendocrinology, and statistics/psychometrics. By engaging a community of experts in an iterative consensus-building procedure, this study aimed to advance thinking about efficacious designs by moving beyond individual research efforts and single-discipline approaches.

Methods

The Delphi procedure is a reliable iterative research method for establishing expert agreement,[53,54] and has been used extensively to address health-related questions, particularly in emerging fields of clinical care.[55–57] In the first round of a two-round Delphi procedure, a key question is presented to experts, who remain anonymous to one another throughout the Delphi process. Each expert provides responses/solutions to the question, which are then combined and organized by the study team. In the Delphi round two, experts rate each proposed statement/solution according to the level of agreement. Responses reaching the *a priori* consensus criterion are included as consensus statements. Given its anonymous iterative nature, the Delphi method avoids problems of typical expert work groups (e.g., adhering to the perspectives of more senior workgroup experts, inflexibly defending ideas) and allows for interaction among larger groups of experts from diverse locations and disciplines through asynchronous communication.[58–60]

We employed a two-round Delphi procedure to obtain expert consensus regarding the most efficacious research design elements to address the following research question: *What, if any, real-world impact does pubertal suppression have on transgender children's cognitive and neural development?* International experts in relevant research fields were identified and invited as follows:

1. An independent advisory panel consisting of five experts across key disciplines (see Acknowledgments section) was formed to identify international experts who, based on knowledge and experience, could best propose a research design to assess neurodevelopmental impacts of pubertal suppression in transgender youth.
2. Thirty-two recommended experts were vetted for their expertise; all met required criteria (i.e., a minimum of 10 first-author publications in relevant fields).
3. These experts were invited to participate in the Delphi procedure and were informed they would be invited to consider being a co-author of the resulting article. Twenty-eight experts responded: 20 agreed to participate, 4 declined due to lack of time, and 4 declined due to self-reported lack of expertise in this research area. Snowball sampling identified an additional 16 recommended experts, who were vetted (as described above) for their experience. Eight met criteria and were invited. Five of these experts participated, yielding a total of 25 experts agreeing to participate, 24 of whom completed the Delphi process. See Table 1 for academic institution locations and areas of expertise represented in the expert panel.

The Ann & Robert H. Lurie Children's Hospital of Chicago Institutional Review Board found that an expert Delphi consensus initiative did not require informed consent since the experts were direct partners in the research product. The first round of Delphi survey was distributed through the REDCap online survey platform and presented an overview of the research question with the following prompt for

Table 1. Institutional Representation and Self-Reported Areas of Expertise

	n
Location of academic institution	
United States	16
The Netherlands	3
Belgium	2
Canada	1
Norway	1
Sweden	1
Self-endorsed areas of expertise[a]	
Brain development	13
Adolescent development	12
Neuroendocrinology	11
Neuroimaging	11
Neuropsychology	8
Cognitive development	7
Developmental assessment	4
Expert in GnRHa	2
Other (write in)	4
Developmental social neuroscience	1
Transgender health	1
Genetics of sex chromosomes	1
Gender development	1

[a]Experts endorsed as many areas of expertise as applicable.
GnRHa, gonadotropin-releasing hormone agonists.

respondents: "What methods and tools should we use to identify clinically meaningful neurodevelopmental impacts of pubertal suppression? What type of longitudinal design and follow-ups are both practical and appropriate? What comparison groups might we consider?" This initial process yielded 131 distinct research design considerations; multiple descriptions of the same concept were collapsed into single statements. In the second Delphi round, each first-round research design consideration was presented back to the experts and rated as follows: a priority idea/approach or not a priority idea/approach. Experts could also select, "cannot rate due to lack of expertise." The first Delphi round also yielded lists of potential comparison groups and assessment domains (29 items). In the second Delphi round, participants were asked to rank order these items according to priority. For the priority rankings of comparison groups, the top-rated comparison group by each expert was given a value of 2 and the second rated comparison group was given a value of 1. A mean was calculated for each comparison group option based on these values and these mean scores were used to identify the overall priority rankings. For the list of priority domains to measure, a parallel approach was taken with the top 6 domains ranked by each expert.

All experts participated in the second Delphi round. Twenty-two of the Delphi experts participated in the construction of the resulting article and are co-authors listed in reverse alphabetical order by last name (authors 5–26). The Results section contains the exact statements endorsed as a "priority" approach by 80% or more of the Delphi panel.

Results

Four of the 131 individually presented statements were excluded from analyses because fewer than 15 experts rated them. Of the remaining 127 statements, 44 met the 80% or higher criterion for consensus and inclusion (see Table 2 for endorsement rates by statement). The average endorsement rate of included statements was 89.4%.

Consensus parameter

Study design considerations. A multicenter design with more than a single clinic will be necessary to recruit a sufficient sample size, as the effect size will likely be small. Meaningful effect sizes must be determined to ensure sufficient recruitment to power multiple expected comparisons accounting for attrition in a longitudinal design. Three time points of measurement are the absolute minimum. It will be necessary to manage the effects of repeated testing with a particular focus on minimizing the practice effects of a longitudinal design with multiple time points. For cognitive assessments, standardized batteries should be employed as: (1) there may be a larger database of norms available that the cohort could be compared to, in addition to a local comparison (control) group(s), (2) general composite scores within test batteries tend to provide more reliable and stable scores than individual tests, and (3) tasks within a category may be swapped in case of worries for learning effects. In any study of cognitive change based on serial assessments, reliability of measures is paramount (the consensus in the field is that tests should have a minimum test-retest reliability of >0.70). It may be pragmatic to use measures and methods from large representative studies, such as the Adolescent Brain Cognitive Development (ABCD) Study.

All processes being studied (e.g., gender identity, mental health, neural structure, and function) display considerable heterogeneity, and methods that fail to capture this will provide distorted findings and lead to biased clinical recommendations. Analyses based on group means (e.g., regression or ANOVAs) are unlikely to generalize to all individuals being treated. Therefore, it is necessary to collect enough data per person to characterize individual trajectories of change over time.

Table 2. Consensus Priority Recommendations Ordered by Consensus Ratings Within Categories

Study design considerations		
1	It would be helpful to follow these youth through and beyond initiation of cross-sex hormone treatment. Some aspects of human adolescent brain development are more related to pubertal hormone status than age *per se*, and to the extent that pubertal suppression may also put some features of brain development on hold; it would be good to know whether these features "catch up" once cross-sex hormone treatment has begun or whether a sensitive window for hormone-dependent brain development has closed.	22/22
2	Follow cohort after GnRHa treatment ends—collect data after the youth transition to GAH (when they complete their GnRHa treatment).	22/23
3	Any neurocognitive effect of GnRHa pubertal suppression may be complicated by the psychosocial and affective aspects of the transgender experience. This means that you would have to include multivariate models of both cognitive and psychosocial functioning.	22/23
4	Need to determine meaningful effect sizes and ensure sufficient statistical power for multiple expected comparisons with attrition.	21/22
5	Across the course of the study, three assessment points is the absolute minimum.	20/21
6	Need to use a multicenter design (not just one clinic).	21/23
7	Effects of GnRHa may not appear for several years. Any difference in brain structure due to GnRHa is likely to be seen over time (long term), rather than immediately.	20/22
8	Social and affective learning process may be affected by pausing puberty. These social and affective learning processes might cause subtle short-term differences that could ultimately cause clinically impactful and meaningful longer-term effects.	17/19
9	Of particular interest would be to also monitor the impact of hormonal therapy. One could then ask, "Does the trajectory change in response to cross-sex hormonal therapy or do they stay on the same trajectory as when they were on GnRHa?"	16/18
10	Assess target and comparison groups before puberty.	20/23
11	Need to manage the effects of repeated testing (i.e., minimize the practice effect of a longitudinal design with multiple time points).	19/22
12	The effect size will likely be small—therefore, you would need a large sample size.	19/23
13	The research design will need to account for the differences between youth who are assumed male versus assumed female as biological sex is differentially related to rate and pattern of cognitive development, connectome distinctiveness, and timing of peak brain volume.	19/23
14	All processes being studied (e.g., gender identity, mental health, and neural structure and function) display huge amounts of heterogeneity, and research methods that fail to capture this will provide distorted findings and lead to biased clinical recommendations. Analyses based on mean levels of these processes are unlikely to generalize to all individuals being treated (e.g., regressions or ANOVAs that compare groups with a slew of covariates). It is, therefore, necessary that enough data are collected per person to capture personalized trajectories of change across time. And the data need to be modeled in ways that reflect the heterogeneity of individual characteristics and trajectories.	18/22
Comparison groups and recruitment		
15	At least one control group should be cisgender participants as this area of research (i.e., hormones and the adolescent brain) is still rather new and more data are needed on all youth during this stage.	20/22
16	Critical to match the groups carefully to allow for evaluation of the effects of repeated testing (practice effects).	20/22
17	Comparison groups should be matched for pubertal stage.	19/21
18	Recruit all gender dysphoric youth across the pubertal age range, including those who are treated with GnRHa and those who are not.	18/21
19	This is not dissimilar from issues of discerning differences in cognitive trajectories in normal aging versus neurodegenerative disorders. The basic question involves cognitive growth curves among cisgender and transgender children overtime. There have been large-scale large-sample studies that have produced trajectories of brain development during the pre-pubertal, pubertal, and adolescent periods that could treated like a "brain growth curve."	15/18
20	Need more than one comparison group to minimize the limitations of any one comparison group (no single comparison group is ideal).	18/22
Pubertal staging/measurement		
21	Measure gonadal hormone levels.	23/23
22	Collect information on menstrual cycle and contraceptive use for female adolescents involved in the study.	23/23
23	Measure Tanner staging (i.e., secondary sex characteristics).	21/23
24	Measure height/weight.	18/22
Domains to measure		
25	Use white matter microstructure scans (diffusion tensor imaging)—and use a longitudinal imaging pipeline (which exists) for processing these data with scientific rigor.	15/15
26	A pragmatic methodological implication is to consider: (1) not only relying solely on measures of performance and behavior but also measures of learning and motivation, and (2) not only relying solely on measures of cognitive capacities but also on social, affective, and value-based learning processes.	19/20
27	If MRI is included, consider imaging approaches focused on the following domains: social-emotional processing, executive functioning, risk and reward processing, and self-concept.	20/22
28	Studies in laboratory rodents show that testosterone, acting during puberty, programs the ability to adapt behavior as a function of social experience—therefore, include instruments that evaluate social proficiency.	19/21
29	Use diffusion tensor imaging to analyze white matter at the microstructural level.	17/19

(*continued*)

Table 2. (Continued)

Study design considerations		
30	Studies in laboratory rodents show that ovarian hormones, acting during puberty, program cognitive flexibility by exerting long-lasting effects on excitatory-inhibitory balance in prefrontal cortex—so include instruments that evaluate behavioral flexibility.	18/21
31	Examine white matter development, which is important for processing speed.	17/20
32	Important to measure emotional functioning because it is bidirectionally related to executive functioning.	16/19
33	Look at white matter characteristics since they seem to develop during puberty under the influence of sex hormones.	15/18
34	One cannot study everything or study everything well. It will be critical to identify the priorities in such a study, as there is a danger of doing too much here. Consider the outcomes that matter most and the hypothesized mediating mechanisms. Focus on the outcomes of interest.	19/23
35	There is no clear evidence that progressing through puberty later than peers is associated with delayed maturation of abstract reasoning, executive function, and social capacities.	18/22
36	Use structural MRI (T1/T2)—and use a longitudinal imaging pipeline (which exists)—for processing these data with scientific rigor.	13/16
37	There is an emerging shift in thinking about the increase in reward sensitivity and sensation-seeking during puberty as related to social value learning. Dopamine release is not primarily a "reward" signal, but rather a learning signal (e.g., prediction error signal)—the natural increased salience of social learning (status, prestige, being admired, respected, liked, etc.) These pubertal changes may have small effects on immediate behavior, yet that could contribute to changes in patterns of behavior over time, which could lead to large individual differences in developmental trajectories for people, such as if they had blocked puberty.	13/16
Measurement approaches		
38	In any study of cognitive change based on serial assessments, reliability of the measure is paramount. The consensus in the field is that tests should have a minimum test-retest reliability of >0.70.	20/20
39	Behavioral measurements should include standardized measures appropriate for repeated assessment with high test-retest reliability.	21/22
40	Match acquisition parameters between imaging sites.	17/18
41	Consider implementing measures and methods from large representative protocols, such as the ABCD.	17/18
42	Neuroimaging should parallel the behavioral study—neural measures should be linked to neurocognitive and behavioral measures.	19/22
43	For cognitive assessment, use a standardized battery for two reasons: (1) there might be a larger database of norms available that the cohort could be compared to, in addition to the likely to be small comparison ("control") group, and (2) tasks within a category may be swapped in case of worries for learning effects.	18/21
44	Use "test batteries" that provide a general composite score as well as specific composites. By virtue of being composites, scores tend to be more reliable and stable than individual test scores.	17/20

The proportion represents the number of experts endorsing an item as a "priority" out of the total number of experts who rated the item as "priority" or "not priority." The denominator represents the number of experts rating an item as a "priority" or "not priority" (as opposed to "cannot rate due to lack of expertise" or skipping the item).

ABCD, Adolescent Brain Cognitive Development Study; GAH, gender-affirming hormones; MRI, magnetic resonance imaging.

Any GnRHa-induced neurocognitive effect may be complicated by psychosocial and affective aspects of the transgender experience. Therefore, multivariate models of both cognitive and psychosocial functioning should be included. Accounting for differences between birth-assigned male youth versus birth-assigned female youth is important, as sex is differentially related to the rate and pattern of cognitive development, connectome distinctiveness, and timing of peak brain volume. Assessments should begin before puberty in both treatment and comparison groups. The effects of pubertal suppression may not appear for several years. Any GnRHa-related difference in brain structure is likely to be observed over the long term, rather than immediately. Shifts in social and affective learning processes might cause subtle short-term differences that could ultimately result in clinically impactful longer-term effects. Therefore, studies should follow GnRHa-treated youth over time, including the time period after GnRHa treatment ends and/or when GAH commence. Some aspects of human adolescent brain development are more related to pubertal hormone status than age *per se*. To the extent that pubertal suppression may also put some features of brain development on hold, it is critical to know whether these features "catch up" (either once GAH treatment is initiated or if the adolescent elects to stop GnRHa and resume endogenous puberty), or whether a sensitive window for hormone-dependent brain development has closed. One way to measure this is to assess whether neurodevelopment shifts in response to initiating GAH following pubertal suppression: Do GnRHa-treated youth stay on the same neurodevelopmental trajectory as when puberty was suspended or does this trajectory change?

Comparison groups. To assess neurodevelopmental trajectories associated with GnRHa treatment, more than one comparison group is needed to minimize the limitations of any one comparison group. No single comparison group is ideal for this study question.

A rank order of possible comparison groups is provided in Table 3. Groups should also be well matched, given the effects of a repeated testing design (e.g., practice effects). Matching for pubertal/developmental stage will be critical, including Tanner staging, gonadal hormone levels, height and weight, and, among youth assigned female at birth, menstrual cycle and contraceptive use. A primary comparison should be between GnRHa-treated transgender youth and untreated transgender youth, but it will also be important to include comparisons with cisgender samples as research on hormones and the adolescent brain is still novel and emerging and more data are needed on all youth during this developmental period. One way to accomplish the latter is to employ existing large-scale databases from studies of brain development during the pre-pubertal, pubertal, and later-adolescent periods, treating them as brain growth curves for comparisons. This approach is similar to the differentiation of cognitive trajectories in normal aging versus neurodegenerative disorders. The basic research question involves comparing cognitive growth curves over time.

Domains to assess. It will be critical to prioritize assessment domains based on hypothesized mediating mechanisms, with the most important domains to measure as follows: mental/behavioral health, pubertal stage, executive function/control, gender identity/dysphoria, and social awareness/functioning. See Table 4 for a complete list of ranked domains. Although we (the Delphi experts) identify executive function/control and social functioning as key domains to measure, it is important to note that there is no clear evidence that progressing through puberty later than peers is associated with delayed maturation of abstract reasoning, executive function, and social capacities. Executive function and emotional functioning are bidirectionally related, and for this reason, the two should be integrated in models/analyses. In addition, cognitive/behavioral flexibility, a component of executive functioning, should be measured, given that studies in rodents show ovarian hormones, acting during puberty, program cognitive flexibility by exerting long-lasting effects on excitatory-inhibitory balance in the prefrontal cortex.[61] Studies in rodents also demonstrate that testosterone, acting during puberty, programs the ability to adapt behavior as a function of social experience.[34] Measurement approaches should extend beyond cognitive capacities alone, embedding social, affective, and value-based learning processes. There is an emerging shift in thinking about increases in reward sensitivity

Table 3. Rank Order of Priority Comparison Groups

Rank order of priority	Comparison group
1	Transgender youth who do not take GnRHa matched on pubertal status at the beginning of the study
2	Cisgender typically developing adolescents matched on pubertal status at the beginning of the study
3	Use a standardized battery and/or a large existing database of norms to compare to (in addition to a smaller comparison group)
4	Transgender youth who commence GnRHa treatment earlier compared to later in puberty
5	Siblings of transgender youth enrolling in the study (to serve as genetic and shared environmental controls)
6	Mixed clinical group of adolescents presenting for MH assessment/treatment in an outpatient setting matched on pubertal status
7[a]	Peers with mood disorders (to control for the overoccurrence of mental health distress in transgender youth) matched on pubertal status
7[a]	Youth with precocious puberty who are given GnRHa to delay puberty

This priority sequence was based on participants' top 2 ranked comparison groups, where the top rated comparison group was given a value of 2 and the second rated comparison group was given a value of 1. A mean score was derived for each comparison group based on participants' ratings and ordered from highest to lowest.

[a]Comparison groups received the same mean score in the ranking.

Table 4. Rank Order of Priority Domains of Characterization and Assessment

Rank order of priority	Domains of characterization and assessment
1	Mental/behavioral health (including suicidality/hopelessness)
2	Pubertal stage/development (Tanner staging/hormone levels)
3	Executive function/control and attention
4	Gender identity/dysphoria
5	Social awareness/functioning
6	Quality of life
7	Brain/functional connectivity
8	Brain structure/volume
9	Emotional awareness/functioning
10	Physical health symptoms and outcomes (especially in adulthood)
11	Adaptive/independence skills
12	General cognitive functioning (IQ)
13	Sensation seeking, risk taking, reward sensitivity, and motivation
14	Genetics (i.e., possible impacts of GnRHa on DNA and RNA expression)
15	Academic functioning
16	Processing speed
17	Memory systems

This priority sequence was based on participants' top 6 ranked domains to measure, where the top rated domain was given a value of 6 and the second rated comparison group was given a value of 5, and so on. A mean score was derived for each domain based on participants' ratings and ordered from highest to lowest.

and sensation-seeking during puberty as related to social-value learning.[18] Dopamine release is not primarily a "reward" signal, but rather a learning signal (e.g., prediction error signal)—the natural increased salience of social learning (e.g., status and prestige, being admired, respected, and liked). The effects of suspending puberty on the salience of social-value learning might produce small near-term effects, but could contribute to changes in patterns of behavior over time, leading to large individual differences in developmental trajectories for GnRHa-treated youth.

If neuroimaging is included, imaging approaches should focus on the following domains: social/emotional processing, executive functioning, risk and reward processing, and self-concept. Neuroimaging should parallel behavioral assessment. Neural measures should be linked to neurocognitive and behavioral measures. Acquisition parameters should be matched between imaging sites. Investigation of white matter development is important as myelination progresses during puberty, likely under the influence of sex hormones,[62] and is related to cognitive processing speed. Both structural MRI and diffusion tensor imaging approaches should be used for white matter imaging and analyzed using a longitudinal imaging pipeline for processing these data with scientific rigor.

Discussion

Puberty suppression has become an increasingly available option for transgender youth, and its benefits have been noted, particularly in the area of mental health. However, puberty is a major developmental process and the full consequences (both beneficial and adverse) of suppressing endogenous puberty are not yet understood. The experts who participated in this procedure believe the effects of pubertal suppression warrant further study, and this Delphi consensus process develops a framework from which future research endeavors can be built.

Expert consensus emphasized a minimum of three measurement time points, inclusion of multiple comparison groups to minimize the limitations of any one group, precision pubertal staging at baseline, accounting for sex in design and analysis, and the use of designs that capture heterogeneity in processes being studied. Focus on longer-term trajectories and outcomes was emphasized, given that effects of pubertal suppression on various processes may not be evident in the near term, and responses to delayed receipt of gonadal hormones may not be comparable to initial potentially organizing effects. Experts also highlighted that accounting for the psychosocial aspects of the transgender experience itself on development will require models that integrate both cognitive and psychosocial functioning. The highest endorsed measurement priorities were mental and behavioral health, executive function/cognitive control, and social awareness/functioning. The importance of interrelations between domains that mature during puberty/adolescence was also emphasized, including bidirectional relationships between cognitive and emotional control and links between reward sensitivity and social value learning. Regarding neuroimaging, experts stressed the importance of linking neural signatures to cognitive and behavioral measures, with attention to white matter development. Notably, while there was consensus in this approach to neuroimaging, there were divergent views as to whether a neuroimaging protocol should be prioritized in a study with limited resources. Some experts noted that insufficient work has been done on neural development during puberty in general and expending resources on an expensive neuroimaging protocol for this subset of youth may be premature, while others felt that defining underlying brain mechanisms by neuroimaging was important. Furthermore, at the final review of the article, four co-authors noted a concern with this specific Delphi consensus recommendation: "Accounting for differences between birth-assigned male youth versus birth-assigned female youth is important, as sex is differentially related to the rate and pattern of cognitive development, connectome distinctiveness, and timing of peak brain volume." The four authors felt that instead of "peak brain volume," a more appropriate measurement concept might be that of "structural brain metrics" (e.g., thickness and regional volumes).

Twelve different comparison groups were proposed in the first round of the Delphi and 8 of the 12 groups were rated as either first or second priority by at least 1 expert in the second Delphi round. This heterogeneity underscores the complexity of selecting comparison groups for this research and lends support to the experts' recommendation to engage more than one comparison group. The highest rated comparison groups were untreated transgender youth matched on pubertal stage, cisgender youth matched on pubertal stage, and a sample from a large-scale quasi-normative database (e.g., from the ABCD study) used as a "brain growth curve." These comparison groups are not without weaknesses. Untreated transgender youth may differ in their

intensity or experience of GD, level of parent support (e.g., are the parents against GnRHa treatment?), and socioeconomic status of the family and access to treatment (e.g., insurance coverage). A cisgender comparison group would lack gender-minority experience and associated stress.

Some statements approached, but did not reach consensus. For example, many experts suggested continuing assessments of transgender youth through young adulthood (mid-20s) when prefrontal development is near completion. Assessing adaptive functioning (everyday skills) over time due to the bidirectional link between executive functioning and adaptive behaviors was also often endorsed.

Not all relevant study considerations were raised by the Delphi panel. Neurodevelopmental impacts of pubertal suppression in transgender youth with neurodevelopmental differences/diagnoses (e.g., attention deficit/hyperactivity disorder and autism spectrum disorder) were not specifically addressed by the experts. Yet, evidence suggests an overoccurrence of neurodiversity characteristics (especially related to autism) among gender-referred youth.[55,63–66] The neurodevelopmental impacts of pubertal suppression on neurodiverse gender-diverse youth might well be different than in neurotypical gender-diverse youth, given variations in neurodevelopmental trajectories observed across neurodevelopmental conditions.[67–69]

This study included experts from a range of relevant disciplines—a strength and also a possible limitation. The varied disciplines allowed for a broader range of ideas and perspectives, but some specialized recommendations might not have been sufficiently understood by Delphi experts from other disciplines. It is possible that some useful recommendations were lost in the process because few experts had backgrounds relevant to them. In fact, four recommendations were dropped from consideration because more than nine experts indicated they could not rate the item or skipped the item. These four items included topics related to advanced growth curve modeling, impact of GnRHa on immune system functioning, multifactorial relationships between GD and neurodevelopment, and challenges associated with using alternative forms of measures in longitudinal designs. The Delphi team included experts across the fields of neuroscience, neurodevelopment, developmental measurement, and gender development; however, most were not specialized in clinical transgender care *per se*. This reflects the dearth of transgender care clinicians/specialists with research productivity in adolescent neurodevelopment. Thus, the experts could comment with authority on neurodevelopment, including gender development/dysphoria aspects of study design, but the real-world clinical care considerations may well be underdeveloped in the proposed research design. For example, the everyday lived experience of transgender youth seeking gender-affirming medical care would be unfamiliar to most neurodevelopmental researchers. After the Delphi procedure was completed, one panelist commented that pubertal hormones might play a role in organizing neurodevelopmental gender-related trajectories, including identity itself, which would be important to consider for a developmental study of gender diverse youth.

Despite these limitations, an international expert team successfully completed an iterative Delphi procedure achieving consensus around priority research design elements to study neurodevelopmental impacts of pubertal suppression in transgender youth. The resulting consensus parameter addresses broad design issues, including comparison groups, longitudinal design, neurodevelopmental targets for assessment, and measurement approaches. While it may not be possible to incorporate all consensus methodologies into a single study, this parameter may serve as a roadmap for a range of research initiatives investigating pubertal suppression treatment in transgender youth.

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Abbreviations Used

ABCD = Adolescent Brain Cognitive Development
GAH = gender-affirming hormones
GD = gender dysphoria
GnRHa = gonadotropin-releasing hormone agonists
MRI = magnetic resonance imaging

The Cass Review

Independent review of gender identity services for children and young people: Interim report

February 2022

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February 2022

Contents

About this report

This interim report represents the work of the independent review of gender identity services for children and young people to date. It reflects a point in time. It does not set out final recommendations; these will be developed over the coming months, informed by our formal research programme.

This Review is forward looking. Its role is to consider how to improve and develop the future clinical approach and service model. However, in order to do this, it is first necessary to understand the current landscape and the reasons why change is needed, so that any future model addresses existing challenges, whilst retaining those features that service users and the professionals supporting them most value.

This report is primarily for the commissioners and providers of services for children and young people needing support around their gender. However, because of the wide interest in this topic, we have included some explanations about how clinical service development routinely takes place in the NHS, which sets the context for some of our interim advice.

The care of this group of children and young people is everyone's business. We therefore encourage the wider clinical community to take note of our work and consider their own roles in providing the best holistic support to this population.

Since the Review began, it has focused on hearing a wide range of perspectives to better understand the challenges within the current system and aspirations for how these could be addressed. This report does not contain all that we have heard during our listening sessions but summarises consistent themes. These conversations will continue throughout the course of the Review and there will be further opportunities for stakeholders to engage and contribute.

It is important to note that the references cited in this report do not constitute a comprehensive literature review and are included only to clarify why specific lines of enquiry are being pursued, and where there are unanswered questions that will be addressed more fully during the life of the Review. A formal literature review is one strand of the Review's commissioned work, and this will be reported in full when complete.

A note about language

There is sometimes no consensus on the best language to use relating to this subject. The language surrounding this area has also changed rapidly and young people have developed varied ways of describing their experiences using different terms and constructs that are relevant to them.

The Review tries as far as possible to use language and terms that are respectful and acknowledge diversity, but that also accurately illustrate the complexity of what we are trying to describe and articulate.

The terms we have used may not always feel right to some; nevertheless, it is important to emphasise that the language used is not an indication of a position being taken by the Review. A glossary of terms is included.

The Review is cognisant of the broader cultural and societal debates relating to the rights of transgender adults. It is not the role of the Review to take any position on the beliefs that underpin these debates. Rather, this Review is strictly focused on the clinical services provided to children and young people who seek help from the NHS to resolve their gender-related distress.

A letter to children and young people

Children and young people accessing the NHS deserve safe, timely and supportive services, and clinical staff with the training and expertise to meet their healthcare needs.

Dr Hilary Cass

I understand that as you read this letter some of you may be anxious because you are waiting to access support from the NHS around your gender identity. Maybe you have tried to get help from your local services, or from the Gender Identity Development Service (GIDS), and because of the long waiting lists they have not yet been able to see you. I hope that some of you have had help – maybe from a supportive GP, a local Child and Adolescent Mental Health Service (CAMHS), or from GIDS.

I have heard that young service users are particularly worried that I will suggest that services should be reduced or stopped. I want to assure you that this is absolutely not the case – the reverse is true. I think that more services are needed for you, closer to where you live. The GIDS staff are working incredibly hard and doing their very best to see you as quickly as possible but providing supportive care is not something that can be rushed – each young person needs enough time and space for their personal needs to be met. So, with the best will in the world, one service is not going to be able to respond to the growing demand in a timely way.

I am advising that more services are made available to support you. But I must be honest; this is not something that can happen overnight, and I can't come up with a solution that will fix the problems immediately. However, we do need to start now.

The other topic that I know is worrying some of you is whether I will suggest that hormone treatments should be stopped. On this issue, I have to share my thoughts as a doctor. We know quite a bit about hormone treatments, but there is still a lot we don't know about the long-term effects.

Whenever doctors prescribe a treatment, they want to be as certain as possible that the benefits will outweigh any adverse effects so that when you are older you don't end up saying 'Why did no-one tell me that that might happen?' This includes understanding both the risks and benefits of having treatment and not having treatment.

Therefore, what we will be doing over the next few months is trying to make sense of all the information that is available, as well as seeing if we can plug any of the gaps in the research. I am currently emphasising the importance of making decisions about prescribing as safe as possible. This means making sure you have all the information you need – about what we do know and what we don't know.

Finally, some of you may want the chance to talk to me and share your thoughts about how services should look in the future. Over the coming months we will need your help and there will be opportunities to get involved with the Review, so please keep an eye on our website (www.cass.independent-review.uk), where we will provide updates on our work.

Dr Hilary Cass, OBE

Introduction from the Chair

Anyone with an interest in the care of gender-questioning children and young people, as well as those with lived experience, may have wondered what qualifies me to take on this Review, and whether I have a pre-existing position on this subject.

I am a paediatrician who was in clinical practice until 2018, my area of specialism being children and young people with disability. I have also held many management and policy roles throughout my career, most notably as President of the Royal College of Paediatrics and Child Health (RCPCH) from 2012-15.

Children's services are often at a disadvantage in healthcare because health services are usually designed around the needs of adults. As President of RCPCH, a key part of my role was to advocate for services to be planned with children and families at their heart.

I have not worked in gender services during my career, but my strong focus on hearing the voice of service users, supporting vulnerable young people, equity of access, and strong clinical standards applies in this area as much as in my other work.

With this in mind, the aim of the Review is to ensure that children and young people who are experiencing gender incongruence or gender-related distress receive a high standard of NHS care that meets their needs and is safe, holistic and effective.

I have previously set out the principles governing this Review process, namely that:

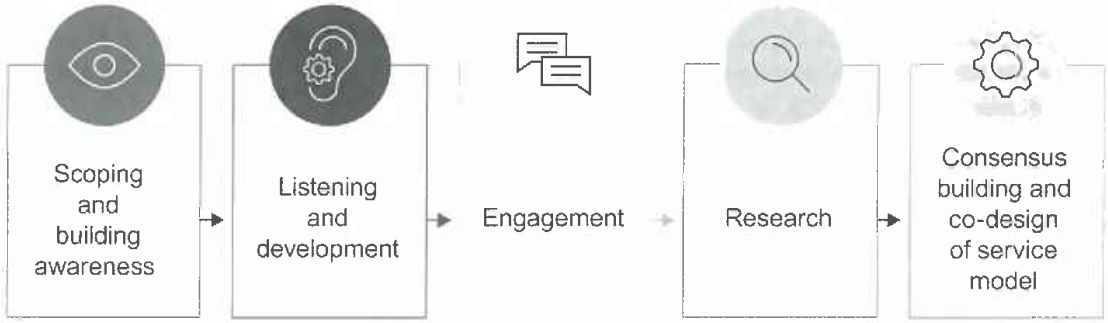
- The welfare of the child and young person will be paramount in all considerations.
- Children and young people must receive a high standard of care that meets their needs.
- There will be extensive and purposeful stakeholder engagement, including ensuring that children and young people can express their own views through a supportive process.
- The Review will be underpinned by research and evidence, including international models of good practice where available.
- There will be transparency in how the Review is conducted and how recommendations are made.
- There are no pre-determined outcomes with regards to the recommendations the Review will make.

The Review's terms of reference (**Appendix 1**) are wide ranging in scope, looking at different aspects of gender identity services across the whole pathway through primary, secondary and specialist services, up to the point of transition to adult services. This includes consideration of referral pathways, assessment, appropriate clinical management and workforce recommendations.

I have also been asked to explore the reasons for the considerable increase in the number of referrals, which have had a significant impact on waiting times, as well as the changing case-mix of gender-questioning children and young people presenting to clinical services.

The Review is taking an investigative approach to understanding what the future service model should look like for children and young people. This means that its outcomes are not being developed in isolation or by committee but rather through an ongoing dialogue aimed at building a shared understanding of the current situation and how it can and should be improved.

The key aspects of the approach to the Review are:



My starting point has been to hear from a variety of experts with relevant expertise and those with lived experience to understand as many perspectives as possible. To date, this has included hearing directly from those with lived experience, from professionals and support and advocacy groups. This listening process will continue.

We have been very fortunate in the generosity of all those who have been prepared to talk to the Review and share their experiences. In addition to some divergent opinions, there are also some themes and views which seem to be widely shared. The commitment of professionals at all levels is striking and I genuinely believe that with collective effort we can improve services for the children and young people who are at the heart of this Review.

These discussions have been valuable to get an in-depth sense of the current situation and different viewpoints on how it may be improved. However, it is essential that this initial understanding is underpinned by more detailed data and an enhanced evidence base, which is being delivered through the Review's academic research programme.

Providing this evidence base for the Review is going to take some time. I recognise there is a pressing need to enhance the services currently available for children, young people, their

parents and carers, some of whom are experiencing considerable distress. Clinicians providing their treatment and care are also under pressure and cannot sustain the current workload. As such, I know the time I am taking to complete this Review and make recommendations will be difficult for some, but it is necessary.

I wrote to NHS England in May 2021 (**Appendix 2**) setting out some more immediate considerations whilst awaiting my full recommendations. This report builds on that letter and looks to provide some further interim advice.

Through our research programme, the Review team will continue to examine the literature and, where possible, will fill gaps in the existing evidence base. However, there will be persisting evidence gaps and areas of uncertainty. We need the engagement of service users, support and advocacy groups, and professionals across the wider workforce to work with us in the coming months in a collaborative and open-minded manner in order to reach a shared understanding of the problems and an agreed way forward that is in the best interests of children and young people.

My measure of success for this Review will be that this group of children and young people receive timely, appropriate and excellent care, not just from specialists but from every healthcare professional they encounter as they take the difficult journey from childhood to adulthood.

1. Summary and interim advice

Summary

1.1. In recent years, there has been a significant increase in the number of referrals to the Gender Identity Development Service (GIDS) at the Tavistock and Portman NHS Foundation Trust. This has contributed to long waiting lists and growing concern about how the NHS should most appropriately assess, diagnose and care for this population of children and young people.

1.2. Within the UK, the single specialist service has developed organically, and the clinical approach has not been subjected to some of the usual control measures that are typically applied when new or innovative treatments are introduced. Many of the challenges and knowledge gaps that we face in the UK are echoed internationally,[1] and there are significant gaps in the research and evidence base.

1.3. This Review was commissioned by NHS England to make recommendations on how to improve services provided by the NHS to children and young people who are questioning their gender identity or experiencing gender incongruence and ensure that the best model for safe and effective services is commissioned (**Appendix 1**).

1.4. This interim report represents the Review's work to date. It sets out what we have heard so far and the approach we are taking moving forward. There is still much evidence to be gathered, questions to be answered, and voices to be heard, and our perspective will evolve as more evidence comes to light. However, there is sufficient clarity on several areas for the Review to be able to offer advice at this stage so that action can be taken more quickly.

1.5. The Review is not able to provide definitive advice on the use of puberty blockers and feminising/masculinising hormones at this stage, due to gaps in the evidence base; however, recommendations will be developed as our research programme progresses.

Every gender-questioning child or young person who seeks help from the NHS must receive the support they need to get on the appropriate pathway for them as an individual.

Children and young people with gender incongruence or dysphoria must receive the same standards of clinical care, assessment and treatment as every other child or young person accessing health services.

[1] Vrouenraets LJ, Fredriks AM, Hannema SE, Cohen-Kettenis PT, de Vries MC (2015). Early medical treatment of children and adolescents with gender dysphoria: an empirical ethical study. J Adolesc Health 57(4): 367-73. DOI: 10.1016/.2015.04.004.

Conceptual understanding and consensus about the meaning of gender dysphoria

1.6. In clinical practice, a diagnosis of gender dysphoria is currently based on an operational definition, using the criteria set out in DSM-5 (**Appendix 3**). Some of these criteria are seen by some as outdated in the context of current understanding about the flexibility of gender expression.

1.7. At primary, secondary and specialist level, there is a lack of agreement, and in many instances a lack of open discussion, about the extent to which gender incongruence in childhood and adolescence can be an inherent and immutable phenomenon for which transition is the best option for the individual. Professionals' experience and position on this spectrum may determine their clinical approach.

1.8. Children and young people can experience this as a 'clinician lottery', and failure to have an open discussion about this issue is impeding the development of clear guidelines about their care.

Service capacity and delivery

1.9. A rapid change in epidemiology and an increase in referrals means that the number of children seeking help from the NHS is now outstripping the capacity of the single national specialist service, the Gender Identity Development Service (GIDS) at The Tavistock and Portman NHS Foundation Trust.

1.10. The mix of young people presenting to the service is more complex than seen previously, with many being neurodiverse and/or having a wide range of psychosocial and mental health needs. The largest group currently comprises birth-registered females first presenting in adolescence with gender-related distress.

1.11. Until very recently, any local professional, including non-health professionals, could refer to GIDS, which has meant that the quality and appropriateness of referrals lacks consistency, and local service provision has remained patchy and scarce.

1.12. The staff working within the specialist service demonstrate a high level of commitment to the population they serve. However, the waiting list pressure and lack of consensus development on the clinical approach, combined with criticism of the service, have all resulted in rapid turnover of staff and inadequate capacity to deal with the increasing workload. Capacity constraints cannot be addressed through financial investment alone; there are some complex workforce (recruitment; retention; and training) and cultural issues to address.

1.13. Our initial work has indicated that many professionals working at primary and secondary level feel that they have the transferable skills and the commitment to offer more robust support to this group of children and young people, but are nervous about doing so, partly because of the lack of formal clinical guidance, and partly due to the broader societal context.

1.14. Primary and secondary care staff have told us that they feel under pressure to adopt an unquestioning affirmative approach and that this is at odds with the standard process of clinical assessment and diagnosis that they have been trained to undertake in all other clinical encounters.

1.15. Children and young people are waiting lengthy periods to access GIDS, during which time some may be at considerable risk. By the time they are seen, their distress may have worsened, and their mental health may have deteriorated.

1.16. Another significant issue raised with us is one of diagnostic overshadowing – many of the children and young people presenting have complex needs, but once they are identified as having gender-related distress, other important healthcare issues that would normally be managed by local services can sometimes be overlooked.

1.17. The current move to adult services at age 17-18 may fall at a critical time in the young person's gender management. In contrast, young people with neurodiversity often remain under children's services until age 19 and some other clinical services continue to mid-20s. Further consideration will be needed regarding the age of transfer to adult services.

Service standards

1.18. The Multi-Professional Review Group (MPRG), set up by NHS England to ensure that procedures for assessment and for informed consent have been properly followed, has stated that the following areas require consideration:

- From the point of entry to GIDS there appears to be predominantly an affirmative, non-exploratory approach, often driven by child and parent expectations and the extent of social transition that has developed due to the delay in service provision.
- From documentation provided to the MPRG, there does not appear to be a standardised approach to assessment or progression through the process, which leads to potential gaps in necessary evidence and a lack of clarity.
- There is limited evidence of mental health or neurodevelopmental assessments being routinely documented, or of a discipline of formal diagnostic or psychological formulation.
- Of 44 submissions received by the MPRG, 31% were not initially assured due to lack of safeguarding information. And in a number of cases there were specific safeguarding concerns. There do not appear to be consistent processes in place to work with other agencies to identify children and young people and families who may be vulnerable, at risk and require safeguarding.

- Appropriate clinical experts need to be involved in informing decision making.

1.19. Many of these issues were also highlighted by the Care Quality Commission (CQC) in 2020.[2]

International comparisons

1.20. The Netherlands was the first country to provide early endocrine interventions (now known internationally as the Dutch Approach). Although GIDS initially reported its approach to early endocrine intervention as being based on the Dutch Approach,[3] there are significant differences in the NHS approach. Within the Dutch Approach, children and young people with neurodiversity and/or complex mental health problems are routinely given therapeutic support in advance of, or when considered appropriate, instead of early hormone intervention. Whereas criteria to have accessed therapeutic support prior to starting hormone blocking treatment do not appear to be integral to the current NHS process.

1.21. NHS endocrinologists do not systematically attend the multi-disciplinary meetings where the complex cases that may be referred to them are discussed, and until very recently did not routinely have direct contact with the clinical staff member who had assessed the child or young person. This is not consistent with some international approaches for this group of children and young people, or in other multi-disciplinary models of care across paediatrics and adult medicine where challenging decisions about life-changing interventions are made.[4,5]

1.22. In the NHS, once young people are started on hormone treatment, the frequency of appointments drops off rather than intensifies, and review usually takes place quarterly. Again, this is different to the Dutch Approach.[6] GIDS staff would recommend more frequent contact during this period, but the fall-off in appointments reflects a lack of service capacity, with the aspiration being for more staff time to remedy this situation.

Existing evidence base

1.23. Evidence on the appropriate management of children and young people with gender incongruence and dysphoria is inconclusive both nationally and internationally.

[2] Care Quality Commission (2021). The Tavistock and Portman NHS Foundation Trust Gender Identity Service Inspection Report. London: CQC.
[3] de Vries ALC, Cohen-Kettenis PT (2012). Clinical management of gender dysphoria in children and adolescents: the Dutch approach. J Homosex 59: 301–320. DOI: 10.1080/00918369.2012.653300.
[4] Ibid.
[5] Kyriakou A, Nicolaides NC, Skordis N (2020). Current approach to the clinical care of adolescents with gender dysphoria. Acta Biomed 91(1): 165–75. DOI: 10.23750/abm.v91i1.9244.
[6] de Vries ALC, Cohen-Kettenis PT (2012). Clinical management of gender dysphoria in children and adolescents: the Dutch approach. J Homosex 59: 301–320. DOI: 10.1080/00918369.2012.653300.

1.24. A lack of a conceptual agreement about the meaning of gender dysphoria hampers research, as well as NHS clinical service provision.

1.25. There has not been routine and consistent data collection within GIDS, which means it is not possible to accurately track the outcomes and pathways that children and young people take through the service.

1.26. Internationally as well as nationally, longer-term follow-up data on children and young people who have been seen by gender identity services is limited, including for those who have received physical interventions; who were transferred to adult services and/or accessed private services; or who desisted, experienced regret or detransitioned.

1.27. There has been research on the short-term mental health outcomes and physical side effects of puberty blockers for this cohort, but very limited research on the sexual, cognitive or broader developmental outcomes.[7]

1.28. Much of the existing literature about natural history and treatment outcomes for gender dysphoria in childhood is based on a case-mix of predominantly birth-registered males presenting in early childhood. There is much less data on the more recent case-mix of predominantly birth-registered females presenting in early teens, particularly in relation to treatment and outcomes.

1.29. Aspects of the literature are open to interpretation in multiple ways, and there is a risk that some authors interpret their data from a particular ideological and/or theoretical standpoint.

The mismatch between service user expectations and clinical standards

1.30. By the time children and young people reach GIDS, they have usually had to experience increasingly long, challenging waits to be seen.[8] Consequently, some feel they want rapid access to physical interventions and find having a detailed assessment distressing.

1.31. Clinical staff are governed by professional, legal and ethical guidance which demands that certain standards are met before a treatment can be provided. Clinicians carry responsibility for their assessment and recommendations, and any harm that might be caused to a patient under their care. This can create a tension between the aspirations of the young person and the responsibilities of the clinician.

[7] National Institute for Health and Care Excellence (2020). Evidence Review: Gonadotrophin Releasing Hormone Analogues for Children and Adolescents with Gender Dysphoria.
[8] Care Quality Commission (2021). The Tavistock and Portman NHS Foundation Trust Gender Identity Service Inspection Report. London: CQC.

Interim advice

1.32. The Review considers that there are some areas where there is sufficient clarity about the way forward and we are therefore offering some specific observations and interim advice. The Review will work with NHS England, providers and the broader stakeholder community to progress action in these areas.

Service model

1.33. It has become increasingly clear that a single specialist provider model is not a safe or viable long-term option in view of concerns about lack of peer review and the ability to respond to the increasing demand.

1.34. Additionally, children and young people with gender-related distress have been inadvertently disadvantaged because local services have not felt adequately equipped to see them. It is essential that they can access the same level of psychological and social support as any other child or young person in distress, from their first encounter with the NHS and at every level within the service.

1.35. A fundamentally different service model is needed which is more in line with other paediatric provision, to provide timely and appropriate care for children and young people needing support around their gender identity. This must include support for any other clinical presentations that they may have.

1.36. The Review supports NHS England's plan to establish regional services, and welcomes the move from a single highly specialist service to regional hubs.

1.37. Expanding the number of providers will have the advantages of:

- creating networks within each area to improve early access and support;
- reducing waiting times for specialist care;
- building capacity and training opportunities within the workforce;
- developing a specialist network to ensure peer review and shared standards of care; and
- providing opportunities to establish a more formalised service improvement strategy.

Service provision

1.38. The primary remit of NHS England's proposed model is for the regional hubs to provide support and advice to referrers and professionals. However, it includes limited provision for direct contact with children and young people and their families.

1: The Review advises that the regional centres should be developed, as soon as feasibly possible, to become direct service providers, assessing and treating children and young people who may need specialist care, as part of a wider pathway. The Review team will work with NHS England and stakeholders to further define the proposed model and workforce implications.

2: Each regional centre will need to develop links and work collaboratively with a range of local services within their geography to ensure that appropriate clinical, psychological and social support is made available to children and young people who are in early stages of experiencing gender distress.

3: Clear criteria will be needed for referral to services along the pathway from primary to tertiary care so that gender-questioning children and young people who seek help from the NHS have equitable access to services.

4: Regional training programmes should be run for clinical practitioners at all levels, alongside the online training modules developed by Health Education England (HEE). In the longer-term, clearer mapping of the required workforce, and a series of competency frameworks will need to be developed in collaboration with relevant professional organisations.

Data, audit and research

1.39. A lack of routine and consistent data collection means that it is not possible to accurately track the outcomes and pathways children and young people take through the service. Standardised data collection is required in order to audit service standards and inform understanding of the epidemiology, assessment and treatment of this group. This, alongside a national network which brings providers together, will help build knowledge and improve outcomes through shared clinical standards and systematic data collection. In the longer-term, formalisation of such a network into a learning health system[9] with an academic host would mean that there was systematised use of data to produce a continuing research programme with rapid translation into clinical practice and a focus on training.

[9] Scobie S, Castle-Clarke S (2019). Implementing learning health systems in the UK NHS: Policy actions to improve collaboration and transparency and support innovation and better use of analytics. Learning Health Systems 4(1): e10209. DOI:10.1002/lrh2.10209.

5: The regional services should have regular co-ordinated national provider meetings and operate to shared standards and operating procedures with a view to establishing a formal learning health system.

6: Existing and future services should have standardised data collection in order to audit standards and inform understanding of the epidemiology, assessment and treatment of this group of children and young people.

7: Prospective consent of children and young people should be sought for their data to be used for continuous service development, to track outcomes, and for research purposes. Within this model, children and young people put on hormone treatment should be formally followed up into adult services, ideally as part of an agreed research protocol, to improve outcome data.

Clinical approach

Assessment processes

1.40. We have heard that there are inconsistencies and gaps in the assessment process. Our work to date has also demonstrated that clinical staff have different views about the purpose of assessment and where responsibility lies for different components of the process within the pathway of care. The Review team has commenced discussions with clinical staff across primary, secondary and tertiary care to develop a framework for these processes.

8: There needs to be agreement and guidance about the appropriate clinical assessment processes that should take place at primary, secondary and tertiary level.

9: Assessments should be respectful of the experience of the child or young person and be developmentally informed. Clinicians should remain open and explore the patient's experience and the range of support and treatment options that may best address their needs, including any specific needs of neurodiverse children and young people.

Hormone treatment

1.41. The issues raised by the Multi-Professional Review Group echo several of the problems highlighted by the CQC. It is essential that principles of the General Medical Council's Good Practice in Prescribing and Managing Medicine's and Devices[10] are closely followed, particularly given the gaps in the evidence base regarding hormone treatment. Standards for decision making regarding endocrine treatment should also be consistent with international best practice.[11,12,13]

10: Any child or young person being considered for hormone treatment should have a formal diagnosis and formulation, which addresses the full range of factors affecting their physical, mental, developmental and psychosocial wellbeing. This formulation should then inform what options for support and intervention might be helpful for that child or young person.

11: Currently paediatric endocrinologists have sole responsibility for treatment, but where a life-changing intervention is given there should also be additional medical responsibility for the differential diagnosis leading up to the treatment decision.

1.42. Paediatric endocrinologists develop a wide range of knowledge within their paediatric training, including safeguarding, child mental health, and adolescent development. Being party to the discussions and deliberations that have led up to the decision for medical intervention supports them in carrying out their legal responsibility for consent to treatment and the prescription of hormones.

12: Paediatric endocrinologists should become active partners in the decision making process leading up to referral for hormone treatment by participating in the multidisciplinary team meeting where children being considered for hormone treatment are discussed.

[10] General Medical Council (2021). Good practice in prescribing and managing medicines and devices (76-78).
[11] Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 102(11): 3869–903. DOI: 10.1210/jc.2017-01658.
[12] Cohen-Kettenis PT, Steensma TD, de Vries ALC (2001). Treatment of adolescents with gender dysphoria in the Netherlands. Child Adolesc Psychiatr Clin N Am 20: 689–700. DOI: 10.1016/j.chc.2011.08.001.
[13] Kyriakou A, Nicolaides NC, Skordis N (2020). Current approach to the clinical care of adolescents with gender dysphoria. Acta Biomed 91(1): 165–75. DOI: 10.23750/abm.v91i1.9244.

1.43. Given the uncertainties regarding puberty blockers, it is particularly important to demonstrate that consent under this circumstance has been fully informed and to follow GMC guidance[14] by keeping an accurate record of the exchange of information leading to a decision in order to inform their future care and to help explain and justify the clinician's decisions and actions.

13: Within clinical notes, the stated purpose of puberty blockers as explained to the child or young person and parent should be made clear. There should be clear documentation of what information has been provided to each child or young person on likely outcomes and side effects of all hormone treatment, as well as uncertainties about longer-term outcomes.

14: In the immediate term the Multi-Professional Review Group (MPRG) established by NHS England should continue to review cases being referred by GIDS to endocrine services.

[14] General Medical Council (2020). Decision making and consent.

2. Context

Transgender, non-binary and gender fluid adults

2.1. NHS clinical services to support transgender adults with hormone treatment and subsequent surgery began in 1966.

2.2. Services were initially established within a mental health model, in conjunction with endocrinology and surgical services.

2.3. Currently, NHS services for transgender adults do not have adequate capacity to cope with demand.[15] In addition, the broader healthcare needs of this group are not well met. This is important in the context of the current generation of gender-questioning children and young people in that there are now two inflows into adult services – individuals transitioning in adulthood, and those moving through from children's services.

2.4. Legal rights and protections for transgender people lagged behind the provision of medical services, with the Gender Recognition Act 2004 coming into force in April 2005. Over the last few years, broader discussions about transgender issues have been played out in public, with discussions becoming increasingly polarised and adversarial. This polarisation is such that it undermines safe debate and creates difficulties in building consensus.

2.5. It is not the role of this Review to take any position on the cultural and societal debates relating to transgender adults. However, in achieving its objectives there is a need to consider the information and support that children and young people access from whatever source, as well as any pressures that they are subject to, before they access clinical services.

Terminology and diagnostic frameworks

2.6. The Office for National Statistics defines sex as "referring to the biological aspects of an individual as determined by their anatomy, which is produced by their chromosomes, hormones and their interactions; generally male or female; something that is assigned at birth".[16]

2.7. The Office for National Statistics defines gender as "a social construction relating to behaviours and attributes based on labels of masculinity and femininity; gender identity is a personal, internal perception of oneself and so the gender category someone identifies with may not match the sex they were assigned at birth".[17]

2.8. Societal attitudes towards gender roles and gender expression are changing. Children, teenagers and younger adults may more commonly see gender as a fluid, multi-faceted phenomenon which

[15] Gender Identity Clinic, The Tavistock and Portman NHS Foundation Trust. Waiting times.
[16] Office for National Statistics (2019). What is the difference between sex and gender?
[17] Ibid.

does not have to be binary, whereas older generations have tended to see gender as binary and fixed. It is not unusual for young people to explore both their sexuality and gender as they go through adolescence and early adulthood before developing a more settled identity. Many achieve this without experiencing significant distress or requiring support from the NHS, but this is not the case for all.

2.9. For those who require support from the NHS, there are two widely used frameworks which provide diagnostic criteria. The International Classification of Diseases (ICD), which is the World Health Organization (WHO) mandated health data standard, and the Diagnostic and Statistical Manual of Mental Disorders (DSM), which is the classification system for mental health disorders produced by the American Psychiatric Association. The current editions of these manuals – ICD-11 and DSM-5 – came into effect in January 2022 and 2013 respectively.

2.10. ICD-11[18] has attempted to depathologise gender diversity, removing the term 'gender identity disorders' from its mental health section and creating a new section for gender incongruence and transgender identities in a chapter on sexual health. These changes are part of a much broader societal drive to remove the stigma previously associated with transgender healthcare. ICD-11 defines gender incongruence as being "characterised by a marked incongruence between an individual's experienced/ expressed gender and the assigned sex." Gender variant behaviour and preferences alone are not a basis for assigning the diagnosis. The full criteria for gender incongruence of childhood and gender incongruence of adolescence or adulthood are listed in **Appendix 3**.

2.11. DSM-5[19] is currently the framework used to diagnose gender dysphoria. This diagnostic category describes gender dysphoria as "the distress that may accompany the incongruence between one's experienced or expressed gender and one's assigned gender". A diagnosis of gender dysphoria is usually deemed necessary before a young person can access hormone treatment, and criteria are listed in **Appendix 3**.

Conceptual understanding of gender incongruence in children and young people

2.12. Children and young people presenting to gender identity services are not a homogeneous group. They vary in their age at presentation, their cultural background, whether they identify as binary, non-binary, or gender fluid, whether they are neurodiverse and in a host of other ways.

[18] World Health Organization (2022). International Classification of Diseases Eleventh Revision.
[19] American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Health Disorders: DSM-5™, 5th ed.

2.13. Some children and young people may thrive during a period of gender-questioning whilst for others it can be accompanied with a level of distress that can have a significant impact on their functioning and development.

2.14. Alongside these very varied presentations, it is highly unlikely that a single cause for gender incongruence will be found. Many authors view gender expression as a result of a complex interaction between biological, cultural, social and psychological factors.

2.15. Despite a high level of agreement about these points, there are widely divergent and, in some instances, quite polarised views among service users, parents, clinical staff and the wider public about how gender incongruence and gender-related distress in children and young people should be interpreted, and this has a bearing on expectations about clinical management.

2.16. These views will be influenced by how each individual weighs the balance of factors that may lead to gender incongruence, and the distress that may accompany it. Beliefs about whether it might be inherent and/or immutable, whether it might be a transient response to adverse experiences, whether it might be highly fluid and/or likely to change in later adolescence/early adulthood, etc will have a profound influence on expectations about treatment options.[20]

2.17. All of these views may be overlaid with strongly held concerns about children's and young people's rights, autonomy, and/or protection.

2.18. The disagreement and polarisation is heightened when potentially irreversible treatments are given to children and young people, when the evidence base underlying the treatments is inconclusive, and when there is uncertainty about whether, for any particular child or young person, medical intervention is the best way of resolving gender-related distress.

2.19. As with many other contemporary polarised disagreements, the situation is exacerbated when there is no space to have open, non-judgemental discussions about these differing perspectives. A key aim of this review process will be to encourage such discussions in a safe and respectful manner so that progress can be made in finding solutions.

[20] Wren B (2019). Notes on a crisis of meaning in the care of gender-diverse children. In: Hertzmann L, Newbigin J (eds) Sexuality and Gender Now: Moving Beyond Heteronormativity. Routledge.

3. Current services

Current service model for gender-questioning children and young people

3.1. Currently there are no locally or regionally commissioned services for children and young people who seek help from the NHS in managing their gender-related distress. Within primary and secondary care, some clinical staff have more interest and expertise in initial management of this group of young people, but such individuals are few and far between.

3.2. The pathway for NHS support around gender identity for children and young people is designated as a highly specialised service.[21] The Gender Identity Development Service (GIDS) at the Tavistock and Portman NHS Foundation Trust is commissioned by NHS England to provide specialist assessment, support and, where appropriate, hormone intervention for children and young people with gender dysphoria. It is the only NHS provider of specialist gender services for children and young people in England. The Trust runs satellite bases in Leeds and Bristol. Until recently GIDS accepted referrals from multiple sources, for example, GPs, secondary care, social care, schools, and support and advocacy groups, which is unusual for a specialist service.

3.3. Children and young people are assessed by two members of the GIDS team who may be any combination of psychologists, psychotherapists, family therapists, or social workers. If there is uncertainty about the right approach, individual cases may be discussed in a complex case meeting. Those deemed appropriate for physical interventions are referred on to the endocrine team; under the current Standard Operating Procedure (SOP), this decision requires a multi-disciplinary team (MDT) discussion within GIDS. A member of the GIDS team attends new appointments in the endocrine clinic, but they will not routinely be the member of staff who saw the young person for assessment. However, very recently a triage meeting has been piloted to enable endocrinologists to discuss upcoming appointments with the clinician who saw the young person for assessment. The young person then attends an education session prior to their endocrine appointment. The endocrinologist will assess any medical contraindications prior to seeking consent from the patient for any hormone treatments.

3.4. For many years, the GIDS approach was to offer assessment and support, and to only start puberty blockers when children reached sexual maturity at about age 15 (Tanner Stage 5) as the first step in the treatment process to feminise or masculinise the young person, with

[21] National Health Service Commissioning Board and Clinical Commissioning Groups (Responsibilities and Standing Rules) Regulations 2012.

oestrogen or testosterone given from age 16. Feminising/masculinising hormones are not given at an earlier stage because of the irreversibility of some of their actions in developing secondary sex characteristics of the acquired gender.[22] [23]

3.5. In 1998, a new protocol was published by the Amsterdam gender identity clinic.[24] It was subsequently named the Dutch Approach.[25] This involved giving puberty blockers much earlier, from the time that children showed the early signs of puberty (Tanner Stage 2), to pause further pubertal changes of the sex at birth. This stage of pubertal development was chosen because it was felt that although many younger children experienced gender incongruence as a transient developmental phenomenon, those who expressed early gender incongruence which continued into puberty were unlikely to desist at that stage.

3.6. It was felt that blocking puberty would buy time for children and young people to fully explore their gender identity and help with the distress caused by the development of their secondary sexual characteristics. The Dutch criteria for treating children with early puberty blockers were: (i) a presence of gender dysphoria from early childhood; (ii) an increase of the gender dysphoria after the first pubertal changes; (iii) an absence of psychiatric comorbidity that interferes with the diagnostic work-up or treatment; (iv) adequate psychological and social support during treatment; and (v) a demonstration of knowledge and understanding of the effects of gonadotropin-releasing hormones (puberty blockers), feminising/masculinising hormones, surgery, and the social consequences of sex reassignment.[26]

3.7. Under the Dutch Approach, feminising/masculinising hormones were started at age 16 and surgery was permitted to be undertaken from age 18, as in England.

3.8. From 2011, early administration of puberty blockers was started in England under a research protocol, which partially paralleled the Dutch Approach (the Early Intervention Study). From 2014, this protocol was adopted by GIDS as routine clinical practice. Results of the Early Intervention Study were published in December 2021.[27]

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[22] Delemarre-van de Wall HA, Cohen-Kettinis PT (2006). Clinical management of gender identity disorder in adolescents: a protocol on psychological and paediatric endocrinology aspects. Eur J Endocrinol 155 (Suppl 1): S131–7. DOI: 10.1530/eje.1.02231.

[23] de Vries ALC, Cohen-Kettenis PT (2012). Clinical management of gender dysphoria in children and adolescents: the Dutch approach. J Homosex 59: 301–320. DOI: 10.1080/00918369.2012.653300.

[24] Cohen-Kettenis PT, Van Goozen S (1998). Pubertal delay as an aid in diagnosis and treatment of a transsexual adolescent. Eur Child Adolesc Psychiatry 7: 246–8. DOI: 10.1007/s007870050073.

[25] de Vries ALC, Cohen-Kettenis PT (2012). Clinical management of gender dysphoria in children and adolescents: the Dutch approach. J Homosex 59: 301–320. DOI: 10.1080/00918369.2012.653300.

[26] Ibid.

[27] Carmichael P, Butler G, Masic U, Cole TJ, De Stavola BL, Davidson S, et al (2021). Short-term outcomes of pubertal suppression in a selected cohort of 12 to 15 year old young people with persistent gender dysphoria in the UK. PLoS One. 16(2):e0243894. DOI:10.1371/journal.pone.0243894.

3.9. However, the Dutch Approach differs from the GIDS approach in having stricter requirements about provision of psychological interventions. For example, under the Dutch Approach, if young people have gender confusion, aversion towards their sexed body parts, psychiatric comorbidities or Autism Spectrum Disorder (ASD) related diagnostic difficulties, they may receive psychological interventions only, or before, or in combination with medical intervention. Of note, in 2011, the Amsterdam team were reporting that up to 10% of their referral base were young people with ASD.[28]

Changing epidemiology

3.10. In the last few years, there has been a significant change in the numbers and case-mix of children and young people being referred to GIDS.[29] From a baseline of approximately 50 referrals per annum in 2009, there was a steep increase from 2014-15, and at the time of the CQC inspection of the Tavistock and Portman NHS Foundation Trust in October 2020 there were 2,500 children and young people being referred per annum, 4,600 children and young people on the waiting list, and a waiting time of over two years to first appointment.[30] This has severely impacted on the capacity of the existing service to manage referrals in the safe and responsive way that they aspire to and has led to considerable distress for those on the waiting list.

3.11. This increase in referrals has been accompanied by a change in the case-mix from predominantly birth-registered males presenting with gender incongruence from an early age, to predominantly birth-registered females presenting with later onset of reported gender incongruence in early teen years. In addition, approximately one third of children and young people referred to GIDS have autism or other types of neurodiversity. There is also an over-representation percentage wise (compared to the national percentage) of looked after children.[31]

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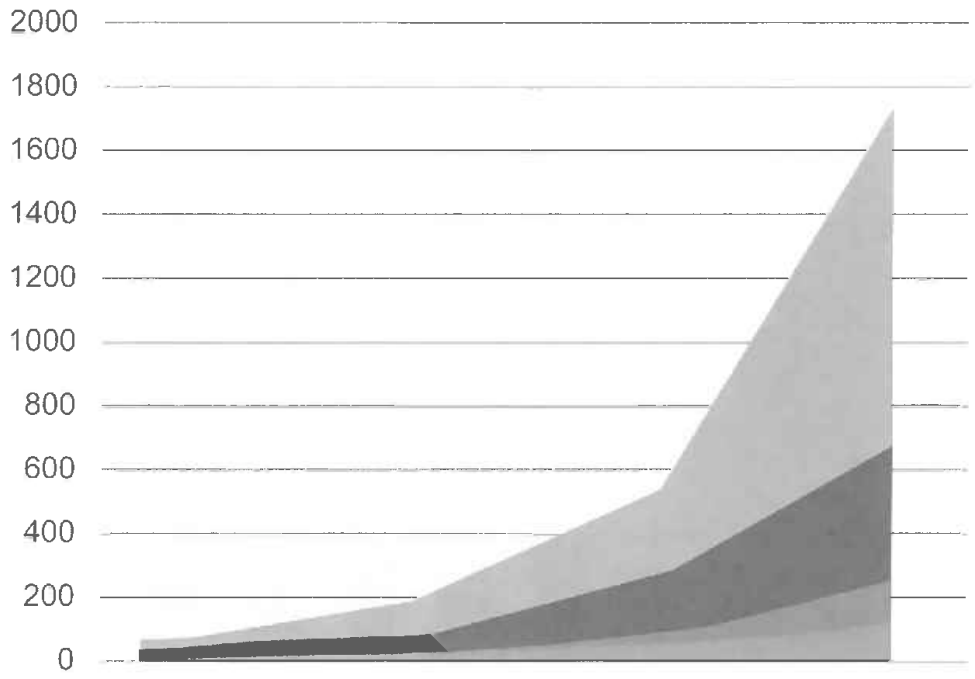
[28] Cohen-Kettenis PT, Steensma TD, de Vries ALC (2001). Treatment of adolescents with gender dysphoria in the Netherlands. Child Adolesc Psychiatr Clin N Am 20: 689–700. DOI: 10.1016/j.chc.2011.08.001.
[29] de Graaf NM, Giovanardi G, Zitz C, Carmichael P (2018). Sex ratio in children and adolescents referred to the gender identity development service in the UK (2009-2016). Arch Sex Behav 47(5): 1301–4.
[30] Care Quality Commission (2021). The Tavistock and Portman NHS Foundation Trust Gender Identity Service Inspection Report. London: CQC.
[31] Matthews T, Holt V, Sahin S, Taylor A, Griksaitis (2019). Gender Dysphoria in looked-after and adopted young people in a gender identity development service. Clinical Child Psychol Psychiatry 24: 112-128. DOI: 10.1177/1359104518791657.

Figure 1: Sex ratio in children and adolescents referred to GIDS in the UK (2009-16)



	2009	2010	2011	2012	2013	2014	2015	2016
Adolescents F	15	48*	78*	141*	221*	314*	689*	1071*
Adolescents M	24	44*	41	77*	120*	185*	293*	426*
Children F	2	7	12	17	22	36	77*	138*
Children M	10	19	29	30	31	55*	103*	131

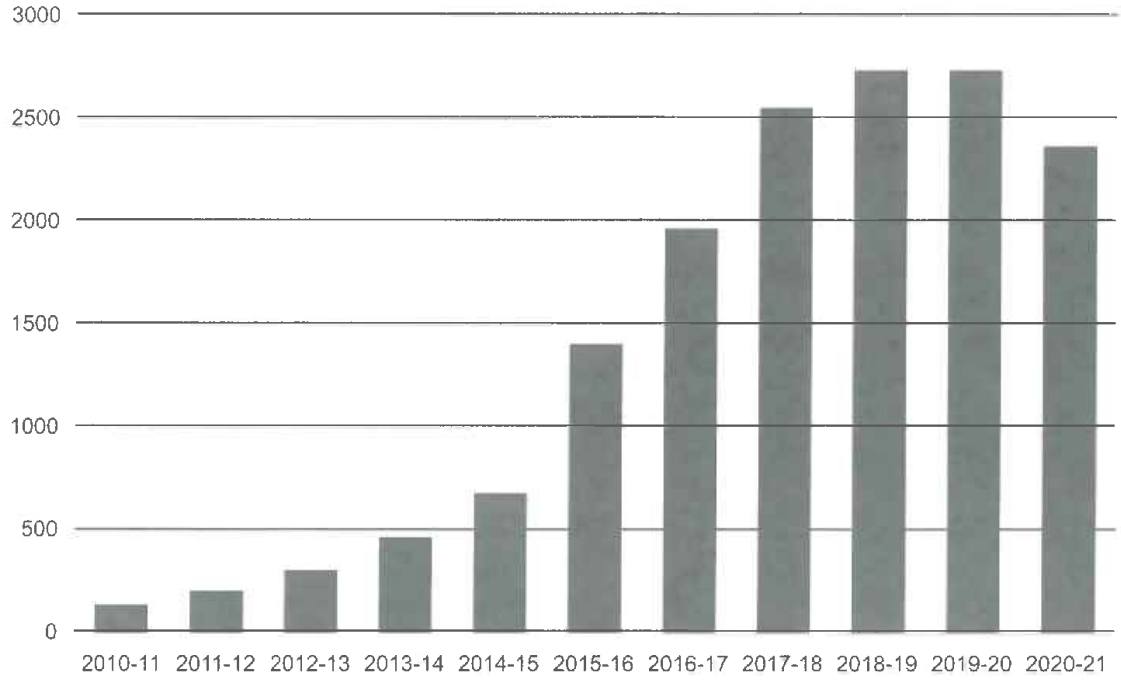
AFAB = assigned female at birth; AMAB = assigned male at birth

***Indicates** $p<.05$ which shows a significant increase of referrals compared to the previous year

Source: de Graaf NM, Giovanardi G, Zitz C, Carmichael P (2018).[32]

[32] de Graaf NM, Giovanardi G, Zitz C, Carmichael P (2018). Sex ratio in children and adolescents referred to the gender identity development service in the UK (2009-2016). Arch Sex Behav 47(5): 1301–4.

Figure 2: Referrals to GIDS, 2010-11 to 2020-21



Source: Gender Identity Development Service.[33]

3.12. In 2019, GIDS reported that about 200 children and young people from a referral base of 2,500 were referred on to the endocrine pathway. There is no published data on how the other children and young people from this referral baseline were managed, for example if: their gender dysphoria was resolved; they were still being assessed or receiving ongoing psychological support and input; they were not eligible for puberty blockers due to age; they were referred to endocrine services at a later stage; they were transferred to adult services; or they accessed private services.

Challenges to the service model and clinical approach

3.13. Over a number of years, in parallel with the increasing numbers of referrals, GIDS faced increasing challenges, both internally and externally. There were different views held within the staff group about the appropriate clinical approach, with some more strongly affirmative and some more cautious and concerned about the use of physical intervention. The complexity of the cases had also increased, so clinical decision making had become more difficult. There was also a high staff

[33] Gender Identity Development Service. Referrals to GIDS, financial years 2010-11 to 2020-21.

turnover, and accounts from staff concerned about the clinical care, which were picked up in both mainstream and social media. This culminated in 2018 with an internal report by a staff governor.

3.14. Following that report, a review was carried out in 2019 by the Trust's medical director. This set out the need for clearer processes for the service's referral management, safeguarding, consent, and clinical approach, and an examination of staff workload and support, and a new Standard Operating Procedure (SOP) was put in place.

NHS England Policy Working Group

3.15. In January 2020, a Policy Working Group (PWG) was established by NHS England to undertake a review of the published evidence on the use of puberty blockers and feminising/masculinising hormones in children and young people with gender dysphoria to inform a policy position on their future use. Given the increasingly evident polarisation among clinical professionals, Dr Cass was asked to chair the group as a senior clinician with no prior involvement or fixed views in this area. The PWG comprised an expert group including endocrinologists, child and adolescent psychiatrists and paediatricians representing their respective Royal Colleges, an ethicist, a GP, senior clinicians from the NHS GIDS, a transgender adult and parents of gender-questioning young people. The process was supported by a public health consultant and policy, pharmacy and safeguarding staff from NHS England.

3.16. NHS England uses a standardised protocol for developing clinical policies. The first step of this involves defining the PICO (the Population being treated, the Intervention, a Comparator treatment, and the intended Outcomes). This of itself was challenging, with a particular difficulty being definition of the intended outcomes of puberty blockers, and suitable comparators for both hormone interventions. However, agreement was reached on what should be included in the PICO and subsequently the National Institute for Health and Care Excellence (NICE) was commissioned to review the published evidence,[34,35] again following a standardised protocol which has strict criteria about the quality of studies that can be included.

3.17. Unfortunately, the available evidence was not strong enough to form the basis of a policy position. Some of the challenges and outstanding uncertainties are summarised as follows.

[34] National Institute for Health and Care Excellence (2020). Evidence Review: Gonadotrophin Releasing Hormone Analogues for Children and Adolescents with Gender Dysphoria.
[35] National Institute for Health and Care Excellence (2020). Evidence review: gender-affirming hormones for children and adolescents with gender dysphoria.

Feminising/masculinising hormones

3.18. Sex hormones have been prescribed for transgender adults for several decades, and the long-term risks and side effects are well understood. These include increased cardiovascular risk, osteoporosis, and hormone-dependent cancers.

3.19. In young people, consideration also needs to be given to the impact on fertility, with the need for fertility counselling and preservation.

3.20. The additional physical risk of starting these treatments at age 16+ rather than age 18+ is unlikely to add significantly to the total lifetime risk, although data on this will not be available for many years. However, as evidenced by take-up of treatment with feminising/masculinising hormones, where there is a high level of certainty that physical transition is the right option, the child or young person may be more accepting of these risks, which can seem remote from the immediate gender distress.

3.21. The most difficult question in relation to feminising/masculinising hormones therefore is not about long-term physical risk which is tangible and easier to understand. Rather, given the irreversible nature of many of the changes, the greatest difficulty centres on the decision to proceed to physical transition; this relies on the effectiveness of the assessment, support and counselling processes, and ultimately the shared decision making between clinicians and patients. Decisions need to be informed by long-term data on the range of outcomes, from satisfaction with transition, through a range of positive and negative mental health outcomes, through to regret and/or a decision to detransition. The NICE evidence review demonstrates the poor quality of these data, both nationally and internationally.

3.22. Regardless of the nature of the assessment process, some children and young people will remain fluid in their gender identity up to early to mid-20s, so there is a limit as to how much certainty one can achieve in late teens. This is a risk that needs to be understood during the shared decision making process with the young person.

3.23. It is also important to note that any data that are available do not relate to the current predominant cohort of later-presenting birth-registered female teenagers. This is because the rapid increase in this subgroup only began from around 2014-15. Since young people may not reach a settled gender expression until their mid-20s, it is too early to assess the longer-term outcomes of this group.

Puberty blockers

3.24. The administration of puberty blockers is arguably more controversial than administration of the feminising/ masculinising hormones, because there are more uncertainties associated with their use.

3.25. There has been considerable discussion about whether the treatment is 'experimental'; strictly speaking an experimental treatment is one that is being given as part of a research protocol, and this is not the case with puberty blockers, because the GIDS research protocol was stopped in 2014. At that time, the treatment was experimental and innovative, because the drug was licensed for use in children, but specifically for children with precocious puberty. This was therefore the first time it was used 'off-label' in the UK for children with gender dysphoria. If a drug is used 'off-label' it means it is being used for a condition that is different from the one for which it was licensed. The many uncertainties around the 'off-label' use were recognised, but given that this was not a new drug, it did not need Medicines and Healthcare products Regulatory Agency (MHRA) approval at that time.

3.26. The important question now, as with any treatment, is whether the evidence for the use and safety of the medication is strong enough as judged by reasonable clinical standards.

3.27. One of the challenges that NHS England's PWG faced in considering this question was the lack of clarity about intended outcomes, several of which have been proposed including:

- providing time/space for the young person to make a decision about continuing with transition;
- reducing or preventing worsening of distress;
- improving mental health; and
- stopping potentially irreversible pubertal changes which might later make it difficult for the young person to 'pass' in their intended gender role.

3.28. Proponents for the use of puberty blockers highlight the distress that young people experience through puberty and the risk of self-harm or suicide.[36] However, some clinicians do not feel that distress is actually alleviated until children and young people are able to start feminising/ masculinising hormones. The Review will seek to gain a better understanding of suicide data and the impact of puberty blockers through its research programme.

3.29. On the other hand, it has been asserted that starting puberty blockers at an older age provides children and young people with more time to achieve fertility preservation. In the case of birth-registered males, there is an argument that it also

[36] Turban JL, King D, Carswell JM, et al (2020). Pubertal suppression for transgender youth and risk of suicidal ideation. Pediatrics 145 (2): e20191725. DOI: 10.1542/peds.2019-1725.

allows more time to achieve adequate penile growth for successful vaginoplasty.

3.30. In the short-term, puberty blockers may have a range of side effects such as headaches, hot flushes, weight gain, tiredness, low mood and anxiety, all of which may make day-to-day functioning more difficult for a child or young person who is already experiencing distress. Short-term reduction in bone density is a well-recognised side effect, but data is weak and inconclusive regarding the long-term musculoskeletal impact.[37]

3.31. The most difficult question is whether puberty blockers do indeed provide valuable time for children and young people to consider their options, or whether they effectively 'lock in' children and young people to a treatment pathway which culminates in progression to feminising/ masculinising hormones by impeding the usual process of sexual orientation and gender identity development. Data from both the Netherlands[38] and the study conducted by GIDS[39] demonstrated that almost all children and young people who are put on puberty blockers go on to sex hormone treatment (96.5% and 98% respectively). The reasons for this need to be better understood.

3.32. A closely linked concern is the unknown impacts on development, maturation and cognition if a child or young person is not exposed to the physical, psychological, physiological, neurochemical and sexual changes that accompany adolescent hormone surges. It is known that adolescence is a period of significant changes in brain structure, function and connectivity.[40] During this period, the brain strengthens some connections (myelination) and cuts back on others (synaptic pruning). There is maturation and development of frontal lobe functions which control decision making, emotional regulation, judgement and planning ability. Animal research suggests that this development is partially driven by the pubertal sex hormones, but it is unclear whether the same is true in humans.[41] If pubertal sex hormones are essential to these brain maturation processes, this raises a secondary question of whether there is a critical time window for the processes to take place, or whether catch up is possible when oestrogen or testosterone is introduced later.

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[37] National Institute for Health and Care Excellence (2020). Evidence Review: Gonadotrophin Releasing Hormone Analogues for Children and Adolescents with Gender Dysphoria.
[38] Brik T, Vrouenraets LJJJ, de Vries MC, Hannema SE (2020). Trajectories of adolescents treated with gonadotropin-releasing hormone analogues for gender dysphoria. Arch Sex Behav 49: 2611–8. DOI: 10.1007/ s10508-020-01660-8.
[39] Carmichael P, Butler G, Masic U, Cole TJ, De Stavola BL, Davidson S, et al (2021). Short-term outcomes of pubertal suppression in a selected cohort of 12 to 15 year old young people with persistent gender dysphoria in the UK. PLoS One. 16(2):e0243894. DOI:10.1371/journal.pone.0243894.
[40] Delevichab K, Klinger M, Nana OJ, Wilbrecht L (2021). Coming of age in the frontal cortex: The role of puberty in cortical maturation. Semin Cell Dev Biol 118: 64–72. DOI: 10.1016/j.semcdb.2021.04.021.
[41] Goddings A-L, Beltz A, Jiska S, Crone EA, Braams BR (2019). Understanding the role of puberty in structural and functional development of the adolescent brain. J Res Adolesc 29(1): 32–53. DOI: 10.1111/jora.12408.

3.33. An international interdisciplinary panel[42] has highlighted the importance of understanding the neurodevelopmental outcomes of pubertal suppression and defined an appropriate approach for investigating this further. However, this work has not yet been undertaken.

Initiation of Cass Review

3.34. Dr Cass' own reflections on the PWG process, the available literature, and the issues it highlighted were as follows:

- Firstly, that hormone treatment is just one possible outcome for gender-questioning children and young people. A much better understanding is needed about: the increasing numbers of children and young people with gender-related distress presenting for help; the appropriate clinical pathway for each individual; their support needs; and the full range of potential treatment options.
- Secondly, there is very limited follow-up of the subset of children and young people who receive hormone treatment, which limits our understanding about the long-term outcomes of these treatments and this lack of follow up data should be corrected.
- Thirdly, the assessment process is inconsistent across the published literature. The outcome of hormone treatment is highly influenced by whether the assessment process accurately selects those children and young people most likely to benefit from medical treatment. This makes it difficult to draw conclusions from published studies.

3.35. In light of the above, NHS England commissioned this independent review to make recommendations on how the clinical management and service provision for children and young people who are experiencing gender incongruence or gender-related distress can be improved.

CQC inspection

3.36. In October and November 2020, the Care Quality Commission (CQC) inspectors carried out an announced, focused inspection of GIDS due to concerns reported to them by healthcare professionals and the Children's Commissioner for England. Concerns related to clinical practice, safeguarding procedures, and assessments of capacity and consent to treatment.

[42] Chen D, Strang JF, Kolbuck VD, Rosenthal SM, Wallen K, Waber DP, et al (2020). Consensus parameter: research methodologies to evaluate neurodevelopmental effects of pubertal suppression in transgender youth. Transgender Health 5(4). DOI: 10.1089/trgh.2020.0006.

3.37. The CQC report, published in January 2021,[43] gave the service an overall rating of inadequate. The report noted the high level of commitment and caring approach of the staff but identified a series of issues that needed improvement. In addition to the growing waiting list pressures, the CQC identified problems in several other areas including: the assessment and management of risk; the variations in clinical approach; the lack of clarity and consistency of care plans; the lack of any clear written rationale for decision making in individual cases; and shortfalls in the multidisciplinary mix required for some patient groups. Recording of capacity, competency and consent had improved since the new SOP in January 2020; however, there remained a culture in which staff reported feeling unable to raise concerns.

3.38. The CQC reported that when it inspected GIDS, there did not appear to be a formalised assessment process, or standard questions to explore at each session, and it was not possible to tell from the notes why an individual child might have been referred to endocrinology whilst another had not. Current GIDS data demonstrate that a majority of children and young people seen by the service do not get referred for endocrine treatment, but there is no clear information about what other diagnoses they receive, and what help or support they might need.

3.39. Since the CQC report, NHS England and The Tavistock and Portman NHS Foundation Trust management team have been working to address the issues raised. However, whilst some problems require a focused Trust response, the waiting list requires a system-wide response. This was noted in the letter from the Review to NHS England in May 2021 (**Appendix 2**).

Legal background

3.40. This section sets out the chronology of recent case law. In October 2019, a claim for Judicial Review was brought against The Tavistock and Portman NHS Foundation Trust. The claimants' case was summarised by the High Court as follows: "The claimants' case is that children and young persons under 18 are not competent to give consent to the administration of puberty blocking drugs. Further, they contend that the information given to those under 18 by the defendant [GIDS] is misleading and insufficient to ensure such children or young persons are able to give informed consent. They further contend that the absence of procedural safeguards, and the inadequacy of the information provided, results in an infringement of the rights of such children and young persons under Article 8 of the European Convention

[43] Care Quality Commission (2021). The Tavistock and Portman NHS Foundation Trust Gender Identity Service Inspection Report. London: CQC.

for the Protection of Human Rights and Fundamental Freedoms."[44]

3.41. In December 2020, three judges in the High Court of England and Wales handed down judgment in *Bell v Tavistock*.[45] (Most cases in the High Court are heard by a single judge sitting alone, and when a case is heard by more than one judge in the High Court, it is described as the Divisional Court.) The Divisional Court recognised that the Tavistock's policies and practices as set out in the service specification were not unlawful. However, the Court made a declaration that set out in detail a series of implications of treatment that a child would need to understand to be *Gillick* competent[46] to consent to puberty blockers. Specifically, because most children put on puberty blockers go on to have feminising/ masculinising hormones, the judgment said a child would need to understand not only the full implications of puberty blocking drugs, but also the implications of the full pathway of medical and surgical transition. The judges concluded that it will be "very doubtful" that 14-15 year-olds have such competence, and "highly unlikely" that children aged 13 or under have competence for that decision. Under the Mental Capacity Act 2005, 16-17 year-olds are presumed to have capacity, and they are effectively treated as adults for consent to medical treatment under the Family Law Reform Act 1969 section 8, but the judges suggested that it would be appropriate for clinicians to involve the court in any case where there were doubts as to whether the proposed treatment would be in the long term best interests of a 16-17 year-old.

3.42. Following the Divisional Court judgment in *Bell v Tavistock*, a claim was brought against the Tavistock in the High Court Family Division by the mother of a child for a declaration that she and the child's father had the ability in law to consent on behalf of their child to the administration of puberty blockers *(AB v CD)*.[47] The Court concluded that "the parents' right to consent to treatment on behalf of the child continues even when the child is *Gillick* competent to make the decision, save where the parents are seeking to override the decision of the child" [para 114] and that there is no "general rule that puberty blockers should be placed in a special category by which parents are unable in law to give consent" [para 128].

[44] Bell v Tavistock, [2020] EWHC 3274 (Admin).
[45] Ibid.
[46] Gillick v West Norfolk and Wisbech AHA [1986] AC 112.
[47] AB v CD & Ors [2021] EWHC 741

3.43. Subsequently, the Tavistock appealed the Divisional Court's earlier decision in *Bell v Tavistock* and was successful.[48] The Court of Appeal held that it was not appropriate for the Divisional Court to provide the guidance about the likelihood of having *Gillick* competence at particular ages, or about the need for court approval [para 91]. The Court of Appeal went on to say "The Divisional Court concluded that Tavistock's policies and practices (as expressed in the service specification and the SOP) were not unlawful and rejected the legal criticism of its materials. In those circumstances, the claim for judicial review is dismissed." [para 91]. However, clinicians should "take great care before recommending treatment to a child and be astute to ensure that the consent obtained from both child and parents is properly informed" [para 92].

3.44. The Court of Appeal in *Bell v Tavistock* recognised the lawfulness of treating children for gender dysphoria in this jurisdiction. Recognising the divergences in medical opinion, morality and ethics, it indicated that the question of whether treatment should be made available is a matter of policy "for the National Health Service, the medical profession and its regulators and Government and Parliament" [para 3].

3.45. Following the Divisional Court decision in *Bell v Tavistock*, new referrals for puberty blockers were suspended and a requirement was put in place that children currently on puberty blockers were reviewed with a view to court proceedings for a judge to determine the best interests for children in whom these medications were considered essential. This requirement was changed following *AB v CD*, with the reinstatement of the hormone pathway in March 2021. However, an external panel, the Multi Professional Review Group (MPRG), was established to ensure that procedures for assessment and for informed consent had been properly followed. The outcome of the *Bell* appeal has not changed this requirement, which is contingent not just on the legal processes but on the concerns raised by CQC regarding consent, documentation and clarity about decision making within the service.[49]

[48] EWCA [2021] Civ 1363.
[49] Care Quality Commission (2021). The Tavistock and Portman NHS Foundation Trust Gender Identity Service Inspection Report. London: CQC.

42

The Multi-Professional Review Group

3.46. NHS England has established a Multi-Professional Review Group (MPRG) to review whether the agreed process has been followed for a child to be referred into the endocrinology clinic and to be prescribed treatment. The Review has spoken directly to the MPRG, which has reported its observations of current practice.

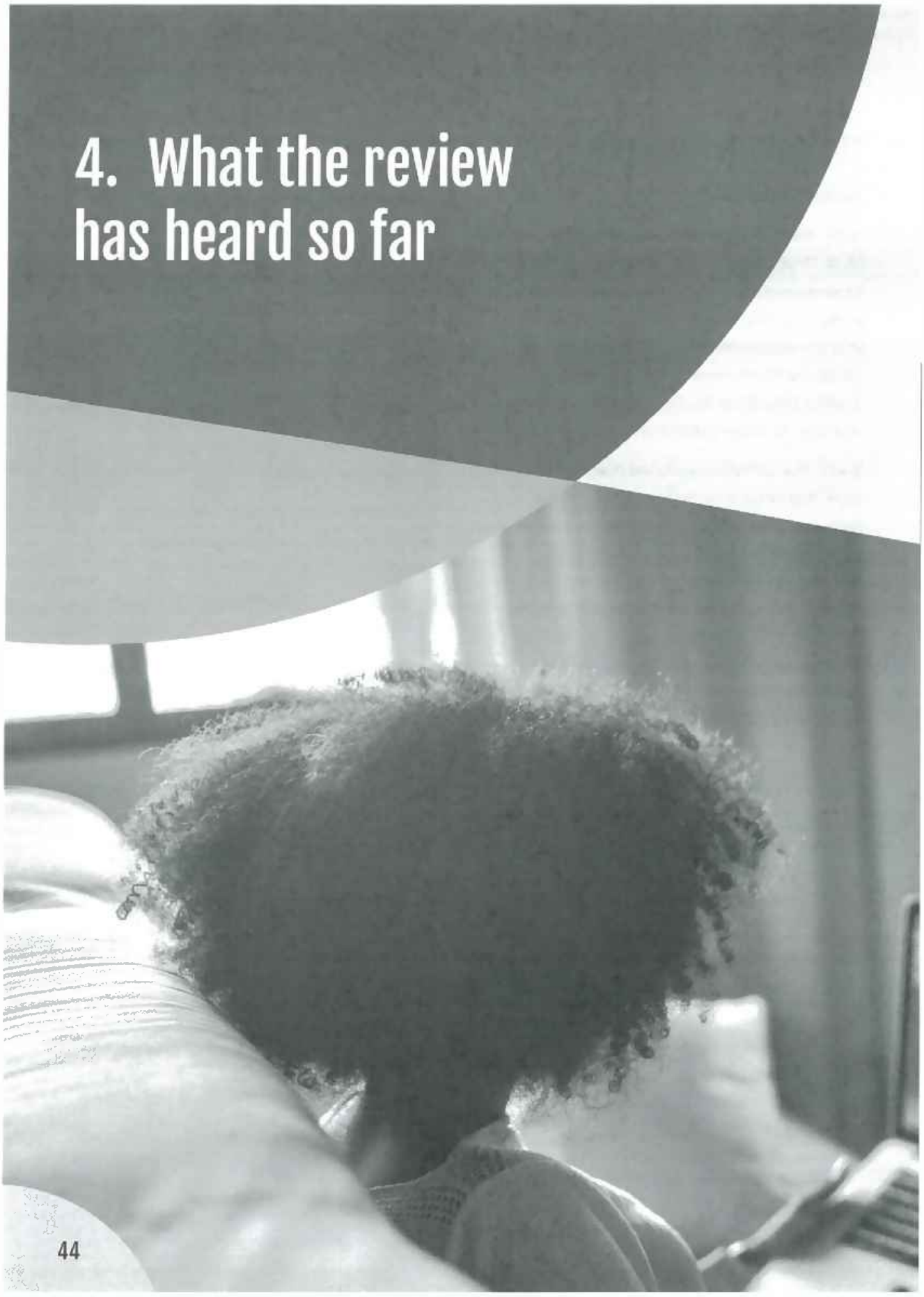
3.47. The MPRG has stated that its work has been impeded by delays in the provision of clinical information, the lack of structure in the documentation received, and gaps in the necessary evidence. This means that when reviewing the documents provided it is not always easy to determine if the process for referral for endocrine treatment has been fully or safely followed for a particular child or young person.

3.48. The MPRG indicates that there does not appear to be a standardised approach to assessment. They are particularly concerned about safeguarding shortfalls within the assessment process. There is also limited evidence of systematic, formal mental health or neurodevelopmental assessments being routinely documented, or of a discipline of formal diagnostic formulation in relation to co-occurring mental health difficulties. This issue was also highlighted by the Care Quality Commission (CQC).[50]

3.49. Additionally, there is concern that communications to GPs and parents regarding prescribed treatment with puberty blockers sometimes come from non-medical staff.

[50] Care Quality Commission (2021). The Tavistock and Portman NHS Foundation Trust Gender Identity Service Inspection Report. London: CQC.

4. What the review has heard so far



Listening sessions

4.1. Since its establishment, the Review has met with an extensive range of stakeholders, including professionals, their respective governing organisations and those with lived experience, both directly and through support and advocacy groups, to understand the broad range of views and experiences surrounding the delivery of gender identity services.

What we have heard from service users, their families and support and advocacy groups

Issues for children and young people

4.2. What we understand most clearly from all we have heard is that at the centre of a difficult and complex debate are children, young people and families in great distress. We have heard concerns about children and young people facing the stress of being on a prolonged waiting list with limited support available from statutory services, lack of certainty about when and if they might reach the top of that list and subsequent impacts on mental health. Also, the particular issues that have followed the *Bell v Tavistock* litigation.

4.3. We have heard about the anxiety that birth-registered males face as they come closer to the point where they will grow facial hair and their voice drops, and the fear that it will make it harder for them to pass as a transgender woman in later life. We have also heard about the distress experienced by birth-registered females as they reach puberty, including the use of painful, and potentially harmful, binding processes to conceal their breasts.

4.4. When children and young people are able to access the service, there is often a sense of frustration with what several describe as the "gatekeeping" medical model and a "clinician lottery". This can feel like a series of barriers and hurdles designed to add to, rather than alleviate, distress. Most children and young people seeking help do not see themselves as having a medical condition; yet to achieve their desired intervention they need to engage with clinical services and receive a medical diagnosis of gender dysphoria. By the time they are seen in the GIDS clinic, they may feel very certain of their gender identity and be anxious to start hormone treatment as quickly as possible. However, they can then face a period of what can seem like intrusive, repetitive and unnecessary questioning. Some feel that this undermines their autonomy and right to self-determination.

4.5. We have heard that some young people learn through peers and social media what they should and should not say to therapy staff in order to access hormone treatment; for example, that they are advised not to admit to previous abuse or trauma, or uncertainty about their sexual orientation. We have also heard that many of those seeking NHS support identify as non-binary, gender non-conforming, or gender fluid. We understand that some

young people who identify as non-binary feel their needs are not met by clinical services unless they give a binary narrative about their gender preferences.

Issues for parents

4.6. We have also heard about the distress parents may feel as they try to work out how best to support their children and how tensions and conflict may arise where parents and their children have different views. For example, some parents have highlighted the importance of ensuring that children and young people are able to keep their options fluid until such time as it becomes essential to commit to a hormonal course of action, whilst their children may want more rapid hormone intervention.

4.7. We have heard about families trying to balance the risks of obtaining unregulated and potentially dangerous hormone supplies over the internet or from private providers versus the ongoing trauma of prolonged waits for assessment.

4.8. Parents have also raised concerns about the vulnerability of neurodiverse children and young people and expressed that the communication needs of these children and young people are not adequately reflected during assessment processes or treatment planning.

4.9. GIDS has always required consent/assent from both the child and parents/carers and has sought ways to resolve family conflict, which in the worst-case scenario can lead to family breakdown. It has been highlighted to us that the future service model should provide more targeted support for parents and carers.

Service issues

4.10. Another significant issue raised with us is one of diagnostic overshadowing – many of the children and young people presenting have complex needs, but once they are identified as having gender-related distress, other important healthcare issues that would normally be managed by local services can sometimes be subsumed by the label of gender dysphoria. This issue is compounded by the waiting list, which means that there can be a significant period of time without appropriate assessment, treatment or care.

4.11. Stakeholders have spoken of the need for appropriate assessment when first accessing NHS services to aid both the exploration of the child or young person's wellbeing and gender distress and any other challenges they may be facing.

Information

4.12. We have also heard about the lack of access to accurate, balanced information upon which children, young people and their families/carers can inform their decisions.

4.13. We have heard that distress may be exacerbated by pressure to identify with societal stereotyping and concerns over the influence of social media, which can be seen to perpetuate unrealistic images of gender and set unhealthy expectations, especially given how long

children and young people are waiting to access services.

Other issues

4.14. Several issues that were raised with us are not explored further in this interim report, but we have taken note of them. These will be considered further during the lifetime of the Review and include:

- The important role of schools and the challenges they face in responding appropriately to gender-questioning children and young people.
- The complex interaction between sexuality and gender identity, and societal responses to both; for example, we have heard from young lesbians who felt pressured to identify as transgender male, and conversely transgender males who felt pressured to come out as gay rather than transgender. We have also heard from adults who identified as transgender through childhood, and then reverted to their birth-registered gender in teen years.
- The issues faced by detransitioners highlight the need for better services and pathways for this group, many of whom are living with irreversible effects of transition but for whom there is no clear access to services as they fall outside the responsibility of NHS gender identity services.
- The age at which adult gender identity clinics can receive referrals, with concerns about the inclusion of 17-year-olds. The service offer in adult services is perceived to be quite different from that of GIDS, and young people presenting later may therefore not be afforded the same level of therapeutic input under the adult service model. There is also concern about the impact on the young person of changing clinicians at a crucial point in their care. The movement of young people with special educational needs between children's and adult services raises particular concerns.

What we have heard from healthcare professionals

Lack of professional consensus

4.15. Clinicians and associated professionals we have spoken to have highlighted the lack of an agreed consensus on the different possible implications of gender-related distress – whether it may be an indication that the child or young person is likely to grow up to be a transgender adult and would benefit from physical intervention, or whether it may be a manifestation of other causes of distress. Following directly from this is a spectrum of opinion about the correct clinical approach, ranging broadly between those who take a more gender-affirmative approach to those who take a more cautious, developmentally-informed approach.

4.16. Speaking to current and ex-GIDS staff, we have heard about the pressure on GIDS clinicians, many of whom feel overwhelmed by the numbers of children and young people being referred and who are demoralised by the media coverage of their service. Although the clinical team attempt to manage risk on the waiting list by engaging with local services, there is limited capacity and/or capability to respond appropriately to the needs of this group in primary and secondary care. The Review has already referred to this issue as the most pressing priority in its letter to NHS England (**Appendix 2**), alongside potential risks relating to safeguarding and/or mental health issues, and diagnostic overshadowing.

4.17. With respect to GIDS, we have been told that although there are forums for staff to discuss difficult cases with senior colleagues, it is still difficult for staff to raise concerns about the clinical approach. Also that many individuals who are more cautious and advocate the need for an exploratory approach have left the service.

Consistency and standards

4.18. GIDS staff have confirmed that judgements are very individual, with some clinicians taking a more gender-affirmative approach and others emphasising the need for caution and for careful exploration of broader issues. The Review has been told that there is considerable variation in the approach taken between the London, Leeds and Bristol teams.

4.19. Speaking to professionals outside GIDS, we have heard widespread concern about the lack of guidance and evidence on how to manage this group of young people.

4.20. Some secondary care providers told us that their training and professional standards dictate that when working with a child or young person they should be taking a mental health approach to formulating a differential diagnosis of the child or young person's problems. However, they are afraid of the consequences of doing so in relation to gender distress because of the pressure to take a purely affirmative approach. Some clinicians feel that they are not supported by their professional body on this matter. Hence the practice of passing referrals straight through to GIDS is not just a reflection of local service capacity problems, but also of professionals' practical concerns about the appropriate clinical management of this group of children and young people.

4.21. GPs have expressed concern about being pressurised to prescribe puberty blockers or feminising/masculinising hormones after these have been initiated by private providers.

4.22. This also links to professional concerns about parents being anxious for hormone treatment to be initiated when the child or young person does not seem ready.

Other issues

4.23. We have also heard that parents and carers play a huge role and are instrumental in helping young people

to keep open their developmental opportunities. In discussion with social workers, we heard concerns about how looked after children are supported in getting the help and support they need.

4.24. Therapists who work with detransitioners and people with regret have highlighted a lack of services and pathways and a need for services to support this population. There is also the need for more research to understand what factors contribute to the decision to detransition.

4.25. The importance of broad holistic interventions to help reduce distress has been emphasised to the Review, with therapists and other clinicians advocating the importance of careful developmentally informed assessment and of showing children and young people a range of different narratives, experiences and outcomes.

4.26. Clinicians have raised concerns about children and young people's NHS numbers being changed inconsistently, as there is no specific guidance for GPs and others as to when this should be done for this population and under what consent. This has implications for safeguarding and clinical management of these children and young people and it also makes it difficult to do research exploring long-term outcomes.

4.27. As with the comments made by service users, their families and support and advocacy groups, we have heard similar views from professionals about the transition from children's to adult services, and the role of schools.

Structured engagement with primary, secondary and specialist clinicians

4.28. The Review's letter to NHS England (**Appendix 2**) set out some of the immediate issues with the current provision of gender identity services for children and young people and suggested how its work might help with the challenging problem of establishing an infrastructure outside GIDS. This included looking at the capacity, capability and confidence of the wider workforce and how this could be built and sustained, and the establishment of potential assessment frameworks for use in primary and/or secondary care.

Professional panel – primary and secondary care

4.29. In order to understand the challenges and establish a picture of current competency, capacity and confidence among the workforce outside the specialist gender development service, an online professional panel was established to explore issues around gender identity services for children and young people. The role of the panel was aimed at better comprehending how it looks and feels for clinicians and other professionals working with these young people, as well as any broader thoughts about the work, and to start exploring how the care of these

children and young people can be better managed in the future.

4.30. The project was designed to capture a broad mix of professional views and experiences, recruiting from the professional groups that are most likely to have a role in the care pathway – GPs, paediatricians, child psychiatrists, child psychologists and child psychotherapists, nurses and social workers.

4.31. A total of 102 clinicians and other professionals were involved in the panel. The panel represented a balanced professional mix, and participant ages and gender were broadly representative of the overall sector workforce. Participants were self-selecting and were recruited via healthcare professional networks and Royal Colleges.

4.32. Each week the panel was set an independent activity comprised of two or more tasks. Additionally, a sub-set of the panel was invited to participate in focus groups at the midway and endpoint of the project. Activities were designed to capture an understanding of:

- experiences of working with gender-questioning children and young people and panel members' confidence and competence to manage their care;
- changes they may have experienced in the presentation of children and young people with gender-related distress;
- areas where professionals feel they require more information in order to support gender-questioning children and young people;
- where professionals currently go to find that information;
- the role of different professions in the care pathway;
- the role of professionals in the assessment framework; and
- what participants felt should be included in an assessment framework across the whole service pathway.

Gender specialist questionnaire

4.33. Having concluded the professional panel exercise, we wanted to triangulate what we had heard with the thoughts and views of professionals working predominantly or exclusively with gender-questioning children and young people.

4.34. To do this in a systematic way, we conducted an online survey which contained some service-specific questions, but also reflected and sought to test some of what we had heard from primary and secondary care professionals.

Findings

4.35. This structured engagement has yielded valuable insights from clinicians and professionals with experience working with gender-questioning children and young people both within and outside the specialist gender service. It has contributed to the thinking of the Review and informed some of the interim advice set out in this report.

4.36. There are a number of consistent messages arising from these activities:

- The current long waiting lists that gender-questioning children and young people and their families/carers face are unacceptable for all parties involved, including professionals.
- Many professionals in our sample said that not only are gender-questioning children and young people having to wait a long time before receiving treatment, but they also do not receive appropriate support during this waiting period.
- Another impact of the long wait that clinicians reported is that when a child or young person is seen at GIDS, they may have a more fixed view of what they need and are looking for action to be taken quickly. This reportedly can lead to frustration with the assessment process.
- When considering the more holistic support that children and young people may need, gender specialists further highlighted the difficulties that children and young people face accessing local support, for example, from CAMHS, whilst being seen at GIDS.
- It is clear from the professionals who took part in these activities that there is a strong professional commitment to provide quality care to gender-questioning children and young people and their families/carers. However, this research indicates that levels of confidence and competence do vary among primary and secondary care professionals in our sample.
- Concerns were expressed by professionals who took part in this research about the lack of consensus among the clinical community on the right clinical approach to take when working with a gender-questioning child or young person and their families/carers.
- In order to support clinicians and professionals more widely, participants felt there is a need for a robust evidence base, consistent legal framework and clinical guidelines, a stronger assessment process and different pathway options that holistically meet the needs of each gender-questioning child or young person and their families/carers.

4.37. There are also several areas where further discussion and consensus is needed:

- There is not a consistent view among the professionals participating in the panel and questionnaire about the nature of gender dysphoria and therefore the role of assessment for children and young people experiencing gender dysphoria.

- Some clinicians felt that assessment should be focused on whether medical interventions are an appropriate course of action for the individual. Other clinicians believe that assessment should seek to make a differential diagnosis, ruling out other potential causes of the child or young person's distress.
- There are different perspectives on the roles of primary, secondary and specialist services in the care pathway(s) and what support or action might best be provided at different levels.
- While there was general consensus that diagnostic or psychological formulation needs to form part of the assessment process, there were differing views as to whether a mental state assessment is needed, and should it be, where in the pathway and by whom this should be done.

4.38. It is important to note that the information gathered represents the views and insights of the panel participants and survey respondents at a moment in time and findings should be read in the context of a developing narrative on the subject, where perspectives may evolve. This relates to both the experiences of professionals, but also the extent to which this subject matter is discussed in the public sphere.

4.39. The Review is grateful to all the participants for their time and high level of engagement. The Review will build on the work we have undertaken and, alongside our academic research, will continue with a programme of engagement with professionals, service users and their families, which will help to further develop the evidence base.

The full reports from the professional panel and gender specialist questionnaire are on the Review's website (https://cass.independent-review.uk/).

5. Principles of evidence based service development

Evidence based service development

5.1. This chapter integrates the information regarding the development of the current service (see Chapter 3) with the views we have heard to date (see Chapter 4) and sets this in the context of how evidence is routinely used to develop and improve services in the NHS.

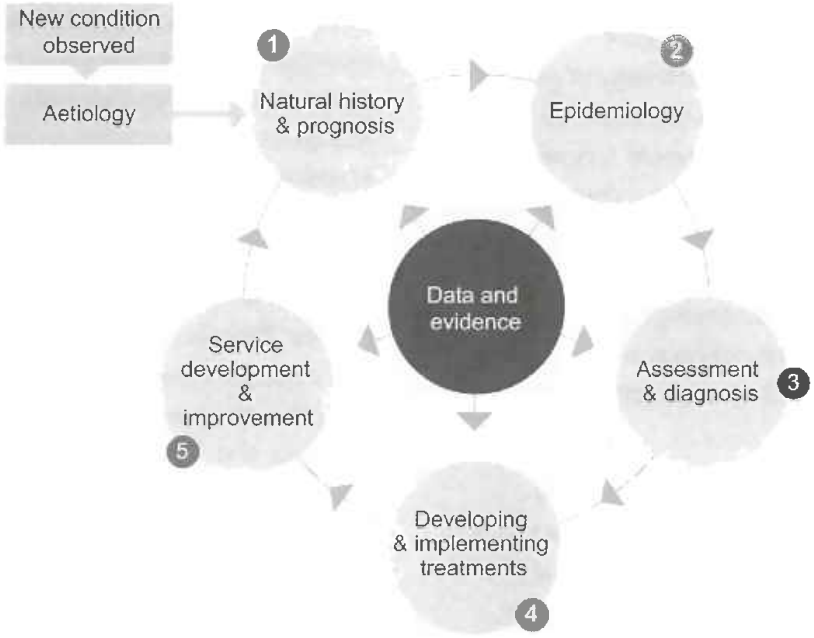
5.2. Some earlier information is necessarily repeated here, but this is with the intention of providing a more accessible explanation of the standards and processes which govern clinical service development. This is essential to an understanding of the rationale for the Review's recommendations.

5.3. Because the specialist service has evolved rapidly and organically in response to demand, the clinical approach and overall service design has not been subjected to some of the normal quality controls that are typically applied when new or innovative treatments are introduced. This Review now affords everyone concerned the opportunity to step back and consider from first principles what this cohort of children and young people now need from NHS services, based on the evidence that exists, or additional evidence that the Review hopes to collect.

5.4. In **Appendix 4** we have described the service development process for three different conditions which may help to illustrate what would be expected to happen at each different stage of developing a clinical service. The steps may proceed in a different sequence for different conditions, but each step is important in the development of evidence based care.

5.5. We recognise that for some of those reading this report it may feel wrong to compare gender incongruence or dysphoria to clinical conditions, and indeed this approach would not be justified if individuals presenting with these conditions did not require clinician intervention. However, where a clinical intervention is given, the same ethical, professional and scientific standards have to be applied as to any other clinical condition.

Key stages of service development



New condition observed: This often begins with a few case reports and then clinicians begin to recognise a recurring pattern and key clinical features, and to develop fuller descriptions of the condition.

Aetiology: Clinicians and scientists try to work out the cause of the condition or the underlying physical or biological basis. Sometimes the answers to this are never found.

Natural history and prognosis: It is important to understand how a condition usually evolves over time, with or without treatment. The latter is important if treatment has limited efficacy and the condition is 'self-limiting' (that is, it resolves without treatment), because otherwise there is a risk that treatments create more difficulties than the condition itself.

5.6. The first UK service for gender-questioning children and young people was established in 1989. At that time there were very few children and young people being seen by medical services internationally. The most common presentation in the early years of the service was of birth-registered

boys who had demonstrated gender incongruence from an early age.[51,52,53]

5.7. There is extensive literature discussing the possible aetiology of gender incongruence. Based on the available evidence, many authors would suggest that it is likely that biological, cultural, social and psychological factors all contribute. The examples in **Appendix 4** show that this is not an uncommon situation; many conditions do not have a single clear causation – they are in other words 'multifactorial'.

5.8. Regardless of aetiology, the more contentious and important question is how fixed or fluid gender incongruence is at different ages and stages of development, and whether, regardless of aetiology, can be an inherent characteristic of the individual concerned. There is a spectrum of academic, clinical and societal opinion on this. At one end are those who believe that gender identity can fluctuate over time and be highly mutable and that, because gender incongruence or gender-related distress may be a response to many psychosocial factors, identity may sometimes change or the distress may resolve in later adolescence or early adulthood, even in those whose early incongruence or distress was quite marked. At the other end are those who believe that gender incongruence or dysphoria in childhood or adolescence is generally a clear indicator of that child or young person being transgender and question the methodology of some of the desistance studies. Previous literature has indicated that if gender incongruence continues into puberty, desistance is unlikely.[54,55] However, it should be noted that these older studies were not based on the current changed case-mix or the different socio-cultural climate of recent years, which may have led to different outcomes. Having an open discussion about these questions is essential if a shared understanding of how to provide appropriate assessment and treatment is to be reached.

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[51] Zucker KJ (2017). Epidemiology of gender dysphoria and transgender identity. Sex Health 14(5): 404–11. DOI:10.1071/SH1.

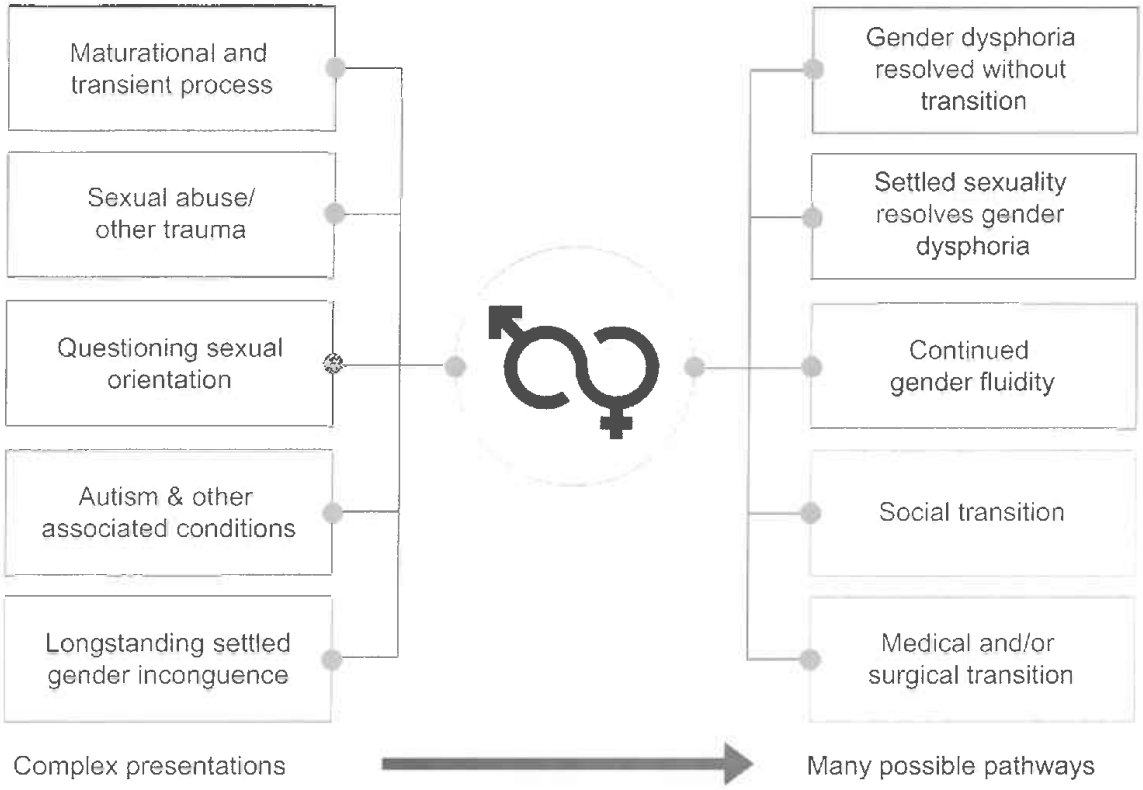
[52] Zucker KJ, Lawrence AA (2009). Epidemiology of gender identity disorder: recommendations for the Standards of Care of the World Professional Association for Transgender Health. Int J Transgend 11(1): 8-18. DOI: 10.1080/15532730902799946.

[53] de Graaf NM, Giovanardi G, Zitz C, Carmichael P (2018). Sex ratio in children and adolescents referred to the gender identity development service in the UK (2009-2016). Arch Sex Behav 47(5): 1301–4.

[54] Steensma TD, Biemond R, de Boer F, Cohen-Kettenis PT (2011). Desisting and persisting gender dysphoria after childhood: a qualitative follow-up study. Clin Child Psychol Psychiatry 16(4): 485-97. DOI: 10.1177/1359104510378037.

[55] Steensma TD, McGuire JK, Kreukels BPC, Beekman AJ, Cohen-Kettenis PT (2013). Factors associated with desistence and persistence of childhood gender dysphoria: a quantitative follow-up study. J Am Acad Child Adolesc Psychiatry 52: 582-590. DOI: 10.1016/j.jaac.2013.03.016.

Complex presentations and complex pathways – exemplars, not comprehensive lists



Epidemiology: Epidemiologists collect data to find out how common a condition is, who is most likely to be affected, what the age distribution is and so on. This allows health service planners to work out how many services are needed, where they should be established, and what staff are needed.

They also report on changes in who is most affected, which may mean that either the disease is changing, or the susceptibility of the population is changing.

5.9. As previously indicated, the epidemiology of gender dysphoria is changing, with an increase in the numbers of birth-registered females presenting in early teens.[56,57] In addition, the majority of children and young people presenting to GIDS have other complex mental health issues and/or neurodiversity.[58] There is also an over-representation of looked after children.[59]

5.10. There are several implications arising from the change in epidemiology:

- Firstly, the speed of change in the numbers presenting means that services have not kept pace with demand.
- Secondly, the cohort that the original Dutch Approach was based on is different from the current more complex NHS cohort, and also from the current case-mix internationally, and therefore it is difficult to extrapolate from older literature to this current group.
- Thirdly, different subgroups may have quite different needs and outcomes, and these must be built into any service design, so that it works for all children and young people.

5.11. At present we have the least information for the largest group of patients – birth-registered females first presenting in early teen years. Since the rapid increase in this group began around 2015, they will not reach late 20s for another 5+ years, which would be the best time to assess longer-term wellbeing.

[56] Steensma TD, Cohen-Kettenis PT, Zucker KJ (2018). Evidence for a change in the sex ratio of children referred for gender dysphoria: Data from the Center of Expertise on Gender Dysphoria in Amsterdam (1988-2016). Journal of Sex & Marital Therapy 44(7): 713–5. DOI: 10.1080/0092623X.2018.1437580.
[57] de Graaf NM, Carmichael P, Steensma TD, Zucker KJ (2018). Evidence for a change in the sex ratio of children referred for Gender Dysphoria: Data from the Gender Identity Development Service in London (2000–2017). J Sex Med 15(10): 1381–3. DOI: 10.1016/j.jsxm.2018.08.002.
[58] Van Der Miesen AIR, Hurley H, De Vries ALC (2016). Gender dysphoria and autism spectrum disorder: A narrative review. Int Rev Psychiatry 28: 70-80. DOI: 10.3109/09540261.2015.1111199.
[59] Matthews T, Holt V, Sahin S, Taylor A, Griksaitis (2019). Gender Dysphoria in looked-after and adopted young people in a gender identity development service. Clinical Child Psychol Psychiatry 24: 112-128. DOI: 10.1177/1359104518791657.

Assessment and diagnosis: Clinicians will usually take a history from (that is, of their symptoms) and examine the patient (that is, for signs and symptoms), and where appropriate undertake a series of investigations or tests, to help them reach an accurate diagnosis.

Sometimes the whole process of making a diagnosis through talking to the patient and asking them to complete formal questionnaires, examining them and/or undertaking investigations is called 'clinical assessment'.

As well as diagnosing and ruling out a particular condition, clinicians often need to consider and exclude other, sometimes more serious, conditions that present in a similar way but may need quite different treatment – this process is called 'differential diagnosis'.

5.12. For children and young people with gender-related distress, many people would dispute the notion that 'making a diagnosis' is a meaningful concept, arguing that gender identity is a personal, internal perception of oneself. However, there are several reasons to why a diagnostic framework is used:

- Firstly, the clinician will seek to determine whether the child or young person has a stable transgender identity, or whether there might be other causes for the gender-related distress.
- Secondly, the clinician will determine whether there are other issues or diagnoses that might be having an impact on the young person's mental health. The Dutch Approach suggesting that these should be addressed prior to or alongside initiation of any medical treatments.
- Thirdly, in any situation where life-altering treatments are being administered, the clinician holds the responsibility for ensuring that they are being administered based on an appropriate decision making process. Therefore, it is usual practice for a diagnosis of gender dysphoria to be made prior to referring for any physical treatments.

5.13. When the word 'diagnosis' is used, people often associate this with the use of blood tests, X-rays, or other laboratory tests. As set out in the **Appendix 4**, the public is very familiar with diagnosis of Covid-19 and understands that there need to be tests that give a high degree of certainty about whether an individual is Covid-19 positive or not. False positive lateral flow tests are rare, but caused problems for schools, while PCR has been treated as the 'gold standard' test for accuracy.

5.14. When it comes to gender dysphoria, there are no blood tests or other laboratory tests, so assessment and diagnosis in children and young people with gender-related distress is reliant on the judgements of experienced clinicians. Because medical, and subsequently possibly surgical treatments will follow, it may be argued that a highly sensitive and specific assessment process is required. The assessment should be able to accurately identify those children or young people for whom physical intervention is going to be the best course of action, but it is equally important that it identifies those who need an alternative pathway or treatment.

5.15. The formal criteria for diagnosing gender dysphoria (DSM-5) are listed in **Appendix 3**. However, there are two problems associated with the use of these criteria:

- Firstly, several of the criteria are based on gender stereotyping which may not be deemed relevant in current society, although the core criteria remain valid.
- Secondly, and more importantly, these criteria give a basis on which to make a diagnosis that a young person is clinically distressed by the incongruence between their birth-registered and their experienced gender, but they do not help in determining which factors may have led to this distress and how they might best be resolved.

5.16. At present, the assessment process varies considerably, dependent on the perceptions, experience and beliefs of different clinicians. There are some existing measurement tools, but it is suggested that these have substantial limitations.[60]

5.17. The challenges are similar to the early difficulties in diagnosing autism, as set out in **Appendix 4**. As with autism, the framework for assessment needs to become formalised so there are clearer criteria for diagnosis and treatment pathways which are shared more widely. These should incorporate not just whether the child or young person meets DSM-5 criteria for gender dysphoria, but how a broader psychosocial assessment should be conducted and evaluated, and what other factors need to be considered to gain a holistic understanding of the child or young person's experience. Professional judgement and experience will still be important, but if the frameworks and criteria for assessment and diagnosis were more consistent and reproducible, there would be a greater likelihood that two different people seeing the same child or young person would come to the same conclusion. This would also mean that any research on interventions or long-term outcomes would be more reliable because the criteria on which a diagnosis was made, and hence the patients within the sample, would have the same characteristics.

[60] Bloom TM, Nguyen TP, Lami F, Pace CC, Poulakis Z, Telfer N (2021). Measurement tools for gender identity, gender expression, and gender dysphoria in transgender and gender-diverse children and adolescents: a systematic review. Lancet Child Adolescent Health. 5: 582-588. DOI: 10.1016/s2352-4642(21)00098-5.

5.18. As outlined above, it is standard clinical practice to undertake a process called differential diagnosis. This involves summarising the main points of the clinical assessment, the most likely diagnosis, other possible diagnoses and the reasons for including or excluding them, as well as any further assessments that may be required to clarify the diagnosis and the treatment options and plan. This is important when a medical intervention is being provided on the basis of the assessment, so the process is robust, explicit and reproducible. These considerations need to be applied to the assessment of children and young people presenting with gender-related distress. In mental health services, practitioners may also undertake a diagnostic or psychological formulation, which is a holistic summary of how the patient is feeling and why, and how to make sense of it, and a plan for moving forward with management or treatment.

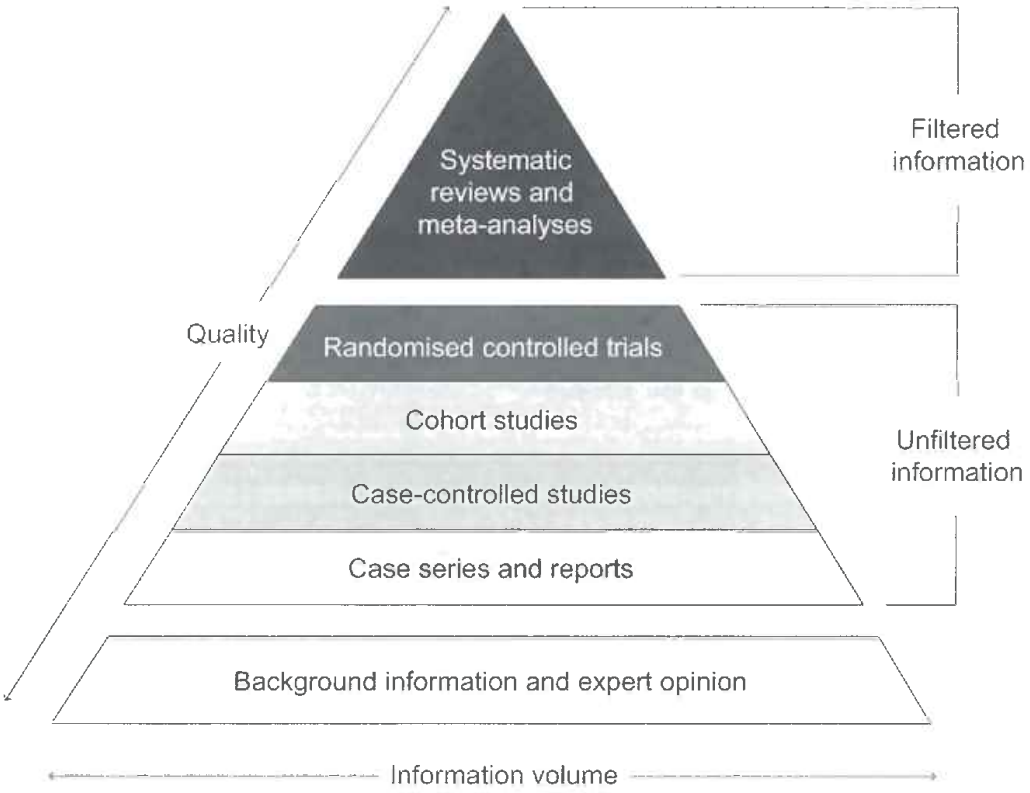
Developing and implementing new treatments: Clinicians and scientists work on developing treatments. This involves clinical trials and, where there are new treatments, comparing them to any existing treatments. Questions include: What are the intended outcomes or benefits of treatment? What are the complications or side effects? What are the costs? To initiate a new treatment, it must be both safe and effective. Questions of affordability can sometimes become controversial.

The best type of single study is considered to be the randomised controlled trial (RCT), but sometimes this is not feasible. Even where RCTs are not available, it is usual to at least have data on the outcomes of sufficient cases or cohorts to understand the risk/benefit of the treatment under consideration. As demonstrated in Fig. 4, the highest level of evidence is when the results of several different studies are pooled, but this is only useful if the individual studies themselves are of high quality.

In many instances, evidence is not perfect and difficult decisions have to be made. Where treatments are innovative or life-changing, the whole multi-disciplinary team will usually meet to consider the available options, and how to advise the child or young person and family so that a shared decision can be made. Sometimes an ethics committee is involved. This is one of the most challenging areas of medicine and is underpinned by GMC guidance.[61,62]

[61] General Medical Council (2020). Decision making and consent.
[62] National Institute for Health and Care Excellence (2021). Shared decision making.

Figure 3: Pyramid of standards of evidence



Source: Levels of evidence pyramid, OpenMD. Reproduced with permission[63]

5.19. There are three types of intervention or treatment for children and young people with gender-related distress, which may be introduced individually or in combination with one another:

- **Social transition** – this may not be thought of as an intervention or treatment, because it is not something that happens within health services. However, it is important to view it as an active intervention because it may have significant effects on the child or young person in terms of their psychological functioning.[64,65] There are different views on the benefits versus the harms of early social transition. Whatever position one

[63] OpenMD (2021). New Evidence in Medical Research.
[64] Sievert EDC, Schweizer K, Barkmann C, Fahrenkrug S, Becker-Hebly I (2020). Not social transition status, but peer relations and family functioning predict psychological functioning in a German clinical sample of children with Gender Dysphoria. Clin Child Psychol Psychiatry 26(1): 79–95. DOI: 10.1177/1359104520964530
[65] Ehrensaft D, Giammattei SV, Storck K, Tishelman AC, Colton K-M (2018). Prepubertal social gender transitions: What we know; what we can learn—A view from a gender affirmative lens. Int J Transgend 19(2): 251–68. DOI: 10.1080/15532739.2017.1414649.

takes, it is important to acknowledge that it is not a neutral act, and better information is needed about outcomes.

- **Counselling, social or psychological interventions** – these may be offered before, instead of, or alongside physical interventions. Again, they should be viewed as active interventions which require robust evaluation in their own right.
- **Physical treatments** – these comprise puberty blockers and feminising/masculinising hormones (administered by endocrinologists) and surgery. The latter is not considered as part of this Review since it is not available to those under age 18.

5.20. It should also be recognised that 'doing nothing' cannot be considered a neutral act.

5.21. The lack of available high-level evidence was reflected in the recent NICE review into the use of puberty blockers and feminising/masculinising hormones commissioned by NHS England, with the evidence being too inconclusive to form the basis of a policy position.[66,67] Assessing treatments for gender dysphoria has many of the same problems as assessing treatment for children with autism – it can take many years to get a full appreciation of outcomes and there may be other complicating factors in the child or young person's life during this period. However, this of itself is not an adequate reason for the major gaps in the international literature.

5.22. It is still common that drugs are not specifically licensed for children because the trials have only taken place on adults. This does not preclude their use or make their use inherently unsafe, particularly if they are used very commonly in children. However, where their use is innovative, patients receiving the drug should ideally do so under trial conditions.

5.23. The same considerations apply to 'off-label' drugs, where the drug is used for a condition different to the one for which it was licensed. This is the case for puberty blockers, which are licensed for use in precocious puberty, but not for puberty suppression in gender dysphoria. Again, it is important that it is not assumed that outcomes for, and side effects in, children treated for precocious puberty will necessarily be the same in children or young people with gender dysphoria.

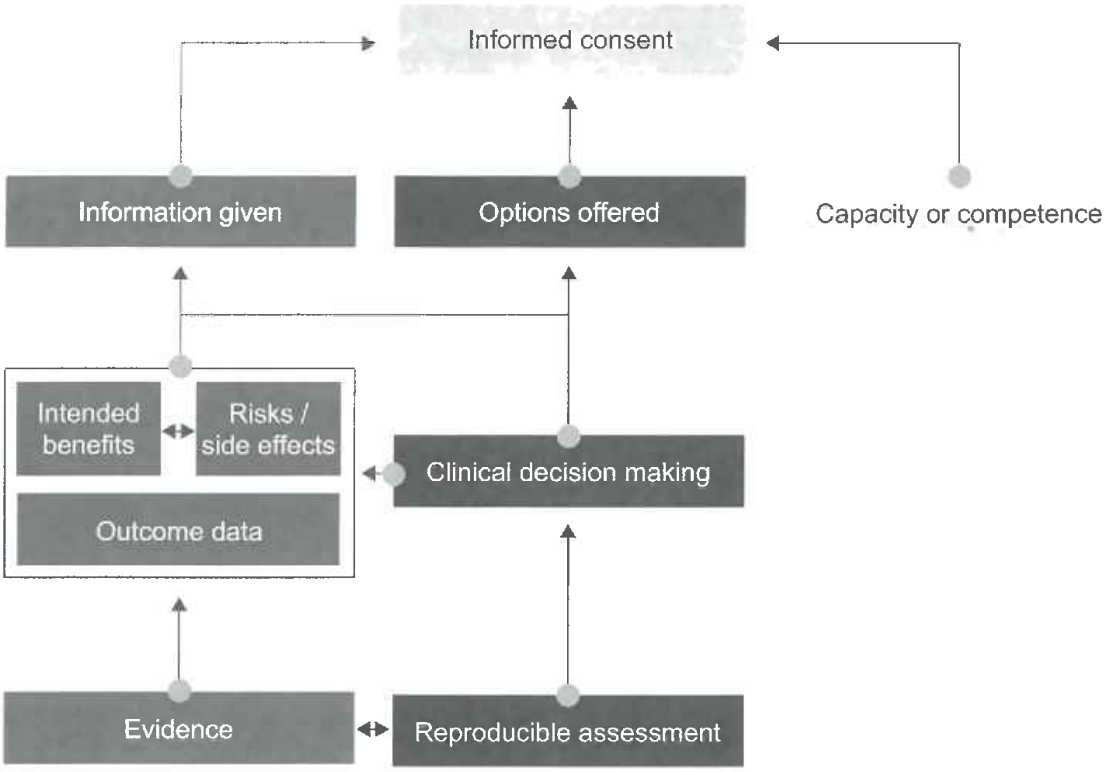
5.24. As outlined above, in other areas of practice where complex or potentially life-altering treatment is being considered for a child or young person, it is usual for the case to be discussed by an MDT including all professionals involved in their care. In gender services for children and young people in the Netherlands, as well as a number of other countries, there are full

[66] National Institute for Health and Care Excellence (2020). Evidence Review: Gonadotrophin Releasing Hormone Analogues for Children and Adolescents with Gender Dysphoria
[67] National Institute for Health and Care Excellence (2020). Evidence review: gender-affirming hormones for children and adolescents with gender dysphoria.

MDT meetings, including psychiatrists and endocrinologists, to make decisions about suitability for hormone intervention and to review progress.[68,69]

5.25. Recent legal proceedings have examined the question of the competence and capacity of children and young people to consent to hormone treatment. However, there are some essential components that underpin informed consent; the robustness of the options offered to the patient, the information provided to them about those options, and their competence and capacity to consider them. The courts have given consideration to competence and capacity, and it is incumbent on this Review to consider the soundness of the decision making which underpins the options offered, and the quality and accuracy of the information provided about those options.

Elements of informed consent



[68] Kyriakou A, Nicolaides NC, Skordis N (2020). Current approach to the clinical care of adolescents with gender dysphoria. Acta Biomed 91(1): 165–75. DOI: 10.23750/abm.v91i1.9244.
[69] Cohen-Kettenis PT, Steensma TD, de Vries ALC. Treatment of adolescents with gender dysphoria in the Netherlands. Child Adolesc Psychiatr Clin N Am 20. 689–700. 2001. DOI: 10.1016/j.chc.2011.08.001

Service development and service improvement: Central to any service improvement is the systematic and consistent collection of data on outcomes of treatment. There is a process of continuous service improvement as new presentations or variations on the original condition are recognised, diagnosis or screening improves and/or trials on new treatments or variations on existing treatments are ongoing.

There should be consistent treatment protocols or guidelines in place, in order to make sense of variations in outcomes. Where possible, these should be compared between and across multiple different centres.

As time passes, services need to be changed or extended based on patient need, and on what resources are needed to deliver the available treatments. They need to be accessible where the prevalence of the condition is highest. The relevant workforce to deliver the service needs to be recruited and trained, contingent on the type of treatments or therapy that is required.

5.26. When a pioneering treatment or specialist service starts, it is often delivered in a single centre. Thereafter, additional centres take on the work as increasing numbers of patients need to access the treatment. Current provision of NHS specialist gender identity services for children and young people has remained concentrated within a single organisation, but demand has grown dramatically.

5.27. The situation has been exacerbated because there are not many local services seeing gender-questioning children at an earlier stage in their journey, which means that GIDS is carrying an unsustainable workload of increasingly complex young people.

5.28. As a condition evolves, rigorous data collection and quantitative research is an essential prerequisite to refining understanding and treatment. Historically, The Tavistock and Portman NHS Foundation Trust built its international reputation as the home of psychoanalysis, psychotherapy and family therapy, with a strong track record of publishing qualitative rather than quantitative research; consequently its approach to quantitative data collection about this important group of children and young people has been weak.

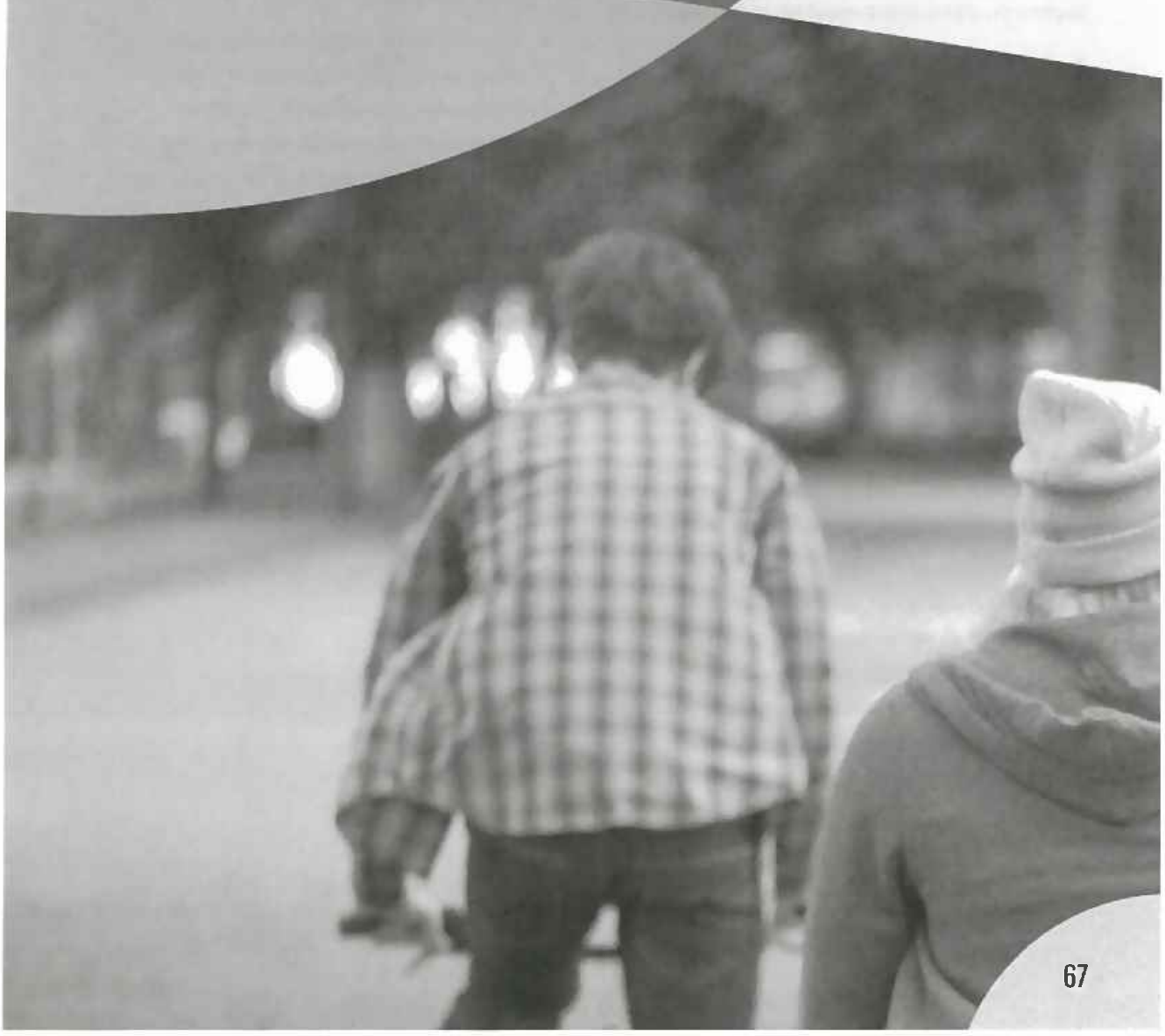
5.29. A further anomaly is a public perception that The Tavistock and Portman NHS Foundation Trust is the responsible organisation for leading the management of children receiving hormone treatment for their gender dysphoria. In reality, the hormone treatment is delivered by paediatric services in University College London Hospitals NHS Foundation Trust and The Leeds Teaching Hospitals NHS Trust.

5.30. In practice, it is important that for children and young people who need physical intervention, paediatric and mental health services are seen as equal partners, with seamless joint working and shared responsibility. When there were very small numbers of patients, it was easier for this to be achieved, but cross-site working with a very large caseload has made this more difficult to achieve, despite the best intentions of the staff.

5.31. Over the last two years there have been strong efforts on the part of The Tavistock and Portman NHS Foundation Trust to make practice within GIDS more consistent, with tighter procedures for case management, consent, and safeguarding. However, although this has resulted in better documentation, variations and inconsistencies in clinical decision making remain. In responding to a changing legal framework, some processes have become more cumbersome and complex, and the team are working hard to streamline the process.

5.32. Overall, GIDS faces a daunting task as a single provider in managing risk on the waiting list, seeing new referrals, reviewing and supporting those on hormone treatment, undertaking an ongoing transformation programme, recruiting and training new staff and trying to retain existing staff. This suggests that the current model is not sustainable and that another model is needed.

6. Interim advice, research programme and next steps



Dealing with uncertainty

6.1. As outlined throughout this report, there are major gaps in the research base underpinning the clinical management of children and young people with gender incongruence and gender dysphoria, including the appropriate approaches to assessment and treatment.

6.2. As with any other area of medicine, where there are gaps in the evidence base and uncertainties about the correct clinical approach, three tasks must be undertaken:

- Clinical services must be run as safely and effectively as possible, within the constraints of current knowledge; treatment options must be weighed carefully; and treatment decisions must be made in partnership between the clinicians and the children, young people and their families and carers, based on our current understanding about outcomes.
- Consistent data must be collected by clinical services, for both audit and research purposes so that knowledge gaps can be filled, alongside an active research programme.
- Where there is not an immediate prospect of filling research gaps, professional consensus should be developed on the correct way to proceed pending clearer research evidence, supported by input from service users.

6.3. The additional problem with the current service model is that safety and access are further compromised by the pace at which referrals have grown and outstripped capacity at tertiary level, and the lack of service availability at local level.

6.4. The Review's approach to these tasks is as follows:

- Our **interim advice** focuses on the issues of capacity, safety, and standards around treatment decisions, as well as data and audit.
- Our **research streams** will provide the Review with an independent collation of published evidence relevant to epidemiology, clinical management, models of care, and outcomes, as well as delivering qualitative and quantitative research relevant to the Terms of Reference of the Review. This offers a real opportunity to contribute to the international evidence base for this service area.
- There will be an ongoing and wide-ranging **programme of engagement** to address areas on which we will not be able to obtain definitive evidence during the lifetime of the Review.

Interim advice

6.5. The Review considers that there are some areas where there is sufficient clarity about the way forward and we are therefore offering some specific observations and interim advice. The Review will work with NHS England, providers and the broader stakeholder community to progress action in these areas.

Service model

6.6. It has become increasingly clear that a single specialist provider model is not a safe or viable long-term option in view of concerns about lack of peer review and the ability to respond to the increasing demand.

6.7. Additionally, children and young people with gender-related distress have been inadvertently disadvantaged because local services have not felt adequately equipped to see them. It is essential that they can access the same level of psychological and social support as any other child or young person in distress, from their first encounter with the NHS and at every level within the service.

6.8. A fundamentally different service model is needed which is more in line with other paediatric provision, to provide timely and appropriate care for children and young people needing support around their gender identity. This must include support for any other clinical presentations that they may have.

6.9. The Review supports NHS England's plan to establish regional services, and welcomes the move from a single highly specialist service to regional hubs.

6.10. Expanding the number of providers will have the advantages of:

- creating networks within each area to improve early access and support;
- reducing waiting times for specialist care;
- building capacity and training opportunities within the workforce;
- developing a specialist network to ensure peer review and shared standards of care; and
- providing opportunities to establish a more formalised service improvement strategy.

Service provision

6.11. The primary remit of NHS England's proposed model is for the regional hubs to provide support and advice to referrers and professionals. However, it includes limited provision for direct contact with children and young people and their families.

1: The Review advises that the regional centres should be developed, as soon as feasibly possible, to become direct service providers, assessing and treating children and young people who may need specialist care, as part of a wider pathway. The Review team will work with NHS England and stakeholders to further define the proposed model and workforce implications.

2: Each regional centre will need to develop links and work collaboratively with a range of local services within their geography to ensure that appropriate clinical, psychological and social support is made available to children and young people who are in early stages of experiencing gender distress.

3: Clear criteria will be needed for referral to services along the pathway from primary to tertiary care so that gender-questioning children and young people who seek help from the NHS have equitable access to services.

4: Regional training programmes should be run for clinical practitioners at all levels, alongside the online training modules developed by Health Education England (HEE). In the longer-term, clearer mapping of the required workforce, and a series of competency frameworks will need to be developed in collaboration with relevant professional organisations.

Data, audit and research

6.12. A lack of routine and consistent data collection means that it is not possible to accurately track the outcomes and pathways children and young people take through the service. Standardised data collection is required in order to audit service standards and inform understanding of the epidemiology, assessment and treatment of this group. This, alongside a national network which brings providers together, will help build knowledge and improve outcomes through shared clinical standards and systematic data collection. In the longer-term, formalisation of such a network into a learning health system[70] with an academic host would mean that there was systematised use of data to produce a continuing research programme with rapid translation into clinical practice and a focus on training.

5: The regional services should have regular co-ordinated national provider meetings and operate to shared standards and operating procedures with a view to establishing a formal learning health system.

6: Existing and future services should have standardised data collection in order to audit standards and inform understanding of the epidemiology, assessment and treatment of this group of children and young people.

[70] Scobie S. Castle-Clarke S (2019). Implementing learning health systems in the UK NHS: Policy actions to improve collaboration and transparency and support innovation and better use of analytics. Learning Health Systems 4(1): e10209. DOI:10.1002/lrh2.10209.

7: Prospective consent of children and young people should be sought for their data to be used for continuous service development, to track outcomes, and for research purposes. Within this model, children and young people put on hormone treatment should be formally followed up into adult services, ideally as part of an agreed research protocol, to improve outcome data.

8: There needs to be agreement and guidance about the appropriate clinical assessment processes that should take place at primary, secondary and tertiary level.

9: Assessments should be respectful of the experience of the child or young person and be developmentally informed. Clinicians should remain open and explore the patient's experience and the range of support and treatment options that may best address their needs, including any specific needs of neurodiverse children and young people.

Clinical approach

Assessment processes

6.13. We have heard that there are inconsistencies and gaps in the assessment process. Our work to date has also demonstrated that clinical staff have different views about the purpose of assessment and where responsibility lies for different components of the process within the pathway of care. The Review team has commenced discussions with clinical staff across primary, secondary and tertiary care to develop a framework for these processes.

Hormone treatment

6.14. The issues raised by the Multi-Professional Review Group echo several of the problems highlighted by the CQC. It is essential that principles of the General Medical Council's Good Practice in Prescribing and Managing Medicine's and Devices[71] are closely followed, particularly given the gaps in the evidence base regarding hormone treatment. Standards for decision making regarding endocrine treatment should also be consistent with international best practice.[72,73,74]

[71] General Medical Council (2021). Good practice in prescribing and managing medicines and devices (76-78).
[72] Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 102(11): 3869–903. DOI: 10.1210/jc.2017-01658.
[73] Cohen-Kettenis PT, Steensma TD, de Vries ALC (2001). Treatment of adolescents with gender dysphoria in the Netherlands. Child Adolesc Psychiatr Clin N Am 20: 689–700. DOI: 10.1016/j.chc.2011.08.001.
[74] Kyriakou A, Nicolaides NC, Skordis N (2020). Current approach to the clinical care of adolescents with gender dysphoria. Acta Biomed 91(1): 165–75. DOI: 10.23750/abm.v91i1.9244.

10: Any child or young person being considered for hormone treatment should have a formal diagnosis and formulation, which addresses the full range of factors affecting their physical, mental, developmental and psychosocial wellbeing. This formulation should then inform what options for support and intervention might be helpful for that child or young person.

11: Currently paediatric endocrinologists have sole responsibility for treatment, but where a life-changing intervention is given there should also be additional medical responsibility for the differential diagnosis leading up to the treatment decision.

6.15. Paediatric endocrinologists develop a wide range of knowledge within their paediatric training, including safeguarding, child mental health, and adolescent development. Being party to the discussions and deliberations that have led up to the decision for medical intervention supports them in carrying out their legal responsibility for consent to treatment and the prescription of hormones.

12: Paediatric endocrinologists should become active partners in the decision making process leading up to referral for hormone treatment by participating in the multidisciplinary team meeting where children being considered for hormone treatment are discussed.

6.16. Given the uncertainties regarding puberty blockers, it is particularly important to demonstrate that consent under this circumstance has been fully informed and to follow GMC guidance[75] by keeping an accurate record of the exchange of information leading to a decision in order to inform their future care and to help explain and justify the clinician's decisions and actions.

13: Within clinical notes, the stated purpose of puberty blockers as explained to the child or young person and parent should be made clear. There should be clear documentation of what information has been provided to each child or young person on likely outcomes and side effects of all hormone treatment, as well as uncertainties about longer-term outcomes.

[75] General Medical Council (2020). Decision making and consent.

14: In the immediate term the Multi-Professional Review Group (MPRG) established by NHS England should continue to review cases being referred by GIDS to endocrine services.

Research programme

6.17. The Review's formal academic research programme, comprising a literature review, quantitative analysis and primary qualitative research, has been based on the identified gaps in the evidence and the feasibility of filling them within the lifetime of the Review.

6.18. Initial work has identified the existing evidence base on epidemiology, natural history, and the treatment and outcomes of children and young people with gender dysphoria/gender-related distress. It has also assessed the feasibility of linking data between local, regional or national datasets in order to assess intermediate and longer-term outcomes.

Literature review

6.19. A literature review is being undertaken, which will interface with evidence gathering from the professional community (see qualitative research section below). Its aim is to systematically identify, collate and synthesise the existing evidence on the changing epidemiology of gender-related distress in children and young people and the appropriate social, clinical, psychological and medical management of that distress.

6.20. The literature review will capture primary studies of any design, including experimental, observational, survey and qualitative, and is looking to answer the following questions:

1. How has the population of children and young people presenting with gender dysphoria and/or gender-related distress changed over time?
2. What are the appropriate referral, assessment and treatment pathways for children and young people with gender dysphoria and/or gender-related distress?
3. What are the short-, medium- and long-term outcomes for children and young people with gender dysphoria and/or gender-related distress?
4. How do children and young people and their families negotiate distress, present this distress to services, and what are their expectations, following presentation?
5. How do children, young people and their families/carers experience referral, assessment and treatment? And how are these negotiated among children and young people, parents/carers, families and healthcare professionals?

6.21. A separate synthesis for each question will be undertaken. The systematic review has been registered on PROSPERO [ID:289659].

Quantitative research

6.22. The National Institute for Health and Care Excellence (NICE) recently published two evidence reviews.[76,77] These highlight shortcomings in the follow-up data collected about children and young people, when they are referred to a specialist gender identity service. The quantitative research will therefore focus on the collection and analysis of data to uncover patterns and quantify problems, thereby helping the Review to address some of these shortcomings.

6.23. The aim of the quantitative study is to supplement the material collected by the literature review, further examining the changing epidemiology of gender-related distress in children and young people, in addition to exploring the appropriate social, clinical, psychological and medical management. Its objectives are to:

a) describe the clinical and demographic characteristics of this population of children and young people and their clinical management in the GIDS service; and

b) assess the intermediate and longer-term outcomes of this population of children and young people utilising national healthcare data.

6.24. This research will provide an evidence base to facilitate informed decision making among children and young people and their families. It will also provide an evidence base for those responsible for commissioning, delivering and managing services.

Qualitative research

6.25. The qualitative research will capture a diverse range of trajectories experienced by gender-questioning children and young people, exploring a range of different experiences and outcomes. This will include talking to children and young people and their families/carers who are currently negotiating gender-related distress, young adults who have gone through the process of resolving their distress and care professionals.

[76] National Institute for Health and Care Excellence (2020). Evidence Review: Gonadotrophin Releasing Hormone Analogues for Children and Adolescents with Gender Dysphoria.
[77] National Institute for Health and Care Excellence (2020). Evidence review: gender-affirming hormones for children and adolescents with gender dysphoria.

The objectives of the qualitative research are to:

1. Explore how children and young people understand, respond and negotiate gender-related distress within the context of their social networks, alongside the perspectives of young adults who experienced gender distress as children.
2. Examine the perspectives, understandings and responses of parents (or carers), including how they support their child.
3. Investigate how children, young people, young adults and their families experience(d) and negotiate(d) referral, assessment and possible treatment and intervention options.
4. Understand the role and experiences of care professionals who offer support, including identifying shared and potentially divergent views among care professionals, children and young people, and parents of what constitutes optimal care.

Progress

6.26. The literature review is already underway and is identifying relevant studies. Initial meetings have also taken place with voluntary organisations and other researchers working in the area to ensure there is no duplication and in recognition of research fatigue among this population.

6.27. Children and young people and young adults who have experienced gender-related distress are involved in the research programme. Their advice has been, and will continue to be, sought throughout this work, including in relation to the focus of the research and interpretation of findings and the design and content of dissemination materials.

6.28. Three research protocols have been produced setting out how the research will be undertaken, and the research team is currently gaining the necessary ethical and governance approvals to progress the study. The systematic review is published on the PROSPERO website and will be published on the Review website in due course, along with the qualitative and quantitative research proposals once ethical and governance approvals have been received.

6.29. The research findings will be subject to peer review through the publication process and various summaries, aimed at different audiences, will be available on the project website and distributed via support organisations. These summaries will also be made available on the Review website.

Ongoing engagement

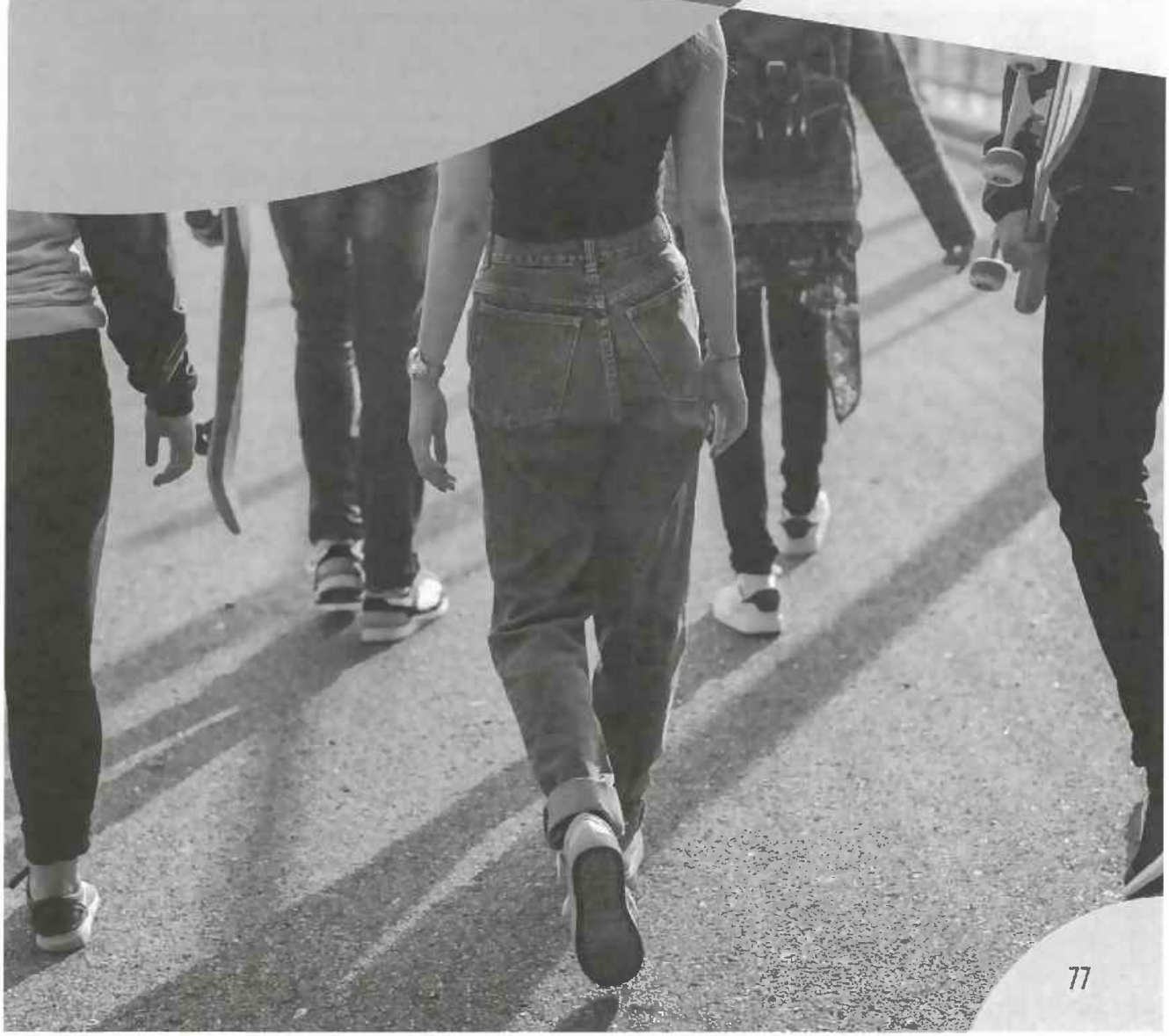
6.30. In recognition that not all the published evidence is likely to be of high enough quality to form the sole basis for our recommendations, a consensus development approach will be used to synthesise the published evidence and research outputs of the academic work with stakeholder submissions and expert opinion.

6.31. Over the coming months, the Review will build on its engagement to date and, alongside the academic research programme, will continue informal and structured engagement with service users, their families, support and advocacy groups and professionals to test emerging thinking, provide opportunities for challenge and further develop the evidence base.

6.32. This review is an iterative process and we will share important findings when they become available. For the latest updates, please visit our website: https://cass.independent-review.uk/

6.33. We thank those who have participated in the Review to date and welcome engagement with us as work progresses towards final recommendations.

Glossary



Glossary

There is sometimes no consensus on the best language to use relating to this subject. The language surrounding this area has also changed rapidly and young people have developed varied ways of describing their experiences using different terms and constructs that are relevant to them.

The Review tries as far as possible to use language and terms that are respectful and acknowledge diversity, but that also accurately illustrate the complexity of what we are trying to describe and articulate.

The terms we have used may not always feel right to some; nevertheless, it is important to emphasise that the language used is not an indication of a position being taken by the Review. The glossary below sets out a description of some of the terms we have used in the Review.

Term		Description
Affirmative model		A model of gender healthcare that originated in the USA[78,79,80,81] which affirms a young person's subjective gender experience while remaining open to fluidity and changes over time. This approach is used in some key child and adolescent clinics across the Western world.
Assent		To agree to or approve of something (idea, plan or request), especially after thoughtful consideration.
Autonomy		Personal autonomy is the ability of a person to make their own decisions. In health this refers specifically to decisions about their care.

[78] Hidalgo MA, Ehrensaft D, Tishelman AC, Clark LF, Garofalo R, Rosenthal SM, et al (2013). The gender affirmative model: What we know and what we aim to learn [Editorial]. Human Dev 56(5): 285–290. DOI:10.1159/000355235.

[79] Chen D, Abrams M, Clark L, Ehrensaft D, Tishelman AC, Chan YM, et al (2021). Psychosocial characteristics of transgender youth seeking gender-affirming medical treatment: baseline findings from the trans youth care study. J Adol Health 68(6): 1104–11.

[80] Olson-Kennedy J, Chan YM, Rosenthal S, Hidalgo MA, Chen D, Clark L, et al (2019). Creating the Trans Youth Research Network: A collaborative research endeavor. Transgend Health 4(12): 304–12. DOI: 10.1089/trgh.2019.0024.

[81] Ehrensaft D, Giammattei SV, Storck K, Tishelman AC, Colton K-M (2018). Prepubertal social gender transitions: What we know; what we can learn—A view from a gender affirmative lens. Int J Transgend 19(2): 251–68. DOI: 10.1080/15532739.2017.1414649.

Term		Description
Best interests		Clinicians and the courts seek to act in the best interests of children and young people. For the Mental Capacity Act (MCA) 2005, decisions for someone who cannot decide for themselves must be made in their best interests. Under the Children Act 1989, in any decision of the court about a child (under 18), the welfare of the child must be paramount. For these purposes, there is little or no material difference between the welfare and best interests, and we have used "best interests" throughout the report. Although there is no standard definition of "best interests of the child," the General Medical Council advises that an assessment of best interests will include what is clinically indicated as well as additional factors such as the child or young person's views, the views of parents and others close to the child or young person and cultural, religious and other beliefs and values of the child or young person.[82]
		The MCA s4,[83] and extensive Court of Protection case law, deals with the approach to best interests under that legislation. Whether in the Court of Protection or the High Court, when the court is asked to make an assessment of a child or young person's best interests, it will consider their welfare/best interests in the widest sense. This will include not just medical factors but also social and psychological factors.
Case-mix		The mix of patients within a particular group.
Child and adolescent mental health services	CAMHS	NHS children and young people's mental health services.[84]

[82] General Medical Council (2018). 0-18 years – guidance for all doctors.
[83] Mental Health Law Online. MCA 2005 s4.
[84] Young Minds. Guide to CAMHS: a guide for young people.

Term		Description
Child and/or young person		In law, everyone under 18 years of age is a child (Children Act 1989) but we recognise that it may be more appropriate to refer to those approaching the age of 18 as a young person, and that such young people may not recognise themselves as a "child". In places, we have referred only to "young person", or only to "child", for example where treatment in question is only given towards the later stages of childhood, closer to the age of 18, or in reference to the parent/child relationship, in which they remain the parents' child, regardless of their age. Otherwise, we have used the phrase "child and/or young person" throughout the report for this reason only, and do not intend there to be a material difference between them other than that.
Cognitive		Relating to, or involving, the process of thinking and reasoning.
Consent		Permission for a clinical intervention (such as an examination, test or treatment) to happen. For consent to be 'informed', information must be disclosed to the person about relevant risks, benefits and alternatives (including the option to take no action), and efforts made to ensure that the information is understood. In legal terms, consent is seen as needing: 1 – capacity (or *Gillick* competence under 16) to make the relevant decision; 2 – to be fully informed (ie the information provided about the available options, the material risks and benefits of each option, and of doing nothing, "material" meaning (per the Montgomery Supreme Court judgment in 2015) what a reasonable patient would want to know, and what this patient actually wants to know, NOT what a reasonable doctor would tell them); and 3 – to be freely given (that is,without coercion).
Contraindications		A condition or circumstance that suggests or indicates that a particular technique or drug should not be used in the case in question.

Term		Description
Court of Appeal		(England and Wales) The Court of Appeal hears appeals against both civil and criminal judgments from the Crown Courts, High Court and County Court. It is second only to the Supreme Court.
Detransition/ detransitioners		Population of individuals who experienced gender dysphoria, chose to undergo medical and/or surgical transition and then detransitioned by discontinuing medications, having surgery to reverse the effects of transition, or both.[85]
Diagnostic and Statistical Manual of Mental Disorders Fifth edition	DSM-5	The American diagnostic manual used to diagnose mental health disorders, and commonly used in UK practice. See **Appendix 3**.
Diagnostic formulation		The comprehensive assessment that includes a patient's history, results of psychological tests, and diagnosis of mental health difficulties.
Divisional Court		(England and Wales) When the High Court of Justice of England and Wales hears a case with at least two judges sitting, it is referred to as the Divisional Court. This is typically the case for certain judicial review cases (as well as some criminal cases).
Dutch Approach		Protocol published in 1998 by the Amsterdam child and adolescent gender identity clinic.[86]
Endocrine treatment		In relation to this clinical area, this term is used to describe the use of gonadotropin-releasing hormones (see below) and feminising and masculinising hormones (see below).
Endocrinologist		An endocrinologist is a medical doctor specialising in diagnosing and treating disorders relating to problems with the body's hormones.
Endocrinology		The study of hormones.

[85] Littman L (2021). Individuals treated for gender dysphoria with medical and/or surgical transition who subsequently detransitioned: a survey of 100 detransitioners. Arch Sex Abuse 50: 3353–69. DOI: 10.1007/s10508-021-02163-w

[86] de Vries ALC, Cohen-Kettenis PT (2012). Clinical management of gender dysphoria in children and adolescents: The Dutch approach. J Homosex 59: 301-320. DOI: 10.1080/00918369.2012.653300.

Term		Description
Epidemiology		Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems.[87]
Exploratory approaches		Therapeutic approaches that acknowledge the young person's subjective gender experience, whilst also engaging in an open, curious, non-directive exploration of the meaning of a range of experiences that may connect to gender and broader self-identity.[88,89,90,91]
Feminising and masculinising hormones (also known as cross-sex hormones, and gender affirming hormones).		Hormones given as part of a medical transition for gender dysphoric individuals, where sex hormones (testosterone for transgender males and oestrogen for transgender females).
Gender dysphoria		Diagnostic term used in DSM-5.[92] Gender dysphoria describes "a marked incongruence between one's experienced/expressed gender and assigned gender of at least 6 months duration" which must be manifested by a number of criterion – see **Appendix 3** for further detail.
Gender fluid		An experience of gender that is not fixed, but changes between two or more identities.
Gender identity		This term is used to describe an individual's internal sense of being male or female or something else.
Gender identity development		The developmental experience of a child or young person in seeking to understand their gender identity over time.
Gender Identity Development Service	GIDS	The service that NHS England commissions for children and adolescents with gender dysphoria.

[87] Centers for Disease Control and Prevention (2012). Principles of Epidemiology in Public Health Practice: An introduction to Applied Epidemiology and Biostatistics, 3rd ed.
[88] Di Ceglie D (2009). Engaging young people with atypical gender identity development in therapeutic work: A developmental approach. J Child Psychother 35(1): 3–12. DOI: 10.1080/00754170902764868.
[89] Spiliadis A (2019). Towards a gender exploratory model: Slowing things down, opening things up and exploring identity development. Metalogos Systemic Ther J 35: 1–9.
[90] Churcher Clarke A, Spiliadis A (2019). 'Taking the lid off the box': The value of extended clinical assessment for adolescents presenting with gender identity difficulties. Clin Child Psychol Psychiatry 24(2): 338–52. DOI:10.1177/1359104518825288.
[91] Bonfatto M, Crasnow E (2018). Gender/ed identities: an overview of our current work as child psychotherapists in the Gender Identity Development Service. J Child Psychother 44(1): 29–46. DOI:10.1080/00754 17X.2018.1443150.
[92] American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Health Disorders: DSM-5™ 5th ed.

Term		Description
Gender incongruence		Diagnostic term used in ICD-11.[93] Gender incongruence is characterised by "a marked and persistent incongruence between an individual's experienced gender and the assigned sex". See **Appendix 3** for further detail.
Gender-questioning		A broader term that might describe children and young people who are in a process of working out how they want to present in relation to their gender.
Gender-related distress		A way of describing distress that may arise from a broad range of experiences connected to a child or young person's gender identity development. Often used for young people whereby any formal diagnosis of gender dysphoria has not yet been made.
Gillick competence/ Fraser guidelines		A term derived from *Gillick v West Norfolk And Wisbech AHA*, 1984 that is used to decide whether a child or young person up to the age of 16 years is able to consent to their own medical treatment, without the need for parental permission or knowledge. A child or young person will be 'Gillick competent' for that decision if they have the necessary maturity and understanding to make the decision.
Gonadotropin-releasing hormone analogues (also known as the hormone blocker/s and puberty blocker/s)	GnRH	GnRH analogues competitively block GnRH receptors to prevent the spontaneous release of two gonadotropin hormones, Follicular Stimulating Hormone (FSH) and Luteinising Hormone (LH) from the pituitary gland. This arrests the progress of puberty.
General Practitioner	GP	GPs deal with a whole range of health problems and manage the care of their patients, referring onto specialists as appropriate.[94]
High Court		The third highest court in the UK. It deals with all high value and high importance civil law (non-criminal) cases and appeals of decisions made in lower courts. When the High Court sits with more than one judge, as required for certain kinds of cases, it is called the Divisional Court.
International Classification of Diseases, Version 11	ICD-11	ICD-11[95] is the World Health Organization (WHO) mandated health data standard used for medical diagnosis.

[93] World Health Organization (2022). International Classification of Diseases Eleventh Revision.
[94] NHS. GP services.
[95] World Health Organization (2022). International Classification of Diseases Eleventh Revision.

Term		Description
Looked after children		Children who are in the care of their Local Authority who may be living with foster parents or in a residential care setting.
Multi-disciplinary-team	MDT	The identified group of professional staff who provide a clinical service.
Neurodiverse		Displaying or characterised by autistic or other neurologically atypical patterns of thought or behaviour; not neurotypical.
Non-binary		A gender identity that does not fit into the traditional gender binary of male and female.[96]
Paediatrics		The branch of medicine dealing with children and their medical conditions.
Pass/passing		A person's gender being seen and read in the way they identify.
Precocious puberty		This is when a child's body begins changing into that of an adult (puberty) too soon – before age 8 in girls and before age 9 in boys.
Primary care		Primary care includes general practice, community pharmacy, dental and optometry (eye health) services. This tends to be the first point of access to healthcare.
Psychological formulation		A structured approach to understanding the factors underlying distressing states in a way that informs the changes needed and the therapeutic intervention for these changes to occur.
Psychosocial		Describes the psychological and social factors that encompass broader wellbeing.
Puberty blockers		See gonadotropin-releasing hormone above.
Secondary care		Hospital and community health care services that do not provide specialist care and are usually relatively close to the patient. For children this will include Child and Adolescent Mental Health Services (CAMHS), child development and general paediatric services.
Tanner Stage		Classification of puberty by stage of development. This ranges from Stage 1, before physical signs of puberty appear, to Stage 5 at full maturity.

[96] Twist J, de Graaf NM (2019). Gender diversity and non-binary presentations in young people attending the United Kingdom's National Gender Identity Development Service. Clin Child Psychol Psychiatry 24(2): 277–90. DOI: 10.1177/1359104518804311.

Term		Description
Tertiary care		Tertiary care is the specialist end of the NHS. These services relate to complex or rare conditions. Services are usually delivered in a number of hospitals/centres.
Transgender	trans	This is an umbrella term that includes a range of people whose gender identity is different from the sex they were registered at birth.
Transition		These are the steps a person may take to live in the gender in which they identify. This may involve different things, such as changing elements of social presentation and role and/or medical intervention for some.

Appendix 1

Terms of reference

TERMS OF REFERENCE FOR REVIEW OF GENDER IDENTITY DEVELOPMENT SERVICE FOR CHILDREN AND ADOLESCENTS

INTRODUCTION

1. NHS England is the responsible commissioner for specialised gender identity services for children and adolescents. The Gender Identity Development Service for children and adolescents is currently managed by the Tavistock and Portman NHS Foundation Trust.

2. In recent years there has been a significant increase in the number of referrals to the Gender Identity Development Service, and this has occurred at a time when the service has moved from a psychosocial and psychotherapeutic model to one that also prescribes medical interventions by way of hormone drugs. This has contributed to growing interest in how the NHS should most appropriately assess, diagnose and care for children and young people who present with gender incongruence and gender identity issues.

3. It is in this context that NHS England and NHS Improvement's Quality and Innovation Committee has asked Dr Hilary Cass to chair an independent review, and to make recommendations on how to improve services for children and young people experiencing issues with their gender identity or gender incongruence, and ensure that the best model/s for safe and effective services are commissioned.

REVIEW SCOPE

The independent review, led by Dr Cass, will be wide ranging in scope and will conduct extensive engagement with all interested stakeholders. The review is expected to set out findings and make recommendations in relation to:

i. Pathways of care into local services, including clinical management approaches for individuals with less complex expressions of gender incongruence who do not need specialist gender identity services;
ii. Pathways of care into specialist gender identity services, including referral criteria into a specialist gender identity service; and referral criteria into other appropriate specialist services;
iii. Clinical models and clinical management approaches at each point of the specialised pathway of care from assessment to discharge, including a description of objectives, expected benefits and expected outcomes for each clinical intervention in the pathway;
iv. Best clinical approach for individuals with other complex presentations.
v. The use of gonadotropin-releasing hormone analogues and gender affirming drugs, supported by a review of the available evidence by the National Institute for Health and Care Excellence; any treatment recommendations will include a description of treatment objectives, expected benefits and expected outcomes, and potential risks, harms and effects to the individual;
vi. Ongoing clinical audit, long term follow-up, data reporting and future research priorities;
vii. Current and future workforce requirements;
viii. Exploration of the reasons for the increase in referrals and why the increase has disproportionately been of natal females, and the implications of these matters; and,

TERMS OF REFERENCE FOR REVIEW OF GENDER IDENTITY DEVELOPMENT SERVICE FOR CHILDREN AND ADOLESCENTS

ix. Any other relevant matters that arise during the course of the review

4. In addition, and with support from the Royal College of Paediatrics and Child Health and other relevant professional associations, the Chair will review current clinical practice concerning individuals referred to the specialist endocrine service. It is expected that findings and any recommendations on this aspect of the review will be reported early in 2021 with the review's wider findings and recommendations delivered later in 2021.

5. The review will not immediately consider issues around informed consent as these are the subject of an ongoing judicial review. However, any implications that might arise from the legal ruling could be considered by the review if appropriate or necessary.

Appendix 2

Letter to NHS England from Dr Cass – May 2021

The Cass Review
Independent review into gender identity services for children and young people

Dr Hilary Cass
Chair
Review of GIDS for Children and Young People

John Stewart
National Director
Specialised Commissioning
NHS England and NHS Improvement

Sent by email

10 May 2021

Dear John

INDEPENDENT REVIEW INTO GENDER IDENTITY SERVICES FOR CHILDREN AND YOUNG PEOPLE

I am writing to update you on my current approach to the work of the independent review into gender identity services for children and young people. However, the most pressing issue is how we augment the immediate support for children and young people currently needing assessment and treatment, some of whom have already been waiting for an extended period for an appointment. I will therefore also make some suggestions about interim arrangements and ways in which the review team could help to support and strengthen these.

Commissioned research programme

As you know, a key principle of the review is that it should be evidence-based, and that final conclusions will be developed through a consensus development process contingent on the synthesised evidence.

I am pleased to see that the National Institute for Health and Care Excellence (NICE) evidence reviews of gonadotrophin releasing hormone analogues and gender affirming hormones for children and adolescents with gender dysphoria have now been published. Although this is a helpful starting point, despite following a standard and robust process the NICE review findings are not conclusive enough to inform policy decisions. As part of my review, I am therefore exploring other methodologies to give increased confidence and clarity about the optimal treatment approaches.

My team is commissioning a broader literature review of the existing evidence base on the epidemiology, management and outcomes of children with gender dysphoria. We are also commissioning qualitative and quantitative research, including considering other approaches which might be employed to understand the intermediate and longer-term outcomes of children with gender dysphoria. We intend to include a review of international models and data in this programme of work.

Addressing the immediate situation

Recognising that the outcome of the review is going to take some time, I have been reflecting on the recent court rulings on puberty blockers and consent and the Care Quality Commission (CQC) report on the Gender Identity Development Service (GIDS) run by the Tavistock and Portman NHS Foundation Trust. These significant developments have changed the context in which the review is taking place, and further added to the service pressures.

I note the proposal to establish an independent multidisciplinary professional review group to confirm decision-making has followed a robust process, which seems an appropriate interim measure pending further clarification of the legal situation.

I know that everyone concerned with the delivery of services – both commissioners and providers – are worried about the increasing number of children on the waiting list for assessment by the GIDS service and the resulting distress for the children and young people and their families. The difficulty in managing risk for those on the waiting list is exacerbated by the staff vacancies at GIDS, the increasing volume of new referrals, and the fact that the support and engagement from local services is highly variable and, in some cases, very limited.

Having a single provider may have been a logical position when the GIDS service was first set up, given that this is a highly specialised service that was seeing a relatively small number of cases each year. As the epidemiology has changed and there has been an exponential increase in numbers of children with gender incongruence or dysphoria, concentration of expertise within a single service has become unsustainable. At the same time, local services have not developed the skills and competencies to provide support for children on the waiting list and those with lesser degrees of gender incongruence who may not wish to pursue specialist medical intervention, and / or to provide help for children with additional complex needs.

I know from discussions we have had that your team is working hard to find some practical alternative arrangements, and that you have been in discussion with relevant professional bodies to come up with creative interim solutions while awaiting the outcome of my review.

The review team has also been in discussion with CQC, with the Tavistock and Portman NHS Foundation Trust and with colleagues within and external to NHS England and NHS Improvement to consider which aspects of this situation we can help with in the short to medium term, whilst keeping our focus on the longer-term questions of the appropriate clinical management and whole care pathway for these children and young people. In the past months I have also met with many groups and individuals with expertise and lived experience relevant to the review, including charities and support groups, Royal Colleges and healthcare professionals.

Recommendations to NHS England and NHS Improvement

I would encourage you to consider the following when developing an interim pathway for children and young people experiencing gender dysphoria:

- **Access and referral:** Children and young people need ready access to services. However, it is unusual for a specialist service to take direct referrals. The risk of having a national service as the first point of access is that assessment and treatment of children and young people who have the greatest need for specialist care is delayed because of the lack of differentiation of those on the waiting list. In addition, many children and

young people have complex needs, but once they are identified as having gender dysphoria, other important healthcare issues which would normally be managed by local services can sometimes be overlooked.

- **Assessment and management:** All children and young people who are referred to specialist services should have a competent local multi-disciplinary assessment and should remain under active holistic local management until they are seen at a specialist centre.

  I recognise that developing capacity and capability outside of the existing GIDS service to provide such initial assessment and support will be difficult to achieve at speed and will be incremental. This means that there will likely be a range of different models and options around the country, dependent on local resources, with some of the work being delivered through existing secondary service teams, and some being delivered at regional level. The support of wider services is vital.

- **Data:** The lack of systematic data collection is a significant issue. Therefore, when employing interim measures, I would suggest that particular attention is paid to the gathering of good quality data, which can then be used to inform the evidence base and future model of provision.

Actions for the review team

I would like to suggest how the review team might help with the challenging problem of growing an infrastructure outside of GIDS. From my conversations to date, I believe there are three barriers to the involvement of local services:

- **Capacity** – the staff most appropriately trained to be involved in initial assessment are those who are already most stretched within Child and Adolescent Mental Health Services (CAMHS) and paediatric services, and this situation has been significantly worsened through the impact of the Covid-19 pandemic on children's mental health. However, I know that there is substantial investment in CAMHS services, so close engagement with the relevant national policy teams at NHS England and NHS Improvement and at Health Education England (HEE) will be crucial.

- **Capability and confidence** – clinical teams outside of GIDS do not feel confident in initial assessment and support of children and young people with gender incongruence and dysphoria, in large part because they have not had the necessary training and experience, but also because of the societal polarisation and tensions surrounding the management of this group.

- **Lack of an explicit assessment framework** – currently expertise in assessment of children and young people presenting to GIDS is held in a small body of clinicians and their assessment processes have not been made explicit. The CQC report drew attention to the lack of structured assessment in the GIDS notes, and this is something that the Tavistock and Portman NHS Foundation Trust is already working to address internally. However, it is equally important to develop an initial assessment approach that can be used by first contact professionals, not just those working in the specialist service.

In the first instance, it is important that we test these assumptions with a range of clinical staff and ascertain whether there are other barriers that are preventing local engagement in this work. Then we would plan to prioritise a series of workshops, in collaboration with relevant professional groups, service users and close engagement with HEE. The purpose of these workshops would be to address identified barriers and develop:

- A framework for initial assessment of children and young people presenting with gender dysphoria.
- An approach to training for professionals at local and regional level.
- Some preliminary workforce recommendations, which will be particularly important in meeting the timelines of the three-year Comprehensive Spending Review.

These workshops will serve multiple purposes – firstly to support NHS England and NHS Improvement in the establishment of local and / or regional teams; secondly as an essential component of the work needed to inform the questions that the review is tackling; and thirdly to form the professional networks that will be needed to underpin future service and research networks.

Timelines

As you will recognise, setting up a complex national review is difficult and time consuming at the best of times. It requires a team to support the work and mechanisms for stakeholders to engage safely and with confidence. Starting a review in the midst of a pandemic is even more challenging.

I have committed to a review approach which is participative, consensus-based, evidence-based, transparent, and informed by lived and professional experience. This requires extensive engagement. Pending the appointment of our research team, the review has now launched its website and I have been proactively engaging with the stakeholder community.

It is critical that we get the approach right, particularly the engagement, the evidence review and the quantitative research given the gaps in the evidence highlighted through the NICE review, and this will take time.

My intention is that an interim report will be delivered in the summer, with a report next year setting out my final recommendations.

Yours sincerely

Dr Hilary Cass
Chair, Independent Review into Gender Identity Services for Children and Young People

Cc: Care Quality Commission
Health Education England
Tavistock and Portman NHS Foundation Trust

Appendix 3

Diagnostic criteria for gender dysphoria

DSM-5 diagnostic criteria for gender dysphoria

Gender Dysphoria in Children

A. A marked incongruence between one's experienced/expressed gender and assigned gender, of at least 6 months' duration, as manifested by at least six of the following (one of which must be Criterion A1):

1. A strong desire to be of the other gender or an insistence that one is the other gender (or some alternative gender different from one's assigned gender).
2. In boys (assigned gender), a strong preference for cross-dressing or simulating female attire; or in girls (assigned gender), a strong preference for wearing only typical masculine clothing and a strong resistance to the wearing of typical feminine clothing.
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. In boys (assigned gender), a strong rejection of typically masculine toys, games, and activities and a strong avoidance of rough-and-tumble play; or in girls (assigned gender), a strong rejection of typically feminine toys, games, and activities.
7. A strong dislike of one's sexual anatomy.
8. A strong desire for the primary and/or secondary sex characteristics that match one's experienced gender.

B. The condition is associated with clinically significant distress or impairment in social, school, or other important areas of functioning.

Specify if:

With a disorder of sex development (e.g., a congenital adrenogenital disorder such as congenital adrenal hyperplasia or androgen insensitivity syndrome).

Gender Dysphoria in Adolescents and Adults

A. A marked incongruence between one's experienced/expressed gender and assigned gender, of at least 6 months' duration, as manifested by at least two of the following:

1. A marked incongruence between one's experienced/expressed gender and primary and/or secondary sex characteristics (or in young adolescents, the anticipated secondary sex characteristics).
2. A strong desire to be rid of one's primary and/or secondary sex characteristics because of a marked incongruence with one's experienced/expressed gender (or in young adolescents, a desire to prevent the development of the anticipated 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 (or some alternative gender different from one's assigned gender).
5. A strong desire to be treated as the other gender (or some alternative gender different from one's assigned gender).
6. A strong conviction that one has the typical feelings and reactions of the other gender (or some alternative gender different from one's assigned gender).

B. The condition is associated with clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Specify if:

With a disorder of sex development (e.g., a congenital adrenogenital disorder such as congenital adrenal hyperplasia or androgen insensitivity syndrome).

Specify if:

Post transition: the individual has transitioned to full-time living in the desired gender (with or without legalization of gender change) and has undergone (or is preparing to have) at least one cross-sex medical procedure or treatment regimen – namely, regular cross-sex hormone treatment or gender reassignment surgery confirming the desired gender (e.g., penectomy, vaginoplasty in a natal male; mastectomy or phalloplasty in a natal female).

ICD-11: HA60 Gender incongruence of adolescence or adulthood

Gender Incongruence of Adolescence and Adulthood is characterised by a marked and persistent incongruence between an individual´s experienced gender and the assigned sex, which often leads to a desire to 'transition', in order to live and be accepted as a person of the experienced gender, through hormonal treatment, surgery or other health care services to make the individual´s body align, as much as desired and to the extent possible, with the experienced gender. The diagnosis cannot be assigned prior the onset of puberty. Gender variant behaviour and preferences alone are not a basis for assigning the diagnosis.

Exclusions:

Paraphilic disorders.

ICD-11: HA61 Gender incongruence of childhood

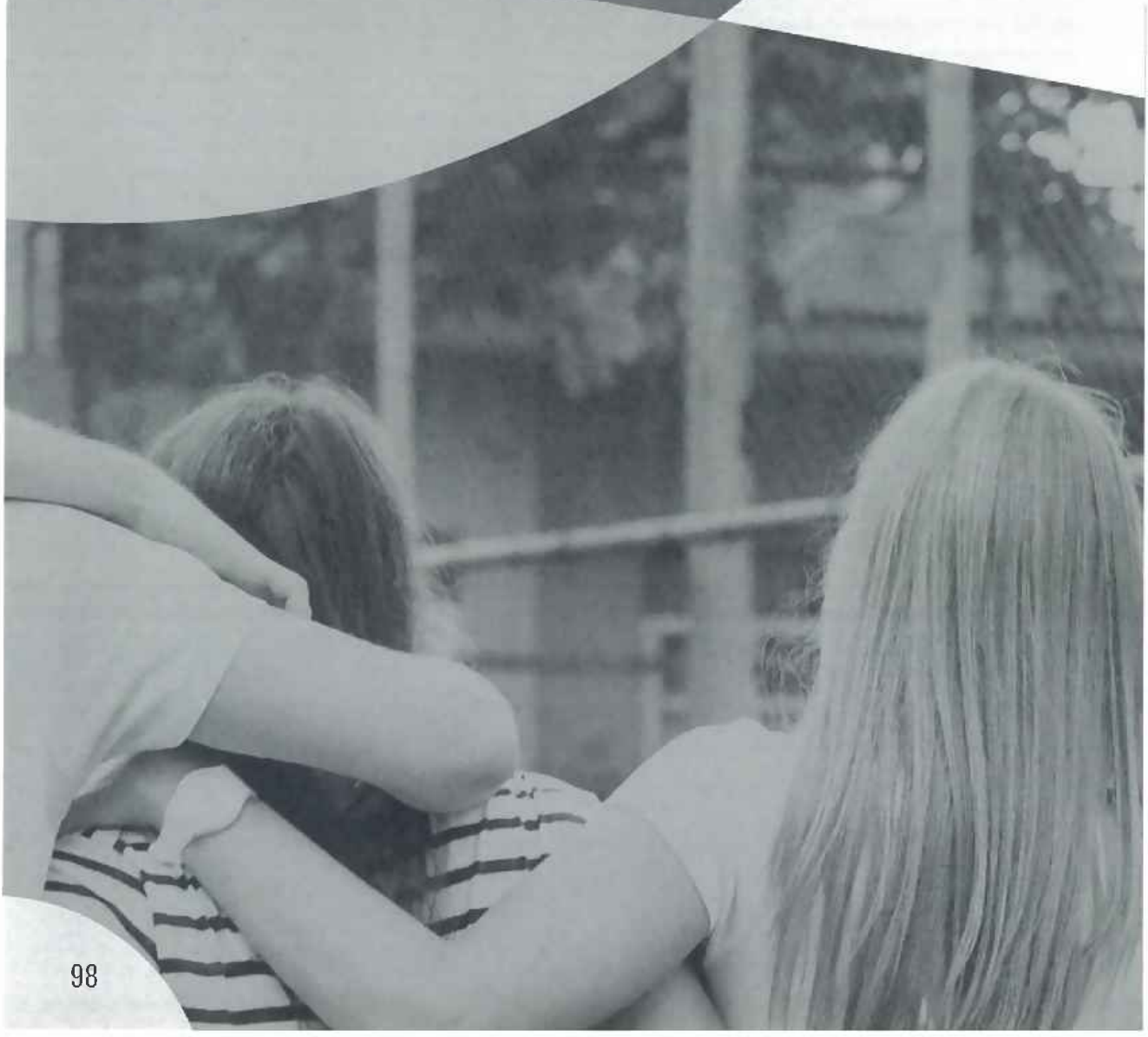
Gender incongruence of childhood is characterised by a marked incongruence between an individual's experienced/expressed gender and the assigned sex in pre-pubertal children. It includes a strong desire to be a different gender than the assigned sex; a strong dislike on the child's part of his or her sexual anatomy or anticipated secondary sex characteristics and/or a strong desire for the primary and/or anticipated secondary sex characteristics that match the experienced gender; and make-believe or fantasy play, toys, games, or activities and playmates that are typical of the experienced gender rather than the assigned sex. The incongruence must have persisted for about 2 years. Gender variant behaviour and preferences alone are not a basis for assigning the diagnosis.

Exclusions:

Paraphilic disorders.

Appendix 4

The standard approach to clinical service development



The standard approach to clinical service development

The three examples below illustrate the usual process of developing a clinical service. Covid-19 is included because this is a new condition that everyone is familiar with; childhood epilepsy because it is a complex condition with physical manifestations; and autism because it is a condition with neuro-behavioural manifestations.

By comparing these examples of clinical service development, it is possible to demonstrate some of the challenges in developing services for children and young people with gender incongruence or dysphoria, and to identify where there are gaps and questions that need to be addressed for this population, in order to ensure any future service model delivers the highest possible standards of care.

The stages below may proceed in a different sequence for different conditions, but each stage is important in the development of evidence based care.

Stage	Covid-19	Childhood Epilepsy	Autism
New condition is observed This often begins with a few case reports and then clinicians begin to recognise a recurring pattern and key clinical features, and to develop fuller descriptions of the condition.	Covid-19 is an example of a recent new condition that we all recognise, and this started with a few unusual cases of respiratory illness being described in Wuhan.	Childhood epilepsy has been recognised for centuries, but over the last century there has been growing understanding of the many different subtypes.	Individuals with autism have probably also existed for an indefinite period, but it wasn't until 1943 and 1944 that Leo Kanner and Hans Asperger wrote the first scientific accounts about the condition.

Stage	Covid-19	Childhood Epilepsy	Autism
Aetiology Clinicians and scientists try to work out the cause of the condition or the underlying physical or biological basis. Sometimes the answers to this are never found.	The cause of Covid-19 was identified at a very early stage as being due to a novel coronavirus, although it remains unclear where and how this originated.	It is now known that there are numerous different types of epilepsy, with many different causes – for example, epilepsy can be caused by specific epilepsy genes, by birth trauma, by metabolic conditions, by brain tumours and many other mechanisms. Epilepsies due to a change in the brain structure which occur after birth are called 'symptomatic' – they are a symptom of something else. Epilepsies for which there is no identified cause are called 'idiopathic'.	The first theory about the aetiology of autism was that it was caused by so called 'refrigerator parents'. This was inaccurate and damaging. It has subsequently been shown that there are many complex genetic and physical or chemical brain changes underpinning this condition.
Natural history and prognosis It is important to understand how a condition usually evolves over time, with or without treatment. The latter is important if treatment has limited efficacy and the condition is 'self-limiting' (that is, it resolves without treatment), because otherwise there is a risk that treatments create more difficulties than the condition itself.	Covid-19 is an example of a condition where there are quite polarised views about management based on its prognosis and natural history. A relatively small proportion of people are seriously affected and need treatment, and for the majority the natural history is that it will get better by itself. This has led some people to question the need for lockdowns, vaccinations and other measures which they see as impacting personal freedoms.	In epilepsy the natural history is very important. Some epilepsies get better through puberty and into adulthood, and some can get worse with hormonal changes. This is important to know when monitoring and reviewing drug treatment.	

Stage	Covid-19	Childhood Epilepsy	Autism
Epidemiology Epidemiologists collect data to find out how common a condition is, who is most likely to be affected, what the age distribution is and so on. This allows health service planners to work out how many services are needed, where they should be established, and what staff are needed. They also report on changes in who is most affected, which may mean that either the disease is changing, or the susceptibility of the population is changing.	Epidemiologists have been crucial in supporting the management of Covid-19 because they have extracted and analysed the data on which patients are at greater risk from the virus. This has been fundamental to planning a vaccination strategy and other protective measures.		The epidemiology of autism has changed considerably, with a dramatic increase in the numbers of children diagnosed over the last 20 years. This has had major implications for service provision. There is ongoing debate about the cause of the increase – whether it is because of greater awareness and better diagnosis, or because there are more children with autism. Current opinion favours the first option.

Stage	Covid-19	Childhood Epilepsy	Autism
Assessment and diagnosis Clinicians will usually take a history from (that is, of their symptoms) and examine the patient (that is, for signs and symptoms), and where appropriate undertake a series of investigations or tests, to help them reach an accurate diagnosis. Sometimes the whole process of making a diagnosis through talking to the patient and asking them to complete formal questionnaires, examining them and/or undertaking investigations is called 'clinical assessment'. As well as diagnosing and ruling out a particular condition, clinicians often need to consider and exclude other, sometimes more serious, conditions that present in a similar way but may need quite different treatment – this process is called 'differential diagnosis'.	PCR has been used as a 'gold standard' test for diagnosis of Covid-19 since the beginning of the pandemic. Lateral flow testing was developed to provide a quicker and cheaper option, but it demonstrates the limitations of testing, it is 99.68% specific, which is a very high specificity. This means there are only a tiny number of false positives. It has lower sensitivity at 76.8%, which means it will miss about a quarter of all cases, so giving many more false negatives, BUT it will only miss 5% of cases with high viral load.	Epilepsy can only be definitively diagnosed by either getting a really clear description of the events from a parent or carer, or seeing the child or young person having a seizure on a video. An EEG (brain wave tracing) and other tests can provide information about the type of epilepsy, but unless a seizure happens during the recording, it does not demonstrate that they actually have seizures – only that they may be susceptible to seizures.	In autism there are no blood tests or X-rays to make the diagnosis. It is a 'clinical' diagnosis, which means it is dependent on taking a standardised history from the parents, and performing standardised assessments on the child or young person to distinguish between autism and other possible diagnoses (for example, language disorder, social anxiety). In the early days, these standardised measures did not exist; the diagnosis was very dependent on experts who were used to diagnosing autism by making a clinical judgement about each child. This made it difficult to teach new people how to do this without a long apprenticeship, and also made it difficult to know whether two different experts would come to the same conclusion about the same child or young person. Standardisation of the questions and process made diagnosis more reliable and consistent, as did an improved evidence base. At the same time, because children with autism all present differently, the assessment had to be flexible enough to accommodate, for example, non-verbal children with severe learning disability, as well as high-functioning children with strong verbal skills.

Stage	Covid-19	Childhood Epilepsy	Autism
Differential diagnosis As well as making a positive diagnosis, clinicians often need to exclude other, sometimes more serious conditions that present in a similar way, but may need quite different treatment.		There are conditions that can be mistaken for epilepsy, so it is important to accurately diagnose whether seizures are happening and exclude other conditions (differential diagnoses) by carrying out relevant tests.	There are many conditions that may be mistaken for autism – for example, children who have language disorders, learning disability, severe social anxiety for other reasons, or ADHD can all appear to have autism. It is important to exclude these other conditions as well as making a positive diagnosis of autism. Sometimes these conditions can exist alongside autism, and management must then be planned to address all the child's difficulties.

Stage	Covid-19	Childhood Epilepsy	Autism
Developing and implementing new treatments Clinicians and scientists work on developing treatments. This involves clinical trials and, where there are new treatments, comparing them to any existing treatments. Questions include: What are the intended outcomes or benefits of treatment? What are the complications or side effects? What are the costs? To initiate a new treatment, it must be both safe and effective. Questions of affordability can sometimes become controversial. The best type of single study is considered to be the randomised controlled trial (RCT), but sometimes this is not feasible. Even where RCTs are not available, it is usual to at least have data on the outcomes of sufficient cases or cohorts to understand the risk/benefit of the treatment under consideration. As demonstrated in Fig. 3, the highest level of evidence is when the results of several different studies are pooled, but this is only useful if the individual studies themselves are of high quality.	Developing treatments for Covid-19 has been possible at speed because of the large numbers of patients, and the fact that outcomes can be observed on each patient within a matter of days to weeks. Because Covid-19 was a new condition, clinicians also started in a position of 'equipoise' which means that they did not have reason to believe any one treatment might be more effective than another; this made it ethical to have one group having a treatment and another group having a different treatment or a placebo. There are also really clear outcome measures, such as whether or not patients survive or need hospitalisation. This has facilitated a high level of evidence through randomised controlled trials (see diagram below).	Similar considerations apply to the treatment of epilepsy in that there are 'hard' outcome measures (for example, frequency of seizures), but it can take several months to determine whether a new drug is better than an existing one for any one patient, and some side effects may be longer-term, so trials can take several years. In addition, children with epilepsy may have very different conditions causing their seizures which can also make trials more challenging. In the most severe cases of epilepsy, surgery may be the best option for controlling seizures. This can be very radical in certain cases and have lifelong implications for how they function. These options, which have a cost as well as a benefit to the child, will only be offered after a multi-disciplinary team meeting, including the paediatricians, therapists, neuropsychologists, radiologists, neurophysiologists and neurosurgeons have all discussed whether the benefits will outweigh the costs.	Evaluating interventions for autism is the most difficult of these three examples. This is because it can take many years to see developmental outcomes; it is hard to get uniform groups of children; outcomes are extremely sensitive to the social (and historical) response of others; and many other things happen in children's lives (such as changes of school, other medications, new diets). Isolating the effect of the target treatment is therefore challenging.

Stage	Covid-19	Childhood Epilepsy	Autism
In many instances, evidence is not perfect and difficult decisions have to be made. Where treatments are innovative or life-changing, the whole multi-disciplinary team will usually meet to consider the available options, and how to advise the child or young person and family so that a shared decision can be made. Sometimes an ethics committee is involved. This is one of the most challenging areas of medicine and is underpinned by GMC guidance.[97, 98]	The UK has been internationally recognised for its Recovery Trial, led by Oxford University. This has recruited over 46,000 participants, and resulted in several treatments being approved. A key factor in this success was the willingness of patients to participate in these studies – with over 46,000 being recruited and consented.		

[97] General Medical Council (2020). Decision making and consent.
[98] National Institute for Health and Care Excellence (2021). Shared decision making.

Stage	Covid-19	Childhood Epilepsy	Autism
Service development and service improvement Central to any service improvement is the systematic and consistent collection of data on outcomes of treatment. There is a process of continuous service improvement as new presentations or variations on the original condition are recognised, diagnosis or screening improves and/or trials on new treatments or variations on existing treatments are ongoing. There should be consistent treatment protocols or guidelines in place, in order to make sense of variations in outcomes. Where possible, these should be compared between and across multiple different centres. As time passes, services need to be changed or extended based on patient need, and on what resources are needed to deliver the available treatments. They need to be accessible where the prevalence of the condition is highest. The relevant workforce to deliver the service needs to be recruited and trained, contingent on the type of treatments or therapy that is required.	Service development to manage Covid-19 has been on a scale unlike any normal new service development ever experienced. It has also demonstrated how other non-Covid services have had to evolve alongside, including the need for isolation, and/or PCR testing prior to routine clinical appointments, use of remote consultation and an array of other changes across the NHS. Continuous audit and monitoring of outcomes has resulted in major improvements in survival – for example, changing ventilation approach to include 'proning' (putting patients on their front while on the ventilator) and delaying fully intubated ventilation by giving mask ventilation for as long as possible.	Paediatric epilepsy is a good example of how a national approach can be taken to service improvement through the Epilepy12 programme.[99] This is a nationally co-ordinated audit which collects a standardised dataset, incorporating NICE standards, and is used to drive up standards of care for children and young people with epilepsy.	Improvement in autism services has been driven by the changing epidemiology, NICE standards, extensive training of the workforce and attempts to improve public understanding. Where previously diagnosis was undertaken in a few specialist centres, the rising waiting times and NICE standards on access, assessment and appropriate multi-professional provision have led to almost every community child development service having an autism assessment clinic or team. Services are able to self-assess against national standards to inform local improvement strategies.

[99] Royal College of Paediatrics and Child Health (2021). Epilepsy 12 – national organisational audit and clinical audit.

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Puberty suppression and executive functioning: An fMRI-study in adolescents with gender dysphoria

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Summary Adolescents with gender dysphoria (GD) may be treated with gonadotropin releasing hormone analogs (GnRHa) to suppress puberty and, thus, the development of (unwanted) secondary sex characteristics. Since adolescence marks an important period for the development of executive functioning (EF), we determined whether the performance on the Tower of London task (ToL), a commonly used EF task, was altered in adolescents with GD when treated with GnRHa. Furthermore, since GD has been proposed to result from an atypical sexual differentiation of the brain, we determined whether untreated adolescents with GD showed sex-atypical brain activations during ToL performance. We found no significant effect of GnRHa on ToL performance scores (reaction times and accuracy) when comparing GnRHa treated

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male-to-females (suppressed MFs, $n=8$) with untreated MFs ($n=10$) or when comparing GnRHa treated female-to-males (suppressed FMs, $n=12$) with untreated FMs ($n=10$). However, the suppressed MFs had significantly lower accuracy scores than the control groups and the untreated FMs. Region-of-interest (ROI) analyses showed significantly greater activation in control boys ($n=21$) than control girls ($n=24$) during high task load ToL items in the bilateral precuneus and a trend ($p<0.1$) for greater activation in the right DLPFC. In contrast, untreated adolescents with GD did not show significant sex differences in task load-related activation and had intermediate activation levels compared to the two control groups. GnRHa treated adolescents with GD showed sex differences in neural activation similar to their natal sex control groups. Furthermore, activation in the other ROIs (left DLPFC and bilateral RLPFC) was also significantly greater in GnRHa treated MFs compared to GnRHa treated FMs. These findings suggest that (1) GnRHa treatment had no effect on ToL performance in adolescents with GD, and (2) pubertal hormones may induce sex-atypical brain activations during EF in adolescents with GD.

1. Introduction

Gender dysphoria (GD) is a condition in which people suffer from an incongruence between their natal sex and their gender identity, *i.e.* their experienced gender (American Psychiatric Association, 2013). For young individuals with GD, puberty is a period that causes great distress because it is characterized by unwanted physical changes, the development of the secondary sex characteristics. Therefore puberty inhibiting hormones may be prescribed such as the gonadotropin releasing hormone analogs (GnRHa) leuprolide or triptorelin (Gooren and Delemarre-van de Waal, 1996).

Some researchers expressed concerns about the possible disadvantages of GnRHa administration during adolescence (Spriggs, 2004; Viner, 2005; Houk and Lee, 2006; Korte et al., 2008). They fear that it may lead to misdiagnosis or that adolescents cannot make complex life decisions. Moreover, some have questioned whether hormonal suppression affects psychological functioning and if it may entail medical risks. Indeed, during adolescence the brain is still developing. Furthermore, puberty has been suggested to represent a second organizational period during brain development in rodents (Juraska et al., 2013) and in humans (Romeo, 2003; Sisk and Zehr, 2005). The prefrontal cortex (PFC) in particular appears to develop much later than other brain areas (Huttenlocher, 1979). Histological studies suggest that there is a second wave of synaptic proliferation in the PFC at the onset of puberty (Huttenlocher, 1979; Bourgeois et al., 1994; Woo et al., 1997), followed by a plateau phase and synaptic pruning. Executive functioning (EF), which is believed to depend heavily on prefrontal activation, also develops relatively slowly. For instance, performance on the Tower of London task (ToL), a frequently used EF task, improves with age until early adulthood (De Luca et al., 2003; Huizinga et al., 2006; Asato et al., 2006; Albert and Steinberg, 2011).

Since puberty marks an important period in the development of EF, the question arises if pubertal suppression affects this development. Therefore, in the present study, adolescents with GD who received GnRHa to suppress their puberty were compared with a group of control adolescents regarding ToL performance and brain activation patterns (using functional magnetic resonance imaging, fMRI). To check whether potential differences between the groups were due to the suppression (and not due to GD), we also compared them with a group of age matched adolescents with GD who were not – yet – using GnRHa but were already in puberty.

Most of the previous ToL neuroimaging studies did not report – or perhaps did not look for – any sex effects (Owen et al., 1996; Baker et al., 1996; Dagher et al., 1999; Lazeron et al., 2000; Rowe et al., 2001; Van den Heuvel et al., 2003; Newman et al., 2003; Schall et al., 2003; Wagner et al., 2006; Boghi et al., 2006). However, one study reported sex differences in precuneus and dorsolateral prefrontal cortex (DLPFC) activation (Boghi et al., 2006). Therefore we examined sex differences as well.

Furthermore, it has been hypothesized that sexual differentiation of the brain might be different in individuals with GD (Cohen-Kettenis and Gooren, 1999; Van Goozen et al., 2002; Swaab, 2004). Functional neuroimaging studies comparing adults with GD (before the start of treatment) to controls demonstrated that MFs differed from their natal sex in parietal activation during a mental rotation task (Schöning et al., 2010) and showed female-like activity during processing of erotic stimuli (Gizewski et al., 2009) and after exposure to androstadienone, an odorous steroid compound (Berglund et al., 2008). In a verbal fluency study with adolescents performed by our group (Soleman et al., 2013), activation levels of untreated FMs and MFs fell in between those of the control groups. Structural neuroimaging studies have also shown intermediate values in adult FMs and MFs compared to control groups (Rametti et al., 2011a,b; Kranz et al., 2014) and several structural studies have shown differences between adults with GD and controls sharing their natal sex (Luders et al., 2009, 2012; Simon et al., 2013; Zubiaurre-Elorza et al., 2013; Hoekzema et al., 2015) although another study reported brain volumes largely in line with their natal sex (Savic and Arver, 2011).

As mentioned above, our group has examined the effect of GD on VF performance and brain activation in untreated adolescents (Soleman et al., 2013). Although the VF task may be considered an executive functioning task, the effect of GnRHa treatment on VF performance and brain activation was not investigated. In this study we examined if ToL-related brain activation of adolescents with GD, before start of GnRHa and while on GnRHa, was more in line with that of individuals of their experienced gender or of their natal sex. We believe that the present study is the first to examine the effects of puberty suppression on executive functioning.

2. Methods

2.1. Subjects

Adolescents who were diagnosed with Gender Identity Disorder according to the DSM-IV-TR (American Psychiatric Association, 2000) at VU University Medical Center in Amsterdam were recruited (Kreukels and Cohen-Kettenis, 2011). During preparation of this manuscript the DSM-5 was published (American Psychiatric Association, 2013), therefore DSM-5 terminology is used throughout this manuscript.

Forty-one adolescents with GD were included in this study; 22 female-to-males, 12 of which were using GnRHa (suppressed FM) and 10 who were not (untreated FM) and 18 male-to-females, of which 8 were using GnRHa (suppressed MF) and 10 were not (untreated MF). The suppressed adolescents with GD had been receiving 3.75 mg Triptorelin (Decapeptyl-CR®) every 4 weeks, subcutaneously or intramuscularly (mean duration ± standard deviation: 1.6 ± 1.0 years). To receive GnRHa, participants had to be at least 12 years old. Furthermore, girls needed to have breast development as described in Tanner stage B2 (Marshall and Tanner, 1969) and the genital development of the boys had to be at Tanner stage G2-G3 (testicular volume of 6–8 ml) (Marshall and Tanner, 1970) with measurable estradiol and testosterone levels, respectively. Relatives and friends of the participants were asked to participate, serving as age-matched controls. Only 3 siblings participated as controls: both a brother and sister of one GnRHa treated FM and one sister of an untreated FM. Thus, the majority of the controls were friends. The control group consisted of 24 girls (F) and 21 boys (M). Subject characteristics are presented in Table 1. According to the Declaration of Helsinki, all participants and their legal guardians gave their informed consent, and the study was approved by the Ethics Committee of the VU University Medical Center Amsterdam.

For all groups exclusion criteria were: (1) insufficient command of the Dutch language, (2) unadjusted endocrine disorders, (3) neurological or psychiatric disorders that could lead to deviant test results, (4) use of psychotropic medication, and (5) contra indications for an MRI scan. Adolescents receiving puberty delaying medication or any form of hormones besides oral contraceptives were excluded as controls. In consultation with the treating clinicians, only adolescents with GD functioning within the normal range were asked to participate in the study. One instrument (amongst other things) to measure psychological functioning was the Dutch translation of the Child Behavior Check List (Achenbach and Edelbrock, 1983; Verhulst et al., 1996), a well-known parent report questionnaire measuring psychological and behavioral problems. Control subjects received the CBCL as part of this study (average CBCL scores of the groups are depicted in Table 1). The gender identity of all controls was in line with their natal sex and was checked by asking them if they felt they belonged to the other gender or wished to be the other gender. All controls had a heterosexual orientation. The adolescents with GD were all sexually attracted to partners of their natal sex.

From the initial selection ten subjects had to be removed from further data analysis due to excessive movement during scanning, two because of scan artifacts (MR signal dropout) due to braces, fifteen due to insufficient mask coverage, two because of performance at chance level, and one due to scanner failure.

2.2. Experimental setup and procedure

In this study an event-related parametric version of the ToL was used (for a detailed description see Van den Heuvel et al., 2003). On each trial, a start configuration (top) and a target configuration (bottom) were displayed simultaneously (see Fig. 1). In the planning condition subjects were asked to work out the minimum number of steps (ranging from 1 to 5) required to reach the target configuration. As a baseline condition, participants had to count the total amount of blue and yellow beads. The task lasted about 12 min and timing of the stimuli was self-paced, with a maximum response duration of 60 s per trial.

Participants practiced the task outside the scanner and performed some practice trials inside the scanner immediately before starting the task. Three other cognitive tasks were performed as well, a verbal fluency task (Soleman et al., 2013), a mental rotation task and a face recognition task (data to be published elsewhere). The four tasks were presented randomly during the scanning session and the entire session lasted 1 h. Prior to the MRI session a physical examination was performed by a clinician and intelligence was estimated with four subscales (arithmetic, vocabulary, picture arrangement, and block design) of the Wechsler Intelligence Scale for Children, third edition (WISC-III®) (Wechsler, 1991) or the Wechsler Adult Intelligence Scale, third edition (WAIS-III®) (Wechsler, 1997), depending on the participant's age. Handedness was measured by means of a Dutch questionnaire (Van Strien, 1992).

2.3. MRI acquisition

Imaging data were acquired on a 3.0 T Philips Intera (Best, The Netherlands) MRI scanner at the Academic Medical Center, Amsterdam, The Netherlands. Axial T2*-weighted whole-brain volumes sensitive to blood oxygen level dependent (BOLD) contrast (Ogawa et al., 1990) were acquired

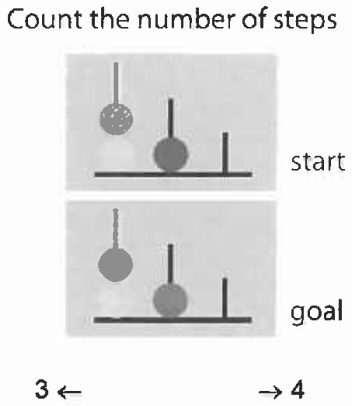

Figure 1 Example of the Tower of London task showing a trial in the planning condition.

Table 1 Sample characteristics and performance data.

Group	*n*	Age (years) (mean ± SD)	IQ* (mean ± SD)	Tanner stage (mean ± SD)	CBCL scores* (mean ± SD)	Accuracy (%)* (mean ± SD)	RT (s) (mean ± SD)
M	21	14.9 ± 1.5	110.7 ± 15.1	4.2 ± 1.2	48.4 ± 10.5■	88.5 ± 6.8	9.6 ± 2.5
F	24	14.4 ± 1.8	103.0 ± 17.3	4.3 ± 0.9	48.4 ± 10.3	87.2 ± 11.9	9.0 ± 1.8
MF (total)	18	15.1 ± 2.4	102.6 ± 18.5	3.9 ± 1.1	57.8 ± 9.2	79.1 ± 10.3	10.4 ± 3.5
suppressed	8	15.4 ± 0.7	94.0 ± 10.3	4.1 ± 1.0	57.4 ± 9.8	73.9 ± 9.1	10.9 ± 4.1
untreated	10	14.6 ± 3.2	109.4 ± 21.2	3.8 ± 1.1	58.2 ± 9.3	83.4 ± 9.5	9.9 ± 3.1
FM (total)	22	15.8 ± 1.9	97.1 ± 15.4	4.5 ± 0.9	60.4 ± 10.2	87.1 ± 10.0	10.0 ± 2.6
suppressed	12	16.1 ± 1.7	95.8 ± 15.6	4.1 ± 1.1	57.5 ± 9.4	85.7 ± 10.5	9.9 ± 3.1
untreated	10	15.4 ± 2.3	98.5 ± 15.9	4.9 ± 0.3	63.9 ± 10.5	88.8 ± 9.7	10.0 ± 2.0

n: number of subjects, SD: standard deviation, accuracy: percentage of correct trials, corrected for task load, RT: reaction times in seconds, corrected for task load, CBCL: Child Behavior Checklist.
* Significantly different between groups ($p < .05$, two-sided).
■ $n = 14$.
◦ $n = 16$.
M: control boys, F: control girls, MF: male adolescents with GD, FM: female adolescents with GD, suppressed: treated with GnRHa, untreated: without GnRHa treatment.

over ±12 min using an echo-planar imaging sequence (repetition time [TR] 2.3 s; echo time [TE] 30 ms; field of view: 22 cm × 22 cm × 10.5 cm; flip angle: 80 degrees; 96 × 96 matrix). A sagittal T1-weighted scan was also performed (repetition time [TR] 9 ms; echo time [TE] 3.5 ms; field of view: 25.6 cm × 23.2 cm × 17.0 cm; flip angle: 8 degrees; 256 × 256 matrix, 170 slices).

2.4. Statistical analysis

Subject characteristics and ToL performance data were analyzed with the Statistical Package for the Social Sciences (SPSS), version 21. Accuracy scores (percentage of correct trials) and reaction times (RT) were corrected for task load by multiplying the scores of category 1–5 with increasing weights (1.0, 2.0, 2.5, 3.0 and 3.5, respectively – analogous to the contrast weights for measuring task load activation during first level SPM analysis) and then dividing the sum by 12. Group differences in age, IQ, and accuracy were tested with a one-way analysis of variance (ANOVA) and *post hoc* comparisons were performed using Games–Howell correction. One-way analysis of covariance (ANCOVA) was performed to examine the effect of IQ on group differences in accuracy. Group differences in RT were examined using Kruskal–Wallis test because the assumption of normality was not met. Tanner stage was examined using Kruskal–Wallis tests and a Chi-square test was used to check for group differences in handedness. A Pearson product-moment correlation was computed to determine the relationship between IQ scores and accuracy scores. The relation between IQ and RT was assessed using Spearman's Rank Order correlation.

The fMRI analysis was carried out with Statistical Parametric Mapping 8 (SPM8, Wellcome Department of Imaging Neuroscience, Institute of Neurology at the University College London, UK) implemented in MATLAB R2011b (MathWorks Inc., Natick, MA, USA). Functional images were slice-timed, realigned to the mean image, and co-registered with the individual anatomical image. Because the participants in this study were adolescents and thus did not have adult-sized brains yet, a DARTEL template of their structural scans was created for optimal spatial normalization into Montreal Neurological Institute (MNI) space (DARTEL: Diffeomorphic Anatomical Registration Through Exponentiated, see Ashburner, 2007). Functional images were smoothed with a 8 mm FWHM Gaussian filter.

First level contrast images for *planning* were calculated by subtracting the baseline condition from the 5 planning categories. Moreover, to identify brain regions that showed signal intensity variation correlated with increasing planning complexity a *task load* contrast was calculated by giving increasing contrast weights to category 1 to 5 (Van den Heuvel et al., 2003). Individual head jerks of more than 1 mm were included in every first-level design matrix (Lemieux et al., 2007) together with the six motion parameters to account for the effects of excessive head motion. Furthermore, error trials were added as regressors of no interest.

Due to the large amount of data, only the results for the *task load* contrast are displayed in Section 3, although analyses were performed for both contrasts. *Task load* was favored because it places a greater emphasis on the more difficult trials and especially those trials (involving 3 or more steps) require EF. The results for *planning* are displayed in the Supplementary Results.

The contrast images for *planning* and *task load* were entered into a second level analysis of variance. IQ scores were added as a covariate because the groups differed in IQ scores. *T*-tests were performed to investigate the activation seen in every group (main effect) and to investigate group differences. The results were examined on whole-brain level first and subsequently region of interest (ROI) analyses were performed. Based on previous ToL neuroimaging studies (see Section 1) the dorsolateral- and rostrolateral prefrontal cortex (DLPFC and RLPFC), and precuneus were chosen as ROIs. These areas were selected from the IBASPM116 atlas and separate left and right masks were created using WFU PickAtlas version 3.0.4. (Maldjian et al., 2003). Because the

frontal ROI did not distinguish between DLPFC and RLPFC, a functional RLPFC ROI from the BrainMap database (Nielsen and Hansen, 2002) was subtracted from the frontal ROI using MarsBar (version 0.43, MRC Cognition and Brain Sciences Unit, Cambridge, UK). Each set of ROIs was masked with the FWE-corrected ($p = 0.05$) main effects for *planning* and *task load* (separately). For examination of the main effect a p-value of 0.05 corrected for multiple comparisons was used (pFWE-corrected = 0.05). For the between group ROI analyses a pFWE-corrected = 0.05 was used as well, corrected for the spatial extent of the ROI.

3. Results

3.1. Sample data

No significant age differences were found between the six groups ($F(5, 79) = 1.52$, NS), but a difference was observed in IQ ($F(5, 79) = 2.32$, $p < .05$). Control boys (M) had significantly higher IQ scores than suppressed MFs ($p = .03$). Tanner stage and handedness did not differ between the groups ($p = 0.207$ and $p = 0.647$, respectively). The means and standard deviations of age, IQ and Tanner stage are presented in Table 1. There was no significant difference in duration of suppression between MFs (mean duration ± standard deviation: 1.8 ± 0.8 years) and FMs (mean duration ± standard deviation: 1.4 ± 1.1 years); $T(18) = 1.03$, *NS*.

3.2. ToL performance data

Accuracy significantly differed between the groups ($F(5, 79) = 3.07$, $p < .05$). *Post hoc* analyses showed that the suppressed MFs had significantly lower accuracy scores than the control groups ($p = .02$ compared to control boys and $p = .04$ compared to control girls) and the untreated FMs ($p = .04$). IQ and accuracy were significantly correlated ($r = 0.31$, $n = 85$, $p < .005$), but even after correcting for IQ, a significant effect of group on accuracy remained ($F(5, 78) = 2.70$, $p < .05$). Additionally, there was a significant negative correlation between IQ and RT ($r_s(85) = -0.31$, $p < .005$). However, RT did not significantly differ between the six groups ($H(5) = 3.92$, NS). No significant correlations between age and the performance scores were found. Means and standard deviations of accuracy and RT are presented in Table 1. For the baseline condition (counting the blue and yellow balls) no significant group differences were found for accuracy ($F(5, 79) = 0.28$, NS) or RT ($F(5, 79) = 1.16$, NS).

3.3. Main effect functional MRI data

The results for the *planning* contrast can be found in the Supplementary Results. *Task load* (Table 2 and Fig. 2) showed a robust activation pattern in the bilateral DLPFC and the left supplementary motor area. The left precentral area was significantly activated as well and activation was seen in the bilateral insular cortices and right pars opercularis. The parietal activation in the left hemisphere was found in the superior gyrus, extending from the precuneus to the more lateral part of the superior parietal cortex. In the right hemisphere significant parietal activation was seen in the supramarginal and inferior parietal gyrus. Task load was also associated with significant activation of bilateral basal ganglia (caudate nucleus, putamen and globus pallidus).

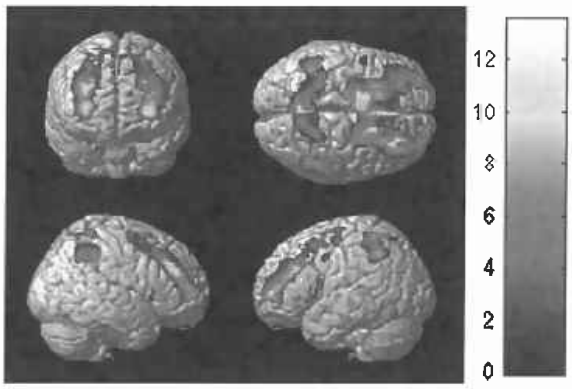

Figure 2 Brain activation pattern in all participants (main effect) for task load, with IQ added as a covariate. *P*-value (FWE-corrected) = 0.05.

3.4. Group effects functional MRI data

3.4.1. Task load activation: sex differences within groups

Whole-brain analyses for *task load* revealed no significant group differences. ROI analyses showed greater activation of the bilateral precuneus and a trend ($p < .10$) for greater activation in the right DLPFC in control boys compared to control girls. In these ROIs similar sex differences were found between the suppressed MFs and FMs. Furthermore, activation in the other ROIs (bilateral RLPFC and left DLPFC) was also greater in suppressed MFs than suppressed FMs. In contrast, no ROIs showed greater activation in the untreated MFs compared to the untreated FMs. The only sex difference between the untreated adolescents with GD was in the opposite direction; the untreated FMs showed a slightly more pronounced ($p < .10$) right DLPFC activation compared to the untreated MFs (An overview of the sex differences reported in this paragraph is given in Table 3.) To explore whether the lack of typical sexual differentiation in untreated adolescents with GD was due to activation levels being in between those of the control groups, we depicted the activation levels of the untreated GD adolescents in those voxels showing the greatest sex difference in controls (Fig. 3). Indeed, plotting effect sizes indicated that activations in untreated GD adolescents were intermediate between the two control groups.

3.4.2. Task load activation in MFs

The suppressed MFs showed greater activation compared to their experienced gender (F) in bilateral DLPFC, left RLPFC, left precuneus and right precuneus (trend), whereas untreated MFs only displayed a trend for greater activation in the right precuneus. The suppressed MFs not only showed a greater left RLPFC activation than Fs, but also relative to their natal sex (M) and untreated MFs. The suppressed MFs

Table 2 Brain regions in main effect of task load.

Brain regions – task load	# Voxels	MNI coordinates			T-value	p (FWE-corrected)
		x	y	z		
Frontal	**27,359**					
Supp_Motor_Area_L		−14	5	66	13.57	<0.001
Frontal_Mid_R		37	38	31	12.25	<0.001
Frontal_Mid_L		−33	32	31	11.89	<0.001
Parietal_L	**7465**					
Parietal_Sup_L		−29	−43	66	9.85	<0.001
Precuneus_L		−6	−60	46	9.85	<0.001
Precuneus_L		−11	−57	63	9.64	<0.001
Parietal_R	**1213**					
Supramarginal_R		54	−42	45	7.81	<0.001
Parietal_Inf_R		52	−54	46	7.14	<0.001
Basal Ganglia_L	**421**					
Putamen_L		−18	0	12	7.26	<0.001
Pallidum_L		−18	−6	0	5.83	<0.01
Basal Ganglia_R						
Caudate_R	103	15	2	16	6.62	<0.001
Frontal						
Precentral_L	56	−50	−1	45	6.04	<0.01
Frontal_Inferior_L	**477**					
Insula_L		−33	15	1	6.04	<0.01
Frontal_Inf_Oper_L		−53	6	12	5.88	<0.01
Insula_L		−29	23	−3	5.44	<0.05
Insula_R						
Insula_R	23	33	18	3	5.32	<0.05

Results are FWE corrected $p < .05$ and cluster size is > 20 voxels. Bold numbers in column 2 represent the number of voxels in the entire cluster.

showed greater left DLPFC activation than the untreated MFs as well.

3.4.3. Task load activation in FMs

Suppressed FMs differed from their experienced gender (M) by showing less bilateral precuneus activation, corresponding with the activation differences between the control groups. The untreated FMs did not show this resemblance to their natal sex (F). Besides lower right precuneus activation than boys (M), suppressed FMs also showed lower activation of this area than girls (F).

The untreated, but not the suppressed, FMs showed a trend for greater right DLPFC activation than their natal sex (F), thus showing a similarity to their experienced gender (M), who also demonstrated this trend compared to Fs. Untreated FMs displayed greater bilateral precuneus activation than suppressed FMs.

4. Discussion

In this study, we aimed to determine whether puberty suppression affected ToL performance. We found no significant effect of GnRHa on ToL performance scores (reaction times and accuracy) in either MFs or FMs when compared to untreated adolescents with GD. However, suppressed MFs had the lowest accuracy scores, which, as the analysis of covariance pointed out, did not just reflect their IQ scores, which were the lowest as well. It is possible that this is just a chance finding due to the small size of this subgroup ($n = 8$). No sex differences in performance were found in the control groups.

ROI analysis did reveal sex differences in brain activations associated with ToL performance. Control boys showed significantly greater activation in the bilateral precuneus and right DLPFC (trend) during high task load compared to control girls. In a previous study (Boghi et al., 2006) adults showed similar sex differences in the precuneus, whereas the sex difference in DLPFC activation was reversed; women exhibited greater DLPFC activation than men. A possible explanation for this discrepancy is that the DLPFC is not yet fully developed in our participants. In a Go-No-Go study children displayed greater activation of the DLPFC than adults, this was explained as resulting from greater network efficiency in adults (Casey et al., 1997). Since frontal gray matter starts developing earlier in girls than in boys (Giedd, 2008) network fine-tuning may start earlier as well. During adolescence the DLPFC may still be under the influence of pubertal hormonal effects, either activational or organizational (Romeo, 2003; Sisk and Zehr, 2005) whereas this is no longer the case for the precuneus, since a strong bilateral sex difference is present both in adolescents (present study) and adults (Boghi et al., 2006).

It has been hypothesized that the sexual differentiation of the brain in individuals with GD may be distinct from other members of their natal sex due to organizational effects

Table 3 Voxels showing sex differences for task load activation (corrected for IQ).

Region of interest	Sex difference	MNI coordinates			*T*-value	*p* (FWE-corrected)
		x	*y*	*z*		
Precuneus L	M > F	−6	−67	51	4.76	<0.01
		−8	−57	46	3.92	<0.05
		−2	−46	46	3.38	<0.10
	MF (s) > FM (s)	−6	−52	48	3.57	<0.05
		−11	−49	46	3.55	<0.05
		−14	−61	60	3.33	<0.10
		−3	−58	48	3.24	<0.10
Precuneus R	M > F	15	−49	60	3.71	<0.05
		15	−43	58	3.63	<0.05
		2	−43	43	3.52	<0.05
		2	−46	48	3.36	<0.10
		8	−43	60	3.31	<0.10
		5	−45	51	3.21	<0.10
	MF (s) > FM (s)	12	−60	45	3.32	<0.10
		8	−54	54	3.30	<0.10
DLPFC L	MF (s) > FM (s)	−18	23	39	5.06	<0.01
		−21	23	51	4.22	<0.05
		−44	38	19	3.96	<0.05
		−17	26	52	3.95	<0.05
		−20	6	49	3.80	<0.10
		−20	18	55	3.65	<0.10
DLPFC R	M > F	27	−1	60	3.76	<0.10
	MF (s) > FM (s)	34	38	25	4.75	<0.01
	FM (u) > MF (u)	*18*	*12*	*45*	*3.59*	*<0.10*
RLPFC L	MF (s) > FM (s)	−27	59	7	4.11	<0.01
		−23	51	10	3.68	<0.05
		−23	54	0	3.46	<0.05
		−26	56	19	3.46	<0.05
		−24	53	16	3.32	<0.05
RLPFC R	MF (s) > FM (s)	30	50	−2	3.26	<0.05
		26	50	15	3.02	<0.10

MNI coordinates are given for the voxels showing a sex difference between males and females within the same group, *e.g.* male *versus* female controls. Indicated in Italic is a reversed sex difference. For these comparisons, ROIs were used (indicated in the first column) since no results were obtained at whole-brain level. All sex differences reported are FWE corrected $p < .10$. M: control boys, F: control girls, MF: male adolescents with GD, FM: female adolescents with GD, (s): suppressed by GnRHa, (u): untreated.

of sex hormones (Cohen-Kettenis and Gooren, 1999; Van Goozen et al., 2002; Swaab, 2004). This was based on findings that the development of the sexual organs and the differentiation of the brain follow separate time courses during prenatal development, implying different time windows during which these processes can be affected. Plotting effect sizes in the present study showed that brain activation levels of the untreated adolescents with GD fell in-between those of the two control groups in the areas that showed significant sex differences in the controls (Fig. 3). Hence, untreated MFs and FMs had a closer resemblance to each other than the control groups and no sex differences were found. Similar results were found in the VF study performed by our group (Soleman et al., 2013), where the controls showed a sex difference in right rolandic operculum activation but the untreated adolescents with GD, who showed intermediate activation compared to the control groups, did not. As proposed by the sexual differentiation hypothesis of GD (Cohen-Kettenis and Gooren, 1999; Van Goozen et al., 2002; Swaab, 2004), the absence of a sex difference in untreated GD might be a result of a different hormonal milieu during prenatal development. However, possible effects of pubertal hormones on establishing atypical differentiation cannot be ruled out based on the results of the untreated participants. To this end, examination of sexual differentiation in puberty suppressed adolescents with GD, as was performed in the present study, provided a useful model. Interestingly, the suppressed MFs showed greater activation than the suppressed FMs in the same ROIs that were more active in control boys than control girls, indicating sex-typical brain activations. This similarity to their natal sex was also observed when comparing the suppressed adolescents with GD to the control groups. Like control boys, suppressed MFs showed greater ROI activation than control

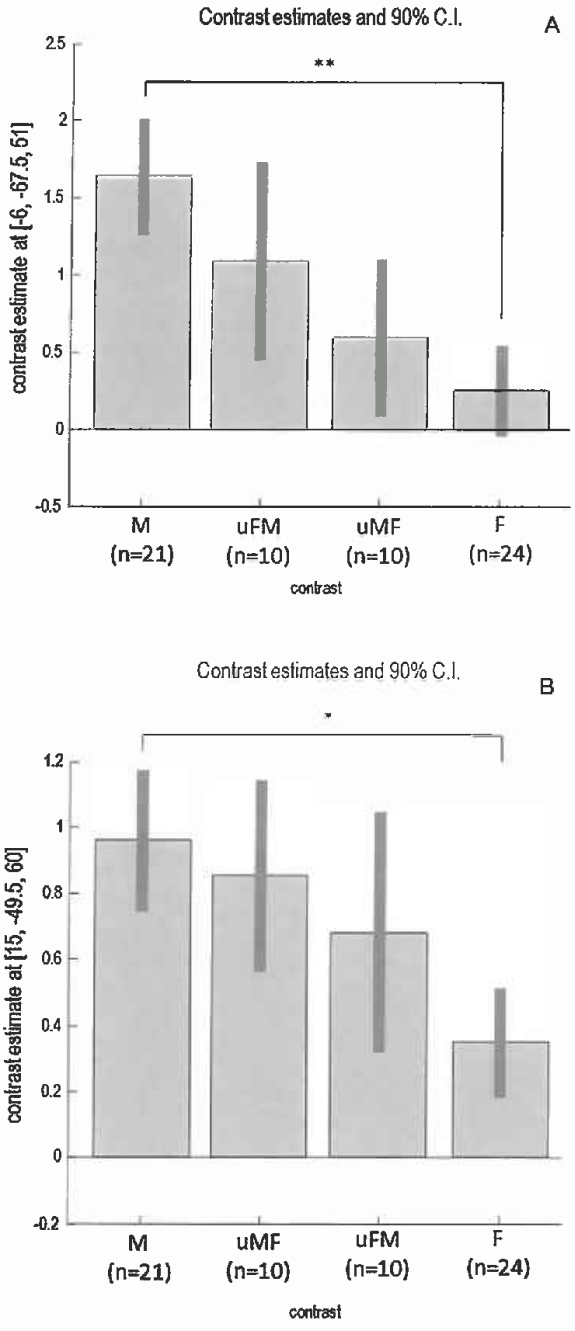

Figure 3 Contrast estimates and 90% confidence intervals (dark gray bars) for task load in the control groups and the untreated adolescents with GD in (A) the left precuneus (MNI coordinates: −6, 67.5, 51) and (B) the right precuneus (MNI coordinates: 15, −49.5, 60). A and B represent the voxels that demonstrated the greatest sex difference in the control groups. *P(FWE) < .05, **P(FWE) < .01. M = control boys, F = control girls, uMF = untreated male adolescents with GD, uFM = untreated female adolescents with GD.

girls. Likewise, suppressed FMs showed lower ROI activation than control boys. These results were not found in the untreated adolescents with GD.

Thus, the present results indicate that the observed atypical sexual differentiation of ToL related brain activation in the untreated individuals with GD was not (solely) due to pre-natal organizing effects. Interestingly, a recent review by Steensma et al. (2013) suggested that the period of adolescence seems to be crucial for the development of a non-normative gender identity. Pubertal hormones might be needed to activate the sex-atypical ToL related brain activations in adolescents with GD, whereas sex-atypical activations are no longer induced when pubertal hormones are suppressed by GnRHa, leading to sex-typical activation.

The GnRHa treated adolescents with GD even appeared to have *exaggerated* sex-typical activation of the ROIs. The suppressed FMs showed a significantly smaller activation of the right precuneus than Fs and the suppressed MFs showed a greater left RLPFC activation than Ms. Furthermore, the suppressed groups showed significant sex differences in every ROI, including ROIs that were not significantly different in the control groups. Interestingly, pre-pubertally administrated GnRHa was also found to modulate the development of cognitive functioning in sheep in a sex-specific manner (Wojniusz et al., 2011). Finally, additional factors might have played a role in the more prominent activation of the RLPFC in suppressed MFs. It is possible that this increase in left RLPFC activity reflects a greater effort of the suppressed MFs in performing the ToL task since they had the lowest IQ scores and made more errors than any other group.

In conclusion, our results suggest that there are no detrimental effects of GnRHa on EF. In addition, we have shed some light on another concern that has been raised among clinicians: whether GnRHa treatment would push adolescents with GD in the direction of their experienced gender. We found no evidence for this and if anything, we found that puberty suppression even seemed to make some aspects of brain functioning more in accordance with the natal sex.

Role of the funding source

The funding sources did not play a role in any component of this study.

Conflicts of interest

The authors report no biomedical financial interest or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.03.007.

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Scientific Statement

Scientific Statement

Considering Sex as a Biological Variable in Basic and Clinical Studies: An Endocrine Society Scientific Statement

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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

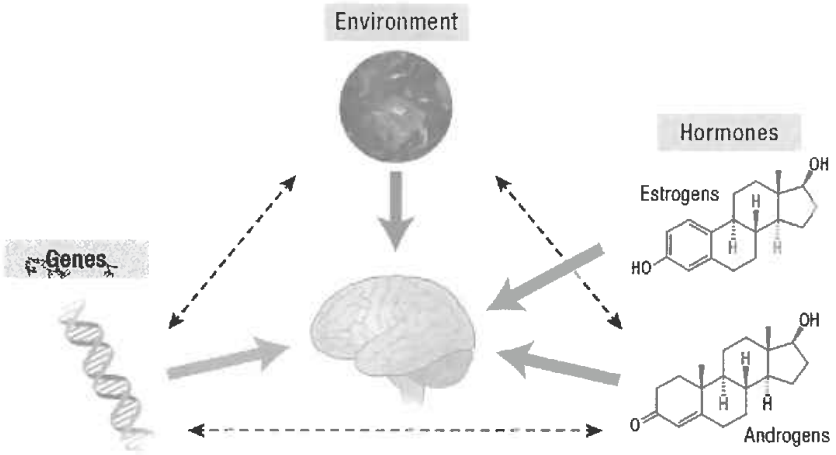

Figure 1. Simplified view of the factors influencing sex differences in the brain. Three broad groups of factors influence the sexually dimorphic brain, as indicated by the broad, colored arrows. 1) Genes and genetic factors that influence the brain include both those on sex chromosomes and autosomes, 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).

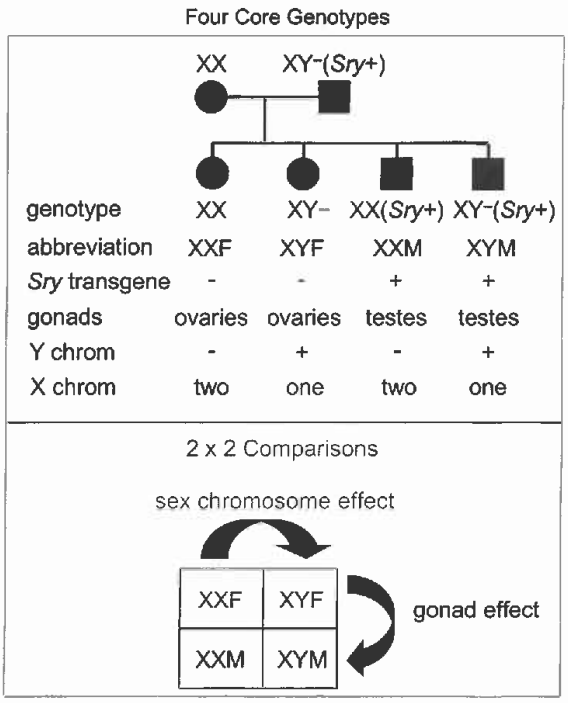

Figure 2. Schematic diagram of the Four Core Genotypes mouse model. The testis-determining gene *Sry* is deleted from the Y chromosome, producing the Y^- chromosome. An *Sry* transgene is inserted onto chromosome 3. Thus, the type of gonad is no longer linked to the sex chromosomes. The model produces 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

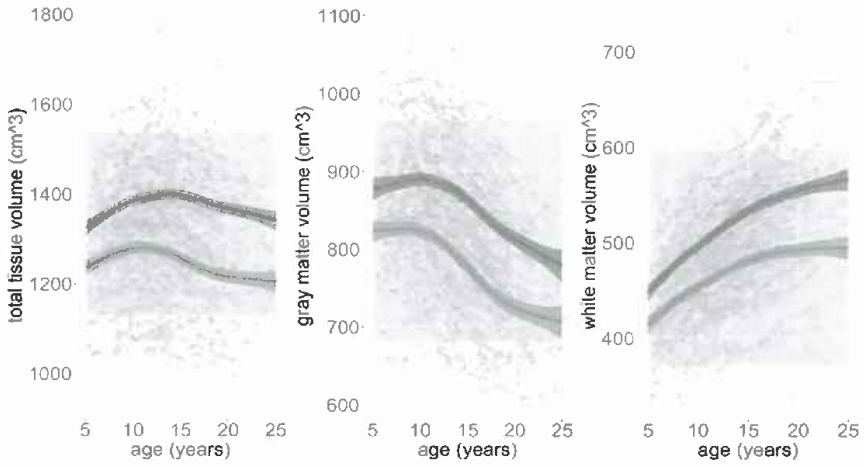

Figure 3. Developmental trajectories for total brain tissue volume, gray matter volume, and white matter volume in men and women over Development. Person-level data are shown for women (red) and men (blue) as points, with lines linking measures from the same person over time. Note the large interindividual 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

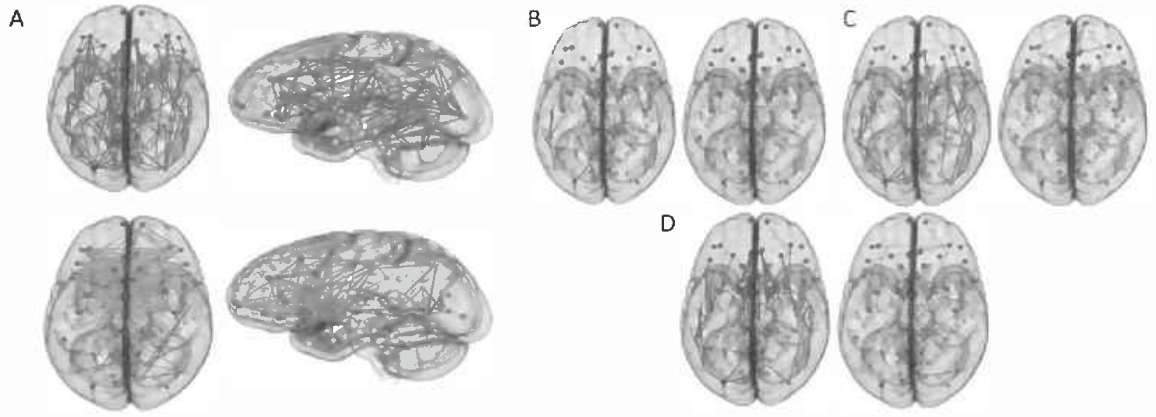

Figure 4. Sex differences in structural connectomes across development. Connectomes representing the white matter structural connectivity in the brain, with nodes indicating the brain regions and edges between the nodes representing the structural connectivity between the nodes. Node colors representing 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 30th centile for male brain volume, and many men have a total brain volume below the 30th 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 mesoanatomical 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

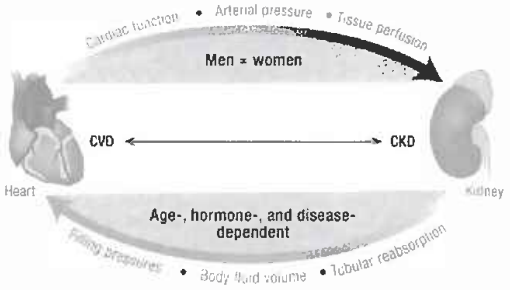

Figure 5. Heart and kidney functions are linked. Sex differences exist in many aspects of heart and kidney function at baseline and in CVD and CKD, as shown. Both organs feed-forward and influence each other's function. Genes, hormones, and age are some known factors that modulate this relationship in 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

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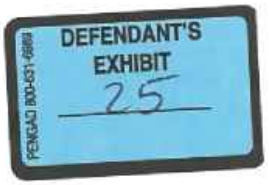

POLICY STATEMENT Organizational Principles to Guide and Define the Child Health Care System and/or Improve the Health of all Children

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

Ensuring Comprehensive Care and Support for Transgender and Gender-Diverse Children and Adolescents

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abstract

FREE

As a traditionally underserved population that faces numerous health disparities, youth who identify as transgender and gender diverse (TGD) and their families are increasingly presenting to pediatric providers for education, care, and referrals. The need for more formal training, standardized treatment, and research on safety and medical outcomes often leaves providers feeling ill equipped to support and care for patients that identify as TGD and families. In this policy statement, we review relevant concepts and challenges and provide suggestions for pediatric providers that are focused on promoting the health and positive development of youth that identify as TGD while eliminating discrimination and stigma.

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Dr Rafferty conceptualized the statement, drafted the initial manuscript, reviewed and revised the manuscript, approved the final manuscript as submitted, and agrees to be accountable for all aspects of the work.

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The guidance in this statement does not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.

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INTRODUCTION

In its dedication to the health of all children, the American Academy of Pediatrics (AAP) strives to improve health care access and eliminate disparities for children and teenagers who identify as lesbian, gay, bisexual, transgender, or questioning (LGBTQ) of their sexual or gender identity.[1,2] Despite some advances in public awareness and legal protections, youth who identify as LGBTQ continue to face disparities that stem from multiple sources, including inequitable laws and policies, societal discrimination, and a lack of access to quality health care, including mental health care. Such challenges are often more intense for youth who do not conform to social expectations and norms regarding gender. Pediatric providers are increasingly encountering such youth and their families, who seek medical advice and interventions, yet they may lack the formal training to care for youth that identify as transgender and gender diverse (TGD) and their families.[3]

This policy statement is focused specifically on children and youth that identify as TGD rather than the larger LGBTQ population, providing brief, relevant background on the basis of current available research

TABLE 1 Relevant Terms and Definitions Related to Gender Care

Term	Definition
Sex	An assignment that is made at birth, usually male or female, typically on the basis of external genital anatomy but sometimes on the basis of internal gonads, chromosomes, or hormone levels
Gender identity	A person's deep internal sense of being female, male, a combination of both, somewhere in between, or neither, resulting from a multifaceted interaction of biological traits, environmental factors, self-understanding, and cultural expectations
Gender expression	The external way a person expresses their gender, such as with clothing, hair, mannerisms, activities, or social roles
Gender perception	The way others interpret a person's gender expression
Gender diverse	A term that is used to describe people with gender behaviors, appearances, or identities that are incongruent with those culturally assigned to their birth sex; gender-diverse individuals may refer to themselves with many different terms, such as transgender, nonbinary, genderqueer,[7] gender fluid, gender creative, gender independent, or noncisgender. "Gender diverse" is used to acknowledge and include the vast diversity of gender identities that exists. It replaces the former term, "gender nonconforming," which has a negative and exclusionary connotation.
Transgender	A subset of gender-diverse youth whose gender identity does not match their assigned sex and generally remains persistent, consistent, and insistent over time; the term "transgender" also encompasses many other labels individuals may use to refer to themselves.
Cisgender	A term that is used to describe a person who identifies and expresses a gender that is consistent with the culturally defined norms of the sex they were assigned at birth
Agender	A term that is used to describe a person who does not identify as having a particular gender
Affirmed gender	When a person's true gender identity, or concern about their gender identity, is communicated to and validated from others as authentic
MTF; affirmed female; trans female	Terms that are used to describe individuals who were assigned male sex at birth but who have a gender identity and/or expression that is asserted to be more feminine
FTM; affirmed male; trans male	Terms that are used to describe individuals who were assigned female sex at birth but who have a gender identity and/or expression that is asserted to be more masculine
Gender dysphoria	A clinical symptom that is characterized by a sense of alienation to some or all of the physical characteristics or social roles of one's assigned gender; also, gender dysphoria is the psychiatric diagnosis in the *DSM-5*, which has focus on the distress that stems from the incongruence between one's expressed or experienced (affirmed) gender and the gender assigned at birth.
Gender identity disorder	A psychiatric diagnosis defined previously in the *DSM-IV* (changed to "gender dysphoria" in the *DSM-5*); the primary criteria include a strong, persistent cross-sex identification and significant distress and social impairment. This diagnosis is no longer appropriate for use and may lead to stigma, but the term may be found in older research.
Sexual orientation	A person's sexual identity in relation to the gender(s) to which they are attracted; sexual orientation and gender identity develop separately.

This list is not intended to be all inclusive. The pronouns "they" and "their" are used intentionally to be inclusive rather than the binary pronouns "he" and "she" and "his" and "her." Adapted from Bonifacio HJ, Rosenthal SM. Gender variance and dysphoria in children and adolescents. *Pediatr Clin North Am*. 2015;62(4):1001–1016. Adapted from Vance SR Jr, Ehrensaft D, Rosenthal SM. Psychological and medical care of gender nonconforming youth. *Pediatrics*. 2014;134(6):1184–1192. DSM-5, *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, FTM, female to male; MTF, male to female.

and expert opinion from clinical and research leaders, which will serve as the basis for recommendations. It is not a comprehensive review of clinical approaches and nuances to pediatric care for children and youth that identify as TGD. Professional understanding of youth that identify as TGD is a rapidly evolving clinical field in which research on appropriate clinical management is limited by insufficient funding.[3,4]

DEFINITIONS

To clarify recommendations and discussions in this policy statement, some definitions are provided. However, brief descriptions of human behavior or identities may not capture nuance in this evolving field. "Sex," or "natal gender," is a label, generally "male" or "female," that is typically assigned at birth on the basis of genetic and anatomic characteristics, such as genital anatomy, chromosomes, and sex hormone levels. Meanwhile, "gender identity" is one's internal sense of who one is, which results from a multifaceted interaction of biological traits, developmental influences, and environmental conditions. It may be male, female, somewhere in between, a combination of both, or neither (ie, not conforming to a binary conceptualization of gender). Self-recognition of gender identity develops over time, much the same way as a child's physical body does. For some people, gender identity can be fluid, shifting in different contexts. "Gender expression" refers to the wide array of ways people display their gender through clothing, hair styles, mannerisms, or social roles. Exploring different ways of expressing gender is common for children and may challenge social expectations. The way others interpret this expression is referred to as "gender perception" (Table 1).[5,6]

These labels may or may not be congruent. The term "cisgender" is used if someone identifies and expresses a gender that is consistent with the culturally defined norms of the sex that was assigned at birth. "Gender diverse" is an umbrella term to describe an ever-evolving array of labels that people may apply when their gender identity, expression, or even perception does not conform

to the norms and stereotypes others expect of their assigned sex. "Transgender" is usually reserved for a subset of such youth whose gender identity does not match their assigned sex and generally remains persistent, consistent, and insistent over time. These terms are not diagnoses; rather, they are personal and often dynamic ways of describing one's own gender experience.

Gender identity is not synonymous with "sexual orientation," which refers to a person's identity in relation to the gender(s) to which they are sexually and romantically attracted. Gender identity and sexual orientation are distinct but interrelated constructs.[8] Therefore, being transgender does not imply a sexual orientation, and people who identify as transgender still identify as straight, gay, bisexual, etc, on the basis of their attractions. (For more information, *The Gender Book*, found at www.thegenderbook.com, is a resource with illustrations that are used to highlight these core terms and concepts.)

EPIDEMIOLOGY

In population-based surveys, questions related to gender identity are rarely asked, which makes it difficult to assess the size and characteristics of the population that is TGD. In the 2014 Behavioral Risk Factor Surveillance System of the Centers for Disease Control and Prevention, only 19 states elected to include optional questions on gender identity. Extrapolation from these data suggests that the US prevalence of adults who identify as transgender or "gender nonconforming" is 0.6% (1.4 million), ranging from 0.3% in North Dakota to 0.8% in Hawaii.[9] On the basis of these data, it has been estimated that 0.7% of youth ages 13 to 17 years (~150 000) identify as transgender.[10] This number is much higher than previous estimates, which were extrapolated from individual states or specialty clinics, and is likely an underestimate given the stigma regarding those who openly identify as transgender and the difficulty in defining "transgender" in a way that is inclusive of all gender-diverse identities.[11]

There have been no large-scale prevalence studies among children and adolescents, and there is no evidence that adult statistics reflect young children or adolescents. In the 2014 Behavioral Risk Factor Surveillance System, those 18 to 24 years of age were more likely than older age groups to identify as transgender (0.7%).[9] Children report being aware of gender incongruence at young ages. Children who later identify as TGD report first having recognized their gender as "different" at an average age of 8.5 years; however, they did not disclose such feelings until an average of 10 years later.[12]

MENTAL HEALTH IMPLICATIONS

Adolescents and adults who identify as transgender have high rates of depression, anxiety, eating disorders, self-harm, and suicide.[13–20] Evidence suggests that an identity of TGD has an increased prevalence among individuals with autism spectrum disorder, but this association is not yet well understood.[21,22] In 1 retrospective cohort study, 56% of youth who identified as transgender reported previous suicidal ideation, and 31% reported a previous suicide attempt, compared with 20% and 11% among matched youth who identified as cisgender, respectively.[13] Some youth who identify as TGD also experience gender dysphoria, which is a specific diagnosis given to those who experience impairment in peer and/or family relationships, school performance, or other aspects of their life as a consequence of the incongruence between their assigned sex and their gender identity.[23]

There is no evidence that risk for mental illness is inherently attributable to one's identity of TGD. Rather, it is believed to be multifactorial, stemming from an internal conflict between one's appearance and identity, limited availability of mental health services, low access to health care providers with expertise in caring for youth who identify as TGD, discrimination, stigma, and social rejection.[24] This was affirmed by the American Psychological Association in 2008[25] (with practice guidelines released in 2015[8]) and the American Psychiatric Association, which made the following statement in 2012:

> *Being transgender or gender variant implies no impairment in judgment, stability, reliability, or general social or vocational capabilities; however, these individuals often experience discrimination due to a lack of civil rights protections for their gender identity or expression.... [Such] discrimination and lack of equal civil rights is damaging to the mental health of transgender and gender variant individuals.*[26]

Youth who identify as TGD often confront stigma and discrimination, which contribute to feelings of rejection and isolation that can adversely affect physical and emotional well-being. For example, many youth believe that they must hide their gender identity and expression to avoid bullying, harassment, or victimization. Youth who identify as TGD experience disproportionately high rates of homelessness, physical violence (at home and in the community), substance abuse, and high-risk sexual behaviors.[5,6,12,27–31] Among the 3 million HIV testing events that were reported in 2015, the highest percentages of new infections were among women who identified as transgender[32] and were also at particular risk for not knowing their HIV status.[30]

GENDER-AFFIRMATIVE CARE

In a gender-affirmative care model (GACM), pediatric providers offer developmentally appropriate care that is oriented toward understanding and appreciating the youth's gender experience. A strong, nonjudgmental partnership with youth and their families can facilitate exploration of complicated emotions and gender-diverse expressions while allowing questions and concerns to be raised in a supportive environment.[5] In a GACM, the following messages are conveyed:

- transgender identities and diverse gender expressions do not constitute a mental disorder;
- variations in gender identity and expression are normal aspects of human diversity, and binary definitions of gender do not always reflect emerging gender identities;
- gender identity evolves as an interplay of biology, development, socialization, and culture; and
- if a mental health issue exists, it most often stems from stigma and negative experiences rather than being intrinsic to the child.[27,33]

The GACM is best facilitated through the integration of medical, mental health, and social services, including specific resources and supports for parents and families.[24] Providers work together to destigmatize gender variance, promote the child's self-worth, facilitate access to care, educate families, and advocate for safer community spaces where children are free to develop and explore their gender.[5] A specialized gender-affirmative therapist, when available, may be an asset in helping children and their families build skills for dealing with gender-based stigma, address symptoms of anxiety or depression, and reinforce the child's overall resiliency.[34,35] There is a limited but growing body of evidence that suggests that using an integrated affirmative model results in young people having fewer mental health concerns whether they ultimately identify as transgender.[24,36,37]

In contrast, "conversion" or "reparative" treatment models are used to prevent children and adolescents from identifying as transgender or to dissuade them from exhibiting gender-diverse expressions. The Substance Abuse and Mental Health Services Administration has concluded that any therapeutic intervention with the goal of changing a youth's gender expression or identity is inappropriate.[33] Reparative approaches have been proven to be not only unsuccessful[38] but also deleterious and are considered outside the mainstream of traditional medical practice.[29,39–42] The AAP described reparative approaches as "unfair and deceptive."[43] At the time of this writing,* conversion therapy was banned by executive regulation in New York and by legislative statutes in 9 other states as well as the District of Columbia.[44]

Pediatric providers have an essential role in assessing gender concerns and providing evidence-based information to assist youth and families in medical decision-making. Not doing so can prolong or exacerbate gender dysphoria and contribute to abuse and stigmatization.[35] If a pediatric provider does not feel prepared to address gender concerns when they occur, then referral to a pediatric or mental health provider with more expertise is appropriate. There is little research on communication and efficacy with transfers in care for youth who identify as TGD, particularly from pediatric to adult providers.

DEVELOPMENTAL CONSIDERATIONS

Acknowledging that the capacity for emerging abstract thinking in childhood is important to conceptualize and reflect on identity, gender-affirmation guidelines are being focused on individually tailored interventions on the basis of the physical and cognitive development of youth who identify as TGD.[45] Accordingly, research substantiates that children who are prepubertal and assert an identity of TGD know their gender as clearly and as consistently as their developmentally equivalent peers who identify as cisgender and benefit from the same level of social acceptance.[46] This developmental approach to gender affirmation is in contrast to the outdated approach in which a child's gender-diverse assertions are held as "possibly true" until an arbitrary age (often after pubertal onset) when they can be considered valid, an approach that authors of the literature have termed "watchful waiting." This outdated approach does not serve the child because critical support is withheld. Watchful waiting is based on binary notions of gender in which gender diversity and fluidity is pathologized; in watchful waiting, it is also assumed that notions of gender identity become fixed at a certain age. The approach is also influenced by a group of early studies with validity concerns, methodologic flaws, and limited follow-up on children who identified as TGD and, by adolescence, did not seek further treatment ("desisters").[45,47] More robust and current research suggests that, rather than focusing on who a child will become, valuing them for who they are, even at a young age, fosters secure attachment and resilience, not only for the child but also for the whole family.[5,45,48,49]

* For more information regarding state-specific laws, please contact the AAP Division of State Government Affairs at stgov@aap.org.

MEDICAL MANAGEMENT

Pediatric primary care providers are in a unique position to routinely inquire about gender development in children and adolescents as part of recommended well-child visits[50] and to be a reliable source of validation, support, and reassurance. They are often the first provider to be aware that a child may not identify as cisgender or that there may be distress related to a gender-diverse identity. The best way to approach gender with patients is to inquire directly and nonjudgmentally about their experience and feelings before applying any labels.[27,51]

Many medical interventions can be offered to youth who identify as TGD and their families. The decision of whether and when to initiate gender-affirmative treatment is personal and involves careful consideration of risks, benefits, and other factors unique to each patient and family. Many protocols suggest that clinical assessment of youth who identify as TGD is ideally conducted on an ongoing basis in the setting of a collaborative, multidisciplinary approach, which, in addition to the patient and family, may include the pediatric provider, a mental health provider (preferably with expertise in caring for youth who identify as TGD), social and legal supports, and a pediatric endocrinologist or adolescent-medicine gender specialist, if available.[6,28] There is no prescribed path, sequence, or end point. Providers can make every effort to be aware of the influence of their own biases. The medical options also vary depending on pubertal and developmental progression.

Clinical Setting

In the past year, 1 in 4 adults who identified as transgender avoided a necessary doctor's visit because of fear of being mistreated.[31] All clinical office staff have a role in affirming a patient's gender identity. Making flyers available or displaying posters related to LGBTQ health issues, including information for children who identify as TGD and families, reveals inclusivity and awareness. Generally, patients who identify as TGD feel most comfortable when they have access to a gender-neutral restroom. Diversity training that encompasses sensitivity when caring for youth who identify as TGD and their families can be helpful in educating clinical and administrative staff. A patient-asserted name and pronouns are used by staff and are ideally reflected in the electronic medical record without creating duplicate charts.[52,53] The US Centers for Medicare and Medicaid Services and the National Coordinator for Health Information Technology require all electronic health record systems certified under the Meaningful Use incentive program to have the capacity to confidentially collect information on gender identity.[54,55] Explaining and maintaining confidentiality procedures promotes openness and trust, particularly with youth who identify as LGBTQ.[1] Maintaining a safe clinical space can provide at least 1 consistent, protective refuge for patients and families, allowing authentic gender expression and exploration that builds resiliency.

Pubertal Suppression

Gonadotrophin-releasing hormones have been used to delay puberty since the 1980s for central precocious puberty.[56] These reversible treatments can also be used in adolescents who experience gender dysphoria to prevent development of secondary sex characteristics and provide time up until 16 years of age for the individual and the family to explore gender identity, access psychosocial supports, develop coping skills, and further define appropriate treatment goals. If pubertal suppression treatment is suspended, then endogenous puberty will resume.[20,57,58]

Often, pubertal suppression creates an opportunity to reduce distress that may occur with the development of secondary sexual characteristics and allow for gender-affirming care, including mental health support for the adolescent and the family. It reduces the need for later surgery because physical changes that are otherwise irreversible (protrusion of the Adam's apple, male pattern baldness, voice change, breast growth, etc) are prevented. The available data reveal that pubertal suppression in children who identify as TGD generally leads to improved psychological functioning in adolescence and young adulthood.[20,57–59]

Pubertal suppression is not without risks. Delaying puberty beyond one's peers can also be stressful and can lead to lower self-esteem and increased risk taking.[60] Some experts believe that genital underdevelopment may limit some potential reconstructive options.[61] Research on long-term risks, particularly in terms of bone metabolism[62] and fertility,[63] is currently limited and provides varied results.[57,64,65] Families often look to pediatric providers for help in considering whether pubertal suppression is indicated in the context of their child's overall well-being as gender diverse.

Gender Affirmation

As youth who identify as TGD reflect on and evaluate their gender identity, various interventions may be considered to better align their gender expression with their underlying identity. This process of reflection, acceptance, and, for some, intervention is known as "gender affirmation." It was formerly referred to as "transitioning," but many view the process as an affirmation and acceptance of who they have always been rather than a transition

TABLE 2 The Process of Gender Affirmation May Include ≥1 of the Following Components

Component	Definition	General Age Range[a]	Reversibility[a]
Social affirmation	Adopting gender-affirming hairstyles, clothing, name, gender pronouns, and restrooms and other facilities	Any	Reversible
Puberty blockers	Gonadotropin-releasing hormone analogues, such as leuprolide and histrelin	During puberty (Tanner stage 2–5)[b]	Reversible[c]
Cross-sex hormone therapy	Testosterone (for those who were assigned female at birth and are masculinizing); estrogen plus androgen inhibitor (for those who were assigned male at birth and are feminizing)	Early adolescence onward	Partially reversible (skin texture, muscle mass, and fat deposition); irreversible once developed (testosterone: Adam's apple protrusion, voice changes, and male pattern baldness; estrogen: breast development); unknown reversibility (effect on fertility)
Gender-affirming surgeries	"Top" surgery (to create a male-typical chest shape or enhance breasts); "bottom" surgery (surgery on genitals or reproductive organs); facial feminization and other procedures	Typically adults (adolescents on case-by-case basis[d])	Not reversible
Legal affirmation	Changing gender and name recorded on birth certificate, school records, and other documents	Any	Reversible

[a] Note that the provided age range and reversibility is based on the little data that are currently available.
[b] There is limited benefit to starting gonadotropin-releasing hormone after Tanner stage 5 for pubertal suppression. However, when cross-sex hormones are initiated with a gradually increasing schedule, the initial levels are often not high enough to suppress endogenous sex hormone secretion. Therefore, gonadotropin-releasing hormone may be continued in accordance with the Endocrine Society Guidelines.[68]
[c] The effect of sustained puberty suppression on fertility is unknown. Pubertal suppression can be, and often is indicated to be, followed by cross-sex hormone treatment. However, when cross-sex hormones are initiated without endogenous hormones, then fertility may be decreased.[68]
[d] Eligibility criteria for gender-affirmative surgical interventions among adolescents are not clearly defined between established protocols and practice. When applicable, eligibility is usually determined on a case-by-case basis with the adolescent and the family along with input from medical, mental health, and surgical providers.[68–71]

from 1 gender identity to another. Accordingly, some people who have gone through the process prefer to call themselves "affirmed females, males, etc" (or just "females, males, etc"), rather than using the prefix "trans-." Gender affirmation is also used to acknowledge that some individuals who identify as TGD may feel affirmed in their gender without pursuing medical or surgical interventions.[7,66]

Supportive involvement of parents and family is associated with better mental and physical health outcomes.[67] Gender affirmation among adolescents with gender dysphoria often reduces the emphasis on gender in their lives, allowing them to attend to other developmental tasks, such as academic success, relationship building, and future-oriented planning.[64] Most protocols for gender-affirming interventions incorporate World Professional Association of Transgender Health[35] and Endocrine Society[68] recommendations and include ≥1 of the following elements (Table 2):

1. Social Affirmation: This is a reversible intervention in which children and adolescents express partially or completely in their asserted gender identity by adapting hairstyle, clothing, pronouns, name, etc. Children who identify as transgender and socially affirm and are supported in their asserted gender show no increase in depression and only minimal (clinically insignificant) increases in anxiety compared with age-matched averages.[48] Social affirmation can be complicated given the wide range of social interactions children have (eg, extended families, peers, school, community, etc). There is little guidance on the best approach (eg, all at once, gradual, creating new social networks, or affirming within existing networks, etc). Pediatric providers can best support families by anticipating and discussing such complexity proactively, either in their own practice or through enlisting a qualified mental health provider.
2. Legal Affirmation: Elements of a social affirmation, such as a name and gender marker, become official on legal documents, such as birth certificates, passports, identification cards, school documents, etc. The processes for making these changes depend on state laws and may require specific documentation from pediatric providers.
3. Medical Affirmation: This is the process of using cross-sex hormones to allow adolescents who have initiated puberty to develop secondary sex characteristics of the opposite biological sex. Some changes are partially reversible if hormones are stopped, but others become

irreversible once they are fully developed (Table 2).

4. Surgical Affirmation: Surgical approaches may be used to feminize or masculinize features, such as hair distribution, chest, or genitalia, and may include removal of internal organs, such as ovaries or the uterus (affecting fertility). These changes are irreversible. Although current protocols typically reserve surgical interventions for adults,[35,68] they are occasionally pursued during adolescence on a case-by-case basis, considering the necessity and benefit to the adolescent's overall health and often including multidisciplinary input from medical, mental health, and surgical providers as well as from the adolescent and family.[69–71]

For some youth who identify as TGD whose natal gender is female, menstruation, breakthrough bleeding, and dysmenorrhea can lead to significant distress before or during gender affirmation. The American College of Obstetrics and Gynecology suggests that, although limited data are available to outline management, menstruation can be managed without exogenous estrogens by using a progesterone-only pill, a medroxyprogesterone acetate shot, or a progesterone-containing intrauterine or implantable device.[72] If estrogen can be tolerated, oral contraceptives that contain both progesterone and estrogen are more effective at suppressing menses.[73] The Endocrine Society guidelines also suggest that gonadotrophin-releasing hormones can be used for menstrual suppression before the anticipated initiation of testosterone or in combination with testosterone for breakthrough bleeding (enables phenotypic masculinization at a lower dose than if testosterone is used alone).[68] Masculinizing hormones in natal female patients may lead to a cessation of menses, but unplanned pregnancies have been reported, which emphasizes the need for ongoing contraceptive counseling with youth who identify as TGD.[72]

HEALTH DISPARITIES

In addition to societal challenges, youth who identify as TGD face several barriers within the health care system, especially regarding access to care. In 2015, a focus group of youth who identified as transgender in Seattle, Washington, revealed 4 problematic areas related to health care:

1. safety issues, including the lack of safe clinical environments and fear of discrimination by providers;
2. poor access to physical health services, including testing for sexually transmitted infections;
3. inadequate resources to address mental health concerns; and
4. lack of continuity with providers.[74]

This study reveals the obstacles many youth who identify as TGD face in accessing essential services, including the limited supply of appropriately trained medical and psychological providers, fertility options, and insurance coverage denials for gender-related treatments.[74]

Insurance denials for services related to the care of patients who identify as TGD are a significant barrier. Although the Office for Civil Rights of the US Department of Health and Human Services explicitly stated in 2012 that the nondiscrimination provision in the Patient Protection and Affordable Care Act includes people who identify as gender diverse,[75,76] insurance claims for gender affirmation, particularly among youth who identify as TGD, are frequently denied.[54,77] In 1 study, it was found that approximately 25% of individuals who identified as transgender were denied insurance coverage because of being transgender.[31] The burden of covering medical expenses that are not covered by insurance can be financially devastating, and even when expenses are covered, families describe high levels of stress in navigating and submitting claims appropriately.[78] In 2012, a large gender center in Boston, Massachusetts, reported that most young patients who identified as transgender and were deemed appropriate candidates for recommended gender care were unable to obtain it because of such denials, which were based on the premise that gender dysphoria was a mental disorder, not a physical one, and that treatment was not medically or surgically necessary.[24] This practice not only contributes to stigma, prolonged gender dysphoria, and poor mental health outcomes,[77] but it may also lead patients to seek nonmedically supervised treatments that are potentially dangerous.[24] Furthermore, insurance denials can reinforce a socioeconomic divide between those who can finance the high costs of uncovered care and those who cannot.[24,77]

The transgender youth group in Seattle likely reflected the larger TGD population when they described how obstacles adversely affect self-esteem and contribute to the perception that they are undervalued by society and the health care system.[74,77] Professional medical associations, including the AAP, are increasingly calling for equity in health care provisions regardless of gender identity or expression.[1,8,23,72] There is a critical need for investments in research on the prevalence, disparities, biological underpinnings, and standards of care relating to gender-diverse populations. Pediatric providers who work with state government and insurance officials can play an essential role in advocating for

stronger nondiscrimination policies and improved coverage.

There is a lack of quality research on the experience of youth of color who identify as transgender. One theory suggests that the intersection of racism, transphobia, and sexism may result in the extreme marginalization that is experienced among many women of color who identify as transgender,[79] including rejection from their family and dropping out of school at younger ages (often in the setting of rigid religious beliefs regarding gender),[80] increased levels of violence and body objectification,[81] 3 times the risk of poverty compared with the general population,[31] and the highest prevalence of HIV compared with other risk groups (estimated as high as 56.3% in 1 meta-analysis).[30] One model suggests that pervasive stigma and oppression can be associated with psychological distress (anxiety, depression, and suicide) and adoption of risk behaviors by such youth to obtain a sense of validation toward their complex identities.[79]

FAMILY ACCEPTANCE

Research increasingly suggests that familial acceptance or rejection ultimately has little influence on the gender identity of youth; however, it may profoundly affect young people's ability to openly discuss or disclose concerns about their identity. Suppressing such concerns can affect mental health.[82] Families often find it hard to understand and accept their child's gender-diverse traits because of personal beliefs, social pressure, and stigma.[49,83] Legitimate fears may exist for their child's welfare, safety, and acceptance that pediatric providers need to appreciate and address. Families can be encouraged to communicate their concerns and questions. Unacknowledged concerns can contribute to shame and hesitation in regard to offering support and understanding,[84] which is essential for the child's self-esteem, social involvement, and overall health as TGD.[48,85–87] Some caution has been expressed that unquestioning acceptance per se may not best serve questioning youth or their families. Instead, psychological evidence suggests that the most benefit comes when family members and youth are supported and encouraged to engage in reflective perspective taking and validate their own and the other's thoughts and feelings despite divergent views.[49,82]

In this regard, suicide attempt rates among 433 adolescents in Ontario who identified as "trans" were 4% among those with strongly supportive parents and as high as 60% among those whose parents were not supportive.[85] Adolescents who identify as transgender and endorse at least 1 supportive person in their life report significantly less distress than those who only experience rejection. In communities with high levels of support, it was found that nonsupportive families tended to increase their support over time, leading to dramatic improvement in mental health outcomes among their children who identified as transgender.[88]

Pediatric providers can create a safe environment for parents and families to better understand and listen to the needs of their children while receiving reassurance and education.[83] It is often appropriate to assist the child in understanding the parents' concerns as well. Despite expectations by some youth with transgender identity for immediate acceptance after "coming out," family members often proceed through a process of becoming more comfortable and understanding of the youth's gender identity, thoughts, and feelings. One model suggests that the process resembles grieving, wherein the family separates from their expectations for their child to embrace a new reality. This process may proceed through stages of shock, denial, anger, feelings of betrayal, fear, self-discovery, and pride.[89] The amount of time spent in any of these stages and the overall pace varies widely. Many family members also struggle as they are pushed to reflect on their own gender experience and assumptions throughout this process. In some situations, youth who identify as TGD may be at risk for internalizing the difficult emotions that family members may be experiencing. In these cases, individual and group therapy for the family members may be helpful.[49,78]

Family dynamics can be complex, involving disagreement among legal guardians or between guardians and their children, which may affect the ability to obtain consent for any medical management or interventions. Even in states where minors may access care without parental consent for mental health services, contraception, and sexually transmitted infections, parental or guardian consent is required for hormonal and surgical care of patients who identify as TGD.[72,90] Some families may take issue with providers who address gender concerns or offer gender-affirming care. In rare cases, a family may deny access to care that raises concerns about the youth's welfare and safety; in those cases, additional legal or ethical support may be useful to consider. In such rare situations, pediatric providers may want to familiarize themselves with relevant local consent laws and maintain their primary responsibility for the welfare of the child.

SAFE SCHOOLS AND COMMUNITIES

Youth who identify as TGD are becoming more visible because gender-diverse expression is increasingly admissible in the media, on social media, and in schools and communities. Regardless of whether a youth with a gender-diverse

identity ultimately identifies as transgender, challenges exist in nearly every social context, from lack of understanding to outright rejection, isolation, discrimination, and victimization. In the US Transgender Survey of nearly 28 000 respondents, it was found that among those who were out as or perceived to be TGD between kindergarten and eighth grade, 54% were verbally harassed, 24% were physically assaulted, and 13% were sexually assaulted; 17% left school because of maltreatment.[31] Education and advocacy from the medical community on the importance of safe schools for youth who identify as TGD can have a significant effect.

At the time of this writing,* only 18 states and the District of Columbia had laws that prohibited discrimination based on gender expression when it comes to employment, housing, public accommodations, and insurance benefits. Over 200 US cities have such legislation. In addition to basic protections, many youth who identify as TGD also have to navigate legal obstacles when it comes to legally changing their name and/or gender marker.[54] In addition to advocating and working with policy makers to promote equal protections for youth who identify as TGD, pediatric providers can play an important role by developing a familiarity with local laws and organizations that provide social work and legal assistance to youth who identify as TGD and their families.

School environments play a significant role in the social and emotional development of children. Every child has a right to feel safe and respected at school, but for youth who identify as TGD, this can be challenging. Nearly every aspect of school life may present safety concerns and require negotiations regarding their gender expression, including name/pronoun use, use of bathrooms and locker rooms, sports teams, dances and activities, overnight activities, and even peer groups. Conflicts in any of these areas can quickly escalate beyond the school's control to larger debates among the community and even on a national stage.

The formerly known Gay, Lesbian, and Straight Education Network (GLSEN), an advocacy organization for youth who identify as LGBTQ, conducts an annual national survey to measure LGBTQ well-being in US schools. In 2015, students who identified as LGBTQ reported high rates of being discouraged from participation in extracurricular activities. One in 5 students who identified as LGBTQ reported being hindered from forming or participating in a club to support lesbian, gay, bisexual, or transgender students (eg, a gay straight alliance, now often referred to as a genders and sexualities alliance) despite such clubs at schools being associated with decreased reports of negative remarks about sexual orientation or gender expression, increased feelings of safety and connectedness at school, and lower levels of victimization. In addition, >20% of students who identified as LGBTQ reported being blocked from writing about LGBTQ issues in school yearbooks or school newspapers or being prevented or discouraged by coaches and school staff from participating in sports because of their sexual orientation or gender expression.[91]

One strategy to prevent conflict is to proactively support policies and protections that promote inclusion and safety of all students. However, such policies are far from consistent across districts. In 2015, GLSEN found that 43% of children who identified as LGBTQ reported feeling unsafe at school because of their gender expression, but only 6% reported that their school had official policies to support youth who identified as TGD, and only 11% reported that their school's antibullying policies had specific protections for gender expression.[91] Consequently, more than half of the students who identified as transgender in the study were prevented from using the bathroom, names, or pronouns that aligned with their asserted gender at school. A lack of explicit policies that protected youth who identified as TGD was associated with increased reported victimization, with more than half of students who identified as LGBTQ reporting verbal harassment because of their gender expression. Educators and school administrators play an essential role in advocating for and enforcing such policies. GLSEN found that when students recognized actions to reduce gender-based harassment, both students who identified as transgender and cisgender reported a greater connection to staff and feelings of safety.[91] In another study, schools were open to education regarding gender diversity and were willing to implement policies when they were supported by external agencies, such as medical professionals.[92]

Academic content plays an important role in building a safe school environment as well. The 2015 GLSEN survey revealed that when positive representations of people who identified as LGBTQ were included in the curriculum, students who identified as LGBTQ reported less hostile school environments, less victimization and greater feelings of safety, fewer school absences because of feeling unsafe, greater feelings of connectedness to their school

* For more information regarding state-specific laws, please contact the AAP Division of State Government Affairs at stgov@ aap.org.

community, and an increased interest in high school graduation and postsecondary education.[91] At the time of this writing,* 8 states had laws that explicitly forbade teachers from even discussing LGBTQ issues.[54]

MEDICAL EDUCATION

One of the most important ways to promote high-quality health care for youth who identify as TGD and their families is increasing the knowledge base and clinical experience of pediatric providers in providing culturally competent care to such populations, as recommended by the recently released guidelines by the Association of American Medical Colleges.[93] This begins with the medical school curriculum in areas such as human development, sexual health, endocrinology, pediatrics, and psychiatry. In a 2009–2010 survey of US medical schools, it was found that the median number of hours dedicated to LGBTQ health was 5, with one-third of US medical schools reporting no LGBTQ curriculum during the clinical years.[94]

During residency training, there is potential for gender diversity to be emphasized in core rotations, especially in pediatrics, psychiatry, family medicine, and obstetrics and gynecology. Awareness could be promoted through the inclusion of topics relevant to caring for children who identify as TGD in the list of core competencies published by the American Board of Pediatrics, certifying examinations, and relevant study materials. Continuing education and maintenance of certification activities can include topics relevant to TGD populations as well.

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RECOMMENDATIONS

The AAP works toward all children and adolescents, regardless of gender identity or expression, receiving care to promote optimal physical, mental, and social well-being. Any discrimination based on gender identity or expression, real or perceived, is damaging to the socioemotional health of children, families, and society. In particular, the AAP recommends the following:

1. that youth who identify as TGD have access to comprehensive, gender-affirming, and developmentally appropriate health care that is provided in a safe and inclusive clinical space;
2. that family-based therapy and support be available to recognize and respond to the emotional and mental health needs of parents, caregivers, and siblings of youth who identify as TGD;
3. that electronic health records, billing systems, patient-centered notification systems, and clinical research be designed to respect the asserted gender identity of each patient while maintaining confidentiality and avoiding duplicate charts;
4. that insurance plans offer coverage for health care that is specific to the needs of youth who identify as TGD, including coverage for medical, psychological, and, when indicated, surgical gender-affirming interventions;
5. that provider education, including medical school, residency, and continuing education, integrate core competencies on the emotional and physical health needs and best practices for the care of youth who identify as TGD and their families;
6. that pediatricians have a role in advocating for, educating, and developing liaison relationships with school districts and other community organizations to promote acceptance and inclusion of all children without fear of harassment, exclusion, or bullying because of gender expression;
7. that pediatricians have a role in advocating for policies and laws that protect youth who identify as TGD from discrimination and violence;
8. that the health care workforce protects diversity by offering equal employment opportunities and workplace protections, regardless of gender identity or expression; and
9. that the medical field and federal government prioritize research that is dedicated to improving the quality of evidence-based care for youth who identify as TGD.

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ABBREVIATIONS

AAP: American Academy of Pediatrics
GACM: gender-affirmative care model
GLSEN: Gay, Lesbian, and Straight Education Network
LGBTQ: lesbian, gay, bisexual, transgender, or questioning
TGD: transgender and gender diverse

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Ensuring Comprehensive Care and Support for Transgender and Gender-Diverse Children and Adolescents

Jason Rafferty, COMMITTEE ON PSYCHOSOCIAL ASPECTS OF CHILD AND FAMILY HEALTH, COMMITTEE ON ADOLESCENCE and SECTION ON LESBIAN, GAY, BISEXUAL, AND TRANSGENDER HEALTH AND WELLNESS

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American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

AACAP Statement Responding to Efforts to ban Evidence-Based Care for Transgender and Gender Diverse Youth

November 8, 2019

Variations in gender expression represent normal and expectable dimensions of human development. They are not considered to be pathological. Health promotion for all youth encourages open exploration of all identity issues, including sexual orientation, gender identity, and/or gender expression according to recognized practice guidelines (1, 2). Research consistently demonstrates that gender diverse youth who are supported to live and/or explore the gender role that is consistent with their gender identity have better mental health outcomes than those who are not (3, 4, 5).

State-based legislation regarding the treatment of transgender youth that directly oppose the evidence-based care recognized by professional societies across multiple disciplines is a serious concern. Many reputable professional organizations, including the American Psychological Association, the American Psychiatric Association, the American Academy of Pediatrics, and the Endocrine Society, which represent tens of thousands of professionals across the United States, recognize natural variations in gender identity and expression and have published clinical guidance that promotes nondiscriminatory, supportive interventions for gender diverse youth based on the current evidence base. These interventions may include, and are not limited to, social gender transition, hormone blocking agents, hormone treatment, and affirmative psychotherapeutic modalities.

The American Academy of Child and Adolescent Psychiatry (AACAP) supports the use of current evidence-based clinical care with minors. AACAP strongly opposes any efforts – legal, legislative, and otherwise – to block access to these recognized interventions. Blocking access to timely care has been shown to increase youths' risk for suicidal ideation and other negative mental health outcomes. Consistent with AACAP's policy against conversion therapy (2), AACAP recommends that youth and their families formulate an individualized treatment plan with their clinician that addresses the youth's unique mental health needs under the premise that all gender identities and expressions are not inherently pathological.

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AMA

March 26, 2021: State Advocacy Update

AMA fights to protect health care for transgender patients

As physicians and leaders in medicine, the AMA is steadfast in its belief that every individual is entitled to high quality evidence-based medical care regardless of gender or sexual orientation and will continue to work diligently to expand access to medical services, reduce stigma for LGBTQ patients and break down discriminatory barriers.

This year, the threat to transgender patients is especially pronounced. More states have filed bills in 2021 that discriminate and harm transgender patients than any year before. These bills drive discrimination, reinforce stigma and erect barriers to care. The AMA's state Advocacy Resource Center remains actively engaged in defeating legislation that would harm transgender patients.

Criminalizing health care for transgender minors

Among the concerning legislation are bills that would criminalize the provision of medically necessary gender transition-related care to minor patients and, in some states, deem such care child abuse. These bills target surgical interventions as well as medications and hormone therapies that delay puberty while the child explores their gender identity.

Legislation of this kind was introduced in 16 states this year. To date, most have been defeated. However, work remains in a few key states, particularly in Alabama (S.B. 10) and Montana (H.B. 427) where bills have passed one chamber and are expected to be brought for a vote in the second chamber.

The AMA views these bills as a dangerous legislative intrusion into the practice of medicine and has been working closely with state medical associations to vigorously oppose them. In letters to legislators (PDF), the AMA has emphasized that it is "imperative that transgender minors be given the opportunity to explore their gender identity under the safe and supportive care of a physician."

Proponents of these disturbing bills often falsely assert that transgender care for minors is extreme or experimental. In fact, clinical guidelines established by professional medical organizations for the care of minors promote supportive interventions based on the current evidence and that enable young

URL: https://www.ama-assn.org/health-care-advocacy/advocacy-update/march-26-2021-state-advocacy-update

people to explore and live as the gender that they choose. Every major medical association in the United States, including the AMA, recognizes the medical necessity of transition-related care for improving the physical and mental health of transgender people.

Unfortunately, if enacted, legislation of this kind could have tragic consequences. Transgender individuals are up to three times more likely than the general population to report or be diagnosed with mental health disorders, with as many as 41.5% reporting at least one diagnosis of a mental health or substance use disorder. Transgender minors also face a significantly heightened risk of suicide. But research has demonstrated that improved body satisfaction and self-esteem following the receipt of gender-affirming care is protective against poorer mental health and supports healthy relationships with parents and peers. Studies also demonstrate dramatic reductions in suicide attempts, as well as decreased rates of depression and anxiety.

Excluding transgender youth from athletics

Another concerning trend are bills that would prohibit transgender women and girls from participating in school athletics consistent with their gender identity. In some states, a health care provider would need to verify a student's sex.

Legislation has been introduced in more than half of all states this year. Though most have not advanced, some states are moving bills forward. Notably, Mississippi recently became the first state this year to enact such a prohibition into law. Legislation is soon expected to be signed in North Dakota and Tennessee as well.

In 2020, Idaho became the first ever state to enact a ban on transgender minors' participation in youth athletics. The law was challenged and blocked by a federal court in August 2020. The AMA, along with the American Academy of Pediatrics and other health care organizations, submitted a friend-of-the-court brief (PDF) with the Ninth Circuit Court of Appeals noting that the law undermines the accepted approach for treating gender dysphoria.

As the AMA's brief stated, barring transgender females from participating in school-sponsored organized sports consistent with their gender identity frustrates the treatment of gender dysphoria by preventing transgender females from living openly in accordance with their true gender. This lack of treatment, in turn, increases the rate of negative mental health outcomes, substance abuse and suicide. In order for transgender females to live their lives fully in accordance with their gender identity, they must be able to publicly identify and compete as female athletes.

The AMA continues to work with state medical associations to oppose legislation that would compound the stigma and discrimination that transgender individuals face.

URL: https://www.ama-assn.org/health-care-advocacy/advocacy-update/march-26-2021-state-advocacy-update

AMA

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- March 26, 2021: Advocacy Update spotlight on progress made to extend sequester moratorium
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- March 26, 2021: Advocacy Update other news

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

REV. PAUL A. EKNES-TUCKER, et al.,
Plaintiffs,
vs.
KAY IVEY, in her official capacity as Governor of the State of Alabama, et al.,
Defendant.

2:22-cv-00184-LCB
May 6, 2022
Montgomery, Alabama
9:00 a.m.

TRANSCRIPT OF PRELIMINARY INJUNCTION HEARING
VOLUME II
BEFORE THE HONORABLE LILES C. BURKE
UNITED STATES DISTRICT JUDGE

Proceedings recorded by OFFICIAL COURT REPORTER, Qualified pursuant to 28 U.S.C. 753(a) & Guide to Judiciary Policies and Procedures Vol. VI, Chapter III, D.2. Transcript produced by computerized stenotype.

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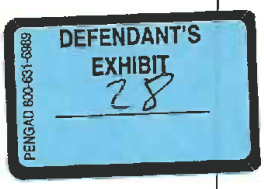

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PROCEEDINGS

(In open court.)

THE COURT: Good morning. Please be seated.

All right. I am going to aim my question at you, Mr. LaCour.

You heard the testimony of Pastor Eknes-Tucker. What, if anything, in his testimony would trip the Alabama statute?

MR. LACOUR: Your Honor, we don't think anything in his testimony would trip the statute, as you said. The key language is does he engage in or causing the prescription or administration of puberty blockers? Is he engaging or causing the prescription or administration of the cross-sex hormones?

Clearly, he is not under the plain text of the statute, so we don't think he has standing, which I think is probably good news and bad news for him. But the good news is he is not going to be prosecuted. The bad news is he doesn't get to -- in his words -- make a difference by continuing in this case.

But that's the State's answer.

THE COURT: All right. Since he has addressed standing, anybody want to touch on that from the plaintiffs' side?

MR. DOSS: Yes, Your Honor.

Our concern remains that under this Act's language, it does capture speech for referrals, for actions by people who are counseling patients, or people who are counseling anyone to

put them in touch with medical providers knowing full well what those medical provisions may or may not be.

I think this does also feed into the void for vagueness argument, Your Honor, because the State is making these post-hoc decisions about when the statute does apply, despite its plain language and when it does not apply.

So we still think that the statute on its face is triggered. We appreciate the State's statement on the record that it doesn't think that the statute is triggered.

On the other hand, it shows just how vague the statute is, that we can't know just by reading it, which violates the Fifth Amendment rights' notice.

THE COURT: All right. One other thing that I will put the parties on notice about when we get to closings.

So I assume everybody has read the Arkansas order and transcript. Would that be a correct statement?

All right. So I would like everybody to be able to address at the conclusion of these proceedings what parts of that order, if any, that they disagree with, why, and to what degree those -- that legal reasoning is applicable here. And I know that we do have some differences in that statute.

So just put that in your back pocket, and let's be prepared to talk about that.

Okay. So I understand the United States has a witness this morning; is that correct?

MR. POWERS: Yes, Your Honor.

Before we get started, I have a quick bit of housekeeping. The United States moves to admit United States Exhibit Numbers 1 through 12.

MR. BOWDRE: No objection, Your Honor.

THE COURT: Be admitted.

MR. POWERS: Thank you. Now, the United States would like to call Dr. Armand Antommaria to the stand.

THE COURT: All right.

ARMAND ANTOMMARIA,

having been first duly sworn by the courtroom deputy clerk, was examined and testified as follows:

DIRECT EXAMINATION

BY MR. POWERS:

Q Good morning.

A **Good morning.**

Q Doctor, could you please introduce yourself for the Court?

A **My name is Armand Herbert Matheny Antommaria. I am a pediatrician and bioethicist. I am employed by Cincinnati Children's Hospital Medical Center where I direct its ethics center. I'm the Lee Ault Carter chair of pediatric ethics and an attending physician in the division of hospital medicine.**

THE COURT: Mr. Powers, I neglected to ask you how long you think this witness will be.

MR. POWERS: Well under half an hour.

THE COURT: Okay. All right.

BY MR. POWERS:

Q Doctor, do you hold an MD from the Washington University School of Medicine?

A **I do.**

Q Do you hold a Ph.D. from the University of Chicago Divinity School?

A **Yes.**

Q Doctor, what are your areas of specialty?

A **As a physician, my area of specialty is pediatric hospital medicine. So I take care of general pediatric patients, patients with asthma or pneumonia, who are admitted to the hospital. And I'm also a bioethicist and specialize in pediatric clinical ethics.**

Q Thank you.

Can you please explain what a bioethicist is?

A **Bioethics is a multidisciplinary field that addresses the ethical issues that arise in medicine and the life sciences.**

Q Doctor, are you board certified?

A **I am. I am board certified in pediatrics and in pediatric hospital medicine. And I'm also certified as a health-care ethics consultant.**

Q Are you part of a multidisciplinary team that provides treatment to adolescent patients with gender dysphoria?

A **Yes. Cincinnati Children's has a clinic that provides**

care for children and adolescents with gender dysphoria, and I participate in their monthly multidisciplinary team meetings, as well as consult on an as-needed basis when special ethical issues arise in the care of the patients that they treat.

09:14:34 Q And are the sorts of ethical issues that do arise when you're consulted regarding the care of transgender patients?

A At times, there are issues regarding who is able to provide informed consent, whether adult patients have medical decision-making capacity or ethical issues when there are

09:14:55 **unusual risks or benefits involved in the care of a particular patient.**

Q Are you involved in the development of treatment protocols related to treating adolescent patients with gender dysphoria?

A Yes, to the extent that they have ethical issues in

09:15:10 **particular. I participated in the development and the periodic review of the clinic's informed consent documents.**

Q Thank you, Doctor.

As part of your duties, do you consult with medical providers on the treatment of infants and children with

09:15:28 differences in sex development?

A Yes. Cincinnati Children's also has a clinic that provides care to individuals with differences of sex development. And I participate in similar ways in that multidisciplinary's team meetings both in terms of patient care

09:15:50 **and in terms of gender policies.**

Q Thank you.

MR. POWERS: Your Honor, the United States moves to have Dr. Antommaria qualified as an expert in bioethics and treatment protocols for adolescents with gender dysphoria.

09:16:05 MR. BOWDRE: No objection, Your Honor.

THE COURT: All right. He will be accepted for that purpose.

MR. POWERS: Thank you.

BY MR. POWERS:

09:16:11 Q Dr. Antommaria, is a diagnosis of gender dysphoria made by physicians and other medical professionals, or is it made by the patient or the parents?

A A diagnosis of gender dysphoria is made by clinicians.

Q And are there external indicators that can be evaluated as

09:16:29 part of that process?

A Yes. There are patient behaviors that can be observed that support the diagnosis such as, you know, missing school or other behaviors which can be observed that support that diagnosis.

09:16:48 Q Doctor, as part of your work, are you familiar with research studies, systematic reviews, and clinical practice guidelines in a variety of areas related to pediatric care?

A Yes, I am.

Q And are you familiar with studies, reviews, and guidelines

09:17:06 regarding treatment specifically for adolescents experiencing

gender dysphoria?

A Yes.

Q And what is the difference between research and clinical care?

09:17:17 **A Research and clinical care are differentiated both in terms of their goals and their methods. So the goal of research is to generate generalizable knowledge. And the methods are the use of a protocol that defines the steps in a study.**

09:17:36 **Clinical care's goal is to provide benefit to individual patients, and its procedures are individualized decision making.**

Q And what is the difference between observational studies and randomly controlled trials?

09:17:51 **A So the two big categories of studies are observational and experimental.**

In observational studies, the investigators don't control who's exposed to the intervention. The most -- one of the common forms of an observational study would be a prospective

09:18:15 **observational study in which individuals who receive a treatment are followed over time to see the effects of that treatment.**

In experimental studies, the investigators control who receives the intervention. Commonly in a randomized controlled

09:18:31 **trial, neither the participant nor the investigator controls**

who receives the treatment or the intervention or the control. People analogize randomization to a coin flip in terms of determining who receives which.

Q And, Doctor, do you have an opinion about the viability of

09:18:53 conducting randomly controlled trials testing the use of treatment, like puberty blockers and hormone therapy, for adolescents with gender dysphoria?

A Yes, I do. I would have concerns that randomized controlled trials of these interventions would be unethical,

09:19:11 **and even if they could be ethically performed, they would have substantial methodological limitations.**

Q And what are the ethical concerns first?

A In order for a research study to be ethical, particularly a randomized controlled trial, there must exist something

09:19:29 **called equipoise. The investigator must believe that the intervention and the control are each likely to be equally efficacious. And many investigators in this field would believe that there is sufficient evidence of the benefit of the use of puberty blockers or gender-affirming hormone therapy**

09:19:51 **that a randomized controlled trial would not be ethical.**

In addition, you would need to be sure that the study could be completed. For example, that you would have enough participants sign up to be in the study to make exposing them to the risks of the study to be beneficial. And there would be

09:20:12 **concerns that not enough participants could be recruited to**

such a trial to be ethical.
Q And do you have any additional methodological concerns regarding randomly controlled trials?
A **Yes. One of the key factors in a randomized controlled trial is they're what's called blinded, but neither the participants nor the investigators know whether the participants is receiving the intervention or the control.**
And in a randomized control trial of this nature, it would be -- not be possible to blind investigators that are participants because they would know which -- what's called an arm, which arm the participant is in by the development or lack of development of secondary sexual characteristics. So such a randomized controlled trial would be of substantially less value.
Q Thank you.
Now, what's the difference between a systematic review of the literature and a clinical practice guideline?
A **So in a systematic review of the literature, the individual will collect all of the evidence and -- relevant to a particular outcome and grade the quality of that evidence.**
Systematic reviews of the literature, however, do not make treatment recommendations. Just because the level of evidence for intervention might be low doesn't mean that that intervention should not be used.
A clinical practice guideline both evaluates the quality

of the evidence, makes treatment recommendations, and grades the quality of those recommendations, because there are many other factors rather than in addition to the quality of the evidence that need to be considered in making treatment recommendations.
Q Doctor, can a clinical practice guideline be based on the results of observational studies?
A **Yes, they can. And frequently in pediatrics, clinical practice guidelines are based on observational studies, because unfortunately there are fewer randomized controlled trials available in pediatrics than in adult medicine.**
So other Endocrine Society guidelines for other pediatric conditions like congenital adrenal hyperplasia or obesity are largely based on observational studies. And even treatment guidelines for important crucial things, such as the American Heart Association's guidelines for performing CPR in children, are largely based on observational studies.
Q I think you might have mentioned one of them already, but what guidelines help establish the standard of care when treating adolescents with gender dysphoria?
A **The two predominant clinical practice guidelines for treating adolescents with gender dysphoria would be the Endocrine Society's and WPATH's.**
Q Thank you.
Is the level of evidence supporting these puberty blockers

and hormone therapy in these guidelines comparable to the level of evidence for other treatments in pediatrics?
A **Yes.**
Q Doctor, are you familiar with the European policies, with respect to treating adolescents diagnosed with gender dysphoria?
A **I am.**
Q And could you please summarize your understanding of that?
A **So in part, particularly reference has been made to the Swedish policy. That policy is only available in an official English translation of a three-page summary.**
So it's difficult to fully evaluate these policies, given the limited amount of material that's available in official English translation.
But my understanding of the policies are that they have reviewed the literature, but they use less robust methods than the Endocrine Society, because they neither grade the evidence nor the strength of their recommendations, and that none of the policies instantiate a ban on gender-affirming health care, the use of puberty blockers or gender-affirming hormone treatment.
Q Thank you.
Doctor, are you familiar with the provisions of Senate Bill 184?
A **I am.**
Q Are the provisions of Senate Bill 184 consistent with the

guidelines issued by any country in Europe?
A **No.**
Q Doctor, once a diagnosis of gender dysphoria has been made, how does the informed content process work in the pediatric context?
A **In the pediatric context, parental consent is required -- in general, parental consent is required for treatment.**
Adolescents should participate in medical decision making to the extent that it is appropriate, and for adolescents, their assent should also be sought.
And the informed consent process requires a discussion of the potential benefits, risks, and alternatives of the treatment.
Q Doctor, do you have an opinion as to whether puberty blockers and hormone therapy treatments have benefits to some adolescents diagnosed with gender dysphoria that outweigh the potential risks?
A **Yes. That for some individuals with gender dysphoria the benefits of treatment outweigh the risks.**
Q And what role does desistance play, or what our friends have referred to as desistance play in your analysis?
A **So in evaluation of the risk, if treatments are discontinued, there may be effects of those treatments, which are only partially reversible. But that is only one of the factors that needs to be weighed in the risks and benefit**

analysis. And that the evidence about the current rates of desistance are that it is sufficiently low, that that would not be in general a reason not to proceed with treatment.

Q Thank you.

09:26:43 Is there high quality evidence supporting the alternative of psychotherapy alone, so without the assistance of puberty blockers and hormone therapy? Is there high quality evidence supporting that as a treatment for gender dysphoria in adolescents?

09:27:03 **A I am not aware of any randomized controlled trials of psychotherapy alone for the treatment of adolescents with gender dysphoria.**

Q As an ethicist, do you have an opinion regarding parents and adolescents' ability to adequately understand the potential 09:27:23 cause and benefits in giving informed consent to the provision of puberty blockers and hormone therapy?

A Although this decision involves a complex set of risks, benefits, and alternatives, it is comparable to other decisions that parents and their children make in pediatric health care 09:27:42 on a frequent basis.

Q And in the instance that there was a medical provider who violated their ethical obligations to their patients with respect to obtaining informed content, are there forms of oversight in place?

09:27:57 **A There would be multiple mechanisms to address those**

potential shortcomings. If that provider worked for a health-care institution, they would be credentialed by that institution. The institution would have a responsibility for oversight of their practice.

09:28:14 **The state medical board could review their practice and potentially discipline them or withdraw their license.**

And although I am not a lawyer, it's my understanding that there would be the potential for malpractice claims for inadequate informed consent.

09:28:32 **So there are multiple mechanisms that exist to address the case in which somebody obtained inadequate informed consent.**

Q So there are other mechanisms in place other than a direct ban on the treatment itself?

A **Yes. I'm sorry. You're correct.**

09:28:48 Q Doctor, I would like you to consider a circumstance where adolescents no longer have access to puberty blockers or hormone treatments. Are there any equally effective alternative medical treatments for adolescents with gender dysphoria?

09:29:03 **A There are not.**

Q Is there an ethical basis for distinguishing the provision of treatment to minors experiencing precocious puberty, from transgender minors experiencing gender dysphoria?

A **There is not.**

09:29:19 **So in particular, the type of evidence for both treatments**

are the same. The evidence supporting the use of puberty blockers for the treatment of central precocious puberty are also prospective observational trials with relatively small numbers of participants.

09:29:42 **There are no randomized controlled trials to support the use of puberty blockers for central precocious puberty.**

Q Compared to treatments in other contexts, is there anything about treatments for adolescents with gender dysphoria that would require prohibition by the State from an ethical

09:29:58 perspective?

A No.

Q And last question, Doctor. What are the ethical implications for medical providers treating minors diagnosed with gender dysphoria if Senate Bill 184 is implemented?

09:30:11 **A They would be unfortunately placed in the untenable position of either violating their ethical obligations to their patients to conform with the law, or fulfilling their professional duties to their patients and being criminally charged.**

09:30:28 MR. POWERS: Thank you. No further questions.

THE COURT: Cross?

CROSS-EXAMINATION

BY MR. BOWDRE:

Q Good morning, Dr. Antommaria. My name is Barrett Bowdre.

09:30:53 I represent the State defendants.

A Good morning.

Q You agree, don't you, that most individuals who experience gender dysphoria in childhood desist?

A The evidence would support that individuals who experience

09:31:18 **gender dysphoria at young ages such as three or four, that the majority of them do desist.**

Q You noted that the goal of clinical practice is the individualized assessment in providing care for the individual patient that you're treating. But no clinician can accurately

09:31:45 predict whether the patient sitting in front of him will persist in their gender dysphoria or will, as the majority do, desist; isn't that correct?

A So at the point of evaluating an adolescent, the desistance rate is substantially smaller than it is for the

09:32:11 **desistance rate of young children, and that there would be the ability to be fairly certain that they are unlikely to desist. But expecting perfection in the practice of medicine and being able to predict with 100 percent certainty is unrealistic because there's nothing in health care that can occur with 100**

09:32:35 **percent certainty.**

Q Can you predict with 80 percent certainty whether the individual patient sitting in front of you will persist or desist in his or her gender dysphoria?

A The evidence of which I am aware would suggest that the

09:32:53 **desistance rate is -- for adolescents is substantially less**

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than 80 percent. If the **desistance** rate for adolescents -- I apologize -- **is substantially less than 20 percent.**

Q Is that for adolescents who are treated with puberty blockers, or adolescents who are not treated with medical interventions?

09 33 11

A The most robust data that is available are adolescents who are treated with gender-affirming health care.

Q So can you tell with 80 percent certainty whether an individual patient, an adolescent who is not treated with

09 33 39

puberty blockers, would desist or persist in the gender dysphoria?

A So the evidence base in that area is less robust, but the evidence of which I'm aware would still suggest that the desistance rate for individuals who are adolescents is less

09 33 52

than 20 percent.

Q And what studies do you rely on to say that it's -- I mean, for adolescents -- we're talking about a 12 or 13 year old who has entered what, Tanner Stage 2 of puberty; is that correct?

09:34:04

A Correct.

Q Okay. So what evidence do you rely on to say that without treating with puberty blockers the group who are not treated there's a more than 80 percent likelihood that the individual patient is going to desist to that point?

09 34 19

A So I would say that that is based on -- so I am not aware

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of a specific prospective observational trial that answers your question, but that experience in the field would suggest that that -- the desistance rate is low.

Q And what is that experience?

09 34 47

A Of the clinicians who provide care to this patient population.

Q I guess my question is -- I'm trying to figure out -- I understand that the majority of children who are started on puberty blockers go on to cross-sex hormones. Is that true?

09 35 02

A Can you restate your question?

Q The majority of children who are -- who start on puberty blockers will then go on to take cross-sex hormones; isn't that right?

A Correct.

09 35 18

Q Okay. And so my question is: If a child does not start on puberty blockers, what degree of certainty can we say that the gender dysphoria would go away? And we are talking about a Tanner Stage 2 adolescent.

A So I would differentiate the likelihood of them desisting

09 35 42

from the quality of evidence that supports that claim. The likelihood of them desisting based on the available evidence would be that it would still be infrequent, the evidence is -- would currently be based on expert opinion of individuals who provide that care.

09:36 01

Q Okay. So there are no studies to support that claim; is

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that right?

A I'm not aware of a study on that specific question.

Q Okay. Would you agree that the combination of puberty blockers and cross-sex hormones -- let me start over.

09 36 24

Because you testified that most children who begin on puberty blockers go on to cross-sex hormones, wouldn't it be reasonable when we're talking about the risks to view those together?

A No.

09 36 42

Q Why is that?

A Because they occur at separate periods of time. So that informed consent is obtained for the use of puberty blockers, there are ongoing conversations about the efficacy of that treatment and the individual symptomology, and a separate

09 37 01

detailed informed consent process is obtained prior to the start of gender-affirming health care.

Q But wouldn't it be relevant to a parent or a child determining whether to start puberty blockers to know that almost everyone who starts on this treatment goes on to

09 37 18

cross-sex hormones?

A It would be relevant for parents to know that the clinical practice guidelines for the treatment of gender dysphoria generally recommend treatment with puberty blockers followed by treatment with gender-affirming hormone therapy.

09 37 42

Q Okay. My question was: Wouldn't it be relevant for them

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to know that almost everyone who starts on puberty blockers then goes on to cross-sex hormones?

A I don't believe that that would -- that category of information would be relevant. I don't know that that specific

09:38 08

framing would be useful and informative to patients.

Q Okay. So you do not think that the --

THE COURT: Hold on a minute, Mr. Bowdre.

Ladies and gentlemen, let me say this: If you are sitting in the audience and you're head nodding or you're mouthing

09 38 23

words and looking at the witness, please stop that, because that could give the appearance that you are trying to influence the witness.

So let me just put that out there. Please follow my guidelines on that.

09 38 38

I am not suggesting that you are being influenced by anyone out here, but it's possible that someone might want to influence you.

So go ahead, Mr. Bowdre.

MR. BOWDRE: Thank you, Your Honor.

09 38 47

Could you read the last question? I'm sorry.

(Whereupon, the Court Reporter read back the pending question.)

BY MR. BOWDRE:

Q Would you agree that there are substantial risks involved

09 39 26

in someone starting puberty blockers and going on to cross-sex

hormones?
A **There are risks involved in the treatment course for the treatment of gender dysphoria.**
Q What are some of those risks?
09:39:39 A **Can you be more specific? Of the entire course of treatment, or particular parts of the treatment?**
Q The entire course of treatment.
A **So I would disaggregate the risks of puberty blockers from the risks of gender-affirming hormone therapy and the risks of**
09:40:06 **testosterone therapy are different from the -- or are somewhat different than the risks of estrogen therapy.**
Would you like me to review all of that.
Q Let me just ask you a couple of those.
Would you agree that some of the risks of puberty blockers
09:40:18 and cross-sex hormones would be loss of fertility?
A **There is a risk of impaired fertility.**
Q Okay. Would you agree that a risk would be loss of sexual function?
A **Particularly the use of testosterone therapy has a risk of**
09:40:42 **changes in sexual function. I apologize. The use of estrogen therapy in -- has a risk of alterations in sexual function.**
Q So if someone cannot predict with very much accuracy whether gender dysphoria will desist, then you cannot predict whether the interventions will help or harm that person; is
09:41:20 that true?

A **No, that is not true.**
Q Why is that not true?
A **Because there is sufficient certainty that gender dysphoria will persist to have a discussion about the potential**
09:41:37 **benefits and risks of treatment.**
Q Okay. So if it were the case that one could not tell with much accuracy whether the, you know, 11 or 12 year old at Tanner Stage 2 sitting in front of you, whether that person's gender dysphoria would desist, assuming that, then is it true
09:41:56 that you would not be able to know whether the intervention treatments of puberty blockers and the cross-sex hormones would be helpful or harmful to that person?
A **So it would depend on how much uncertainty there was, and that would likely be information that was relevant to the**
09:42:24 **informed assent discussion and the parents' decision about whether to proceed with treatment.**
Q What if you were 40 percent sure that the -- that the child would persist, then could you tell whether the interventions would be helpful or harmful?
09:42:52 A **It -- so part of -- so do you mean 40 percent sure that your prediction of their likelihood of persisting was accurate, or do you mean that their likelihood of persisting was 40 percent?**
Q I'm sorry. Let's assume that you are -- that you -- that
09:43:18 it is 40 percent accurate that the person sitting in front of

you is going to persist. The person has a 40 percent chance of persisting.
A **Then in that hypothetical case, there would be less justification for proceeding with that course of treatment.**
09:43:40 **But that is a hypothetical case and not the decision that patients and their families are currently facing.**
Q Okay. Dr. Antommaria, what is a detransitioner?
A **So I don't know that there's a technical -- currently a widely accepted technical definition of that term, because**
09:44:05 **people -- individuals use that term in a variety of different ways to mean different things.**
Q Okay. Would one definition, sort of a common definition be someone who identifies or has been diagnosed with gender dysphoria, has begun puberty blockers, cross-sex hormones, and
09:44:27 then the dysphoria desists, or for whatever other reason they realign with their biological sex and they stop the medical interventions; is that a fair overall description?
A **So that is a potential definition. The one qualification I would make is if it's defined in terms of an individual who**
09:44:53 **discontinues medical therapy, there may be a wide variety of reasons for individuals to discontinue their medical therapy beyond change in their gender identity.**
Q And have you reviewed the literature -- let me be more specific.
09:45:14 Have you reviewed recent surveys of people who identify as

detransitioners, specifically Lisa Littman's and Elie Vandenbussche's? Have you reviewed those two?
A **No, I have not reviewed those two.**
Q Are you -- do -- let me -- I will strike that.
09:45:34 Are you aware that at least, according to one of those studies, only 25 percent of people who detransition ever tell their gender-affirming care doctors that they have detransitioned?
A **I heard you state that yesterday in court. But, no, as I**
09:45:54 **said, I'm not aware of those particular studies.**
I would say that, for example, our clinic's informed consent documents emphasize if individuals discontinue their treatment, it's very important for them to provide that information to their health-care providers.
09:46:11 Q Okay. Does the fact that some people who are diagnosed with gender dysphoria, given puberty blockers and cross-sex hormones, dramatically change their bodies, sometimes permanently, and then divert to identifying with their biological sex give you any pause that we might not be so good
09:46:32 at identifying who are good candidates for these medical interventions and who might not be good candidates?
A **Can I ask what you mean by give me pause?**
Q Does it give you concern?
A **So I think that in this field, all the available data and**
09:46:46 **information should be considered in making treatment decisions.**

That would be potentially relevant information that should be incorporated in an ongoing basis in treatment decisions and revisions of clinical guidelines when they're revised.

Q But you have not reviewed at least these studies on detransitioners to consider whether those would impact your clinical standards; is that true?

A So those -- so I am aware of the discussion about detransition, including the stories of individual patients who have detransitioned. The body of literature is large.

And at this point in time, no, I have not reviewed those two specific studies. If it became relevant, I would make effort to review those studies.

Q Thank you.

You do not touch on this in your testimony, but in your declaration, you spent a couple of pages talking about access to top surgery for gender dysphoric minors; is that right?

A Yes. There's reference to top surgery in my declaration.

Q Okay. In such surgeries -- we're talking about mastectomies usually; is that right?

A That's one way to characterize the procedure.

Q Okay. And they are performed on minors in at least some states in the United States; isn't that true?

A That is true.

Q Okay. At what age do you think that a -- someone can consent to a double mastectomy as part of the gender-affirming

care?

A So I would be unable to answer that in terms of an age. The relevant factor is their decision-making capacity, which only has a correlation with age, but is not specific to age.

Q Okay. You said in your declaration that adolescents generally possess comparable medical decision-making capacity to adults; is that right?

A So part of the question is how you define adolescents. But, yes, older adolescents generally have comparable medical decision-making capacity to adults.

Q So what age are we talking about?

A So the specific study that I cited in my declaration compared 14 year olds to older adults.

Q Okay.

A Or to adults.

Q And you would agree that Tanner Stage 2 puberty normally occurs before age 14?

A **Correct.**

Q Okay. So given that adults can consent to both top and bottom sex-change surgeries, why can't a 14 year old not?

A Can you restate the question?

Q Yeah. Given that adults can consent to both top and bottom sex-change surgeries, why should a 14 year old not be able to consent to those procedures?

A Because in general, adolescents are not permitted to

consent to medical treatment, and we rely on their parents or legal guardians to consent.

Q But why is that? If they -- you just testified that 14 year olds have comparable medical decision-making abilities to adults, is it simply a matter of law that they cannot consent, or is there some basis in the literature that would require the parental consent for 14 year olds?

A So at a minimum, the legal requirement -- so informed consent is in part a legal requirement. And although there are exceptions to permit minor -- some minors to provide consent for certain forms of medical treatment, parental consent is required for a variety of different reasons, not a single reason.

Q I want to read to you a paragraph -- I will go -- I want to read to you a paragraph on an amicus brief to the American Psychological Association, the American Psychiatric Association, and the National Association of Social Workers did in a case called Miller vs. Alabama.

All right. And so this is the amicus brief. And I am going to flip to page 12.

In highlighted portion, paragraph 3, it says, Finally, juveniles differ from adults in their ability to foresee and take into account the consequences of their behavior. By definition, adolescents have less life experience on which to draw, making it less likely that they will fully apprehend the

potential negative consequences of their actions. Moreover, adolescents are less able than adults to envision and plan for the future, a capacity still developing during adolescence. The study of maturity of judgment discussed above found that adolescents' future orientation is weaker than adults'.

I will skip the sentence about the specific subjects.

Then it says, Similarly, studies have shown that among 15 to 17 year olds, realism in thinking about the future increases with age, and that the skills required for future planning continue to develop until the early 20s. The ability to resist and control emotional impulses, to gauge risks and benefits in an adult manner, and to envision the future consequences of one's actions -- even in the case of environmental or peer pressures are critical components of social and emotional maturity necessary in order to make mature, fully considered decisions. Empirical research confirms that even older adolescents have not fully developed these abilities and hence lack an adult's capacity for mature judgment.

Do you disagree with that?

A So you would appreciate having seen this for the first time and not being able to review the evidence on which it's based, it's difficult for me to form a full opinion, but I am happy to provide my initial reaction.

And that would be that informed consent is generally considered to be a threshold at which people need to meet. The

language that I see here refers to optimal capacities which might far exceed that threshold. If you read the language that it continues to mature into the 20s, I don't take it that that's justifying that the age of consent should be moved to 20 or 22 instead of 18.

So I think it's consistent to say that individuals' medical decision-making capacity may continue to mature over time without saying that adolescents lack the sufficient capacity to assent to treatment.

Q Thank you.

THE COURT: How much longer do we have with our cross?

MR. BOWDRE: 20 minutes, maybe 30.

BY MR. BOWDRE:

Q Do you agree that more research is needed to study the efficacy and the cost and benefits of providing gender-affirming care to minors?

A I would say that more research is needed in all areas of health care, and that the State's legislation would prohibit such research.

Q And what are the questions that would need to be answered that the research needs to answer in this area that are left open?

A There are a range of questions that might benefit from further refinement, including issues about the timing of the initiation of therapy, dosing. There are a variety of

considerations that could be further refined and developed. But further refining those treatment protocols would be a refinement.

Q In your declaration, you noted that once the FDA has approved a medication for one indication, thereby agreeing that it is safe and effective for this intended use, prescribers are generally free to prescribe that for other indications; is that correct?

A That is correct.

Q Okay. But that does not mean that an off-label use would always be safe to prescribe to an individual simply because it is an FDA-approved medication for some purpose?

A Correct.

Q So, for instance, a nine-year-old boy with diabetes, the FDA has approved the use of insulin for that purpose, but providing insulin to a nine-year-old boy without diabetes would be very dangerous, wouldn't it?

A Yes.

Q So whether an off-label use is appropriate depends on the proven risks and benefits of that particular use that we're looking at?

A Yes. But the fact that a medication is used off label does not intrinsically mean that that evidence does not exist.

Q Okay. In your direct testimony, you said -- I think you said -- correct me if I'm wrong -- that randomized controlled

trials in this area would be unethical because no equipoise exists between treating someone simply with psychotherapy versus treating someone with psychotherapy and puberty blockers and cross-sex hormones; is that fair?

A Correct.

Q When did that equipoise come into existence?

A I don't know that I can provide you a particular date as to when that lack of equipoise came into existence.

Q For that equipoise or lack of equipoise to come into existence, wouldn't we need studies that, you know -- doesn't there need to be at least one study that shows -- that looks at a group treated only with psychotherapy and one group treated with the medical interventions?

A Can you restate your question?

Q For the lack of equipoise to come into existence, for us to know that, you know, psychotherapy plus puberty blockers and cross-sex hormones are the way to go and that any other treatment would be unethical, don't we first need to have a study that treats someone with psychotherapy and has a controlled group that way versus someone who is treated with all of those interventions?

A No. There are prospective observational trials that demonstrate the efficacy of puberty blockers and gender-affirming hormone therapy, and withhold those treatments from an individual may be considered unethical.

Q Okay. So you were asked whether there were any high quality randomized controlled studies looking only at psychotherapy, which I will note is not the level of evidence that you are relying on.

But doesn't that concern you that we have no idea whether psychotherapy alone versus psychotherapy plus puberty blockers plus cross-sex hormones is doing the work in creating any benefits that we see?

A So there is substantial clinical experience that -- so I will differentiate psychotherapy from psychological and psychiatric treatment given that psychotherapy is a distinct entity. But that there is substantial experience that providing mental health care to adolescents with gender dysphoria in and of itself is not sufficient to resolve individuals' dysphoria and hence the reason for proceeding with medical interventions.

If a patient had gender dysphoria and was -- their gender dysphoria was adequately treated with mental health care, they would not proceed to medical therapy.

Q Do you contend that the Endocrine Society's practice guidelines that were released in 2017 provides a more robust overview of the literature than the UK's recent literature review of looking at puberty blockers and cross-sex hormones?

A Can you be specific as to which British report you're referring to?

Q Yes. And if you want to look at them, they are Defendants' Exhibits 9 and 10. I think you do have the right binder.

A So I can't answer your question because it's asking me what in effect are apples and oranges. One is a systematic review of the literature, and one is a clinical practice guideline, which are different types of material.

Q Okay. I believe you testified that the -- I mean, the clinical practice guidelines you said does a comprehensive review of the literature and then suggests -- suggests, you know, practices. Is that fair?

A So a clinical practice guideline will be based on a systematic review of the literature and grades the quality of the evidence and the strengths and recommendations.

Q Okay. So for that part of the practice guideline analysis, the literature review part, would you say that the Endocrine Society's review was more extensive and is more accurate than the UK's more recent literature reviews that you're looking at in Defendants' Exhibits 9 and 10?

A So I can't answer your question in detail without more thoroughly reviewing the documents.

Based on my understanding of the Endocrine Society's methodology, I would expect them to be comparable, but I can't form a formed opinion based on the information that I currently have.

Q Okay. You have not reviewed closely the UK's recent literature reviews?

A So I've reviewed their conclusions. I haven't reviewed them in the degree of methodological detail that your question would require.

There are a large number of systematic reviews available in the literature. Some of which I know in detail, and others of which I know at less -- a lesser level of detail.

Q What are the prospective observational studies that you claim demonstrate the efficacy of puberty blockers and gender-affirming care?

A So the specific references are included in my report. But in general, they're the studies that are conducted by the Dutch group.

Q And in that study, both the 2011 study that looks only at puberty blockers and then the 2014 report that reported on people who then went on to cross-sex hormones and total surgical interventions, those studies -- so everyone in those studies got psychotherapy and psychiatric help the entire time; is that true?

A Correct.

Q Is it also true that people who had psychological comorbidities, depression, things like that, were excluded from the treatments from the medical interventions?

A So I would have to review their specific inclusion and

exclusion criteria to be able to answer your question.

Q Okay. Do you know if everyone in that study, whether their psychological functioning and improvements went to a new clinical range or not?

A So there were a variety of different outcome variables that were examined in the study, some of which were unchanged, but some -- but others of which showed statistically significant improvement. And so can you clarify what you mean by a new range?

Q I think I will move on, given our time.

If parents of a 14 year old can consent to cross-sex hormones, why cannot parents of -- and the 14 year old consent to a double mastectomy?

A As a legal matter -- can you clarify your question?

Q As a medical ethical matter.

A So I don't believe that there would be an indication to perform a mastectomy on a 14 year old.

Q Why not? Isn't mastectomy a gender-affirming care for a transgender man?

A So in general, the purpose of utilizing puberty blockers would be to prevent the development of those secondary sexual characteristics, and the use of cross-sex hormones would be to promote the development of secondary sexual characteristics that are consistent with an individual's gender identity. And there would be a period of time in which it would be required

for the gender-affirming hormone therapy to take an effect.

The effects develop over a period of years. So it's hard for me to understand the clinical scenario that you're presenting.

Q Well, what if someone did not start on puberty blockers and comes to the clinic as a 14 year old already having developed?

A So in -- so it would be my general understanding that that individual -- would they be -- presumably may be pursuing gender-affirming hormone therapy and would not -- so I'm having trouble understanding.

Are you suggesting that they're not starting gender-affirming hormone therapy and are simply moving to top surgery?

Q Either that, or -- I mean, my understanding is that if, you know, if a biological woman has already developed breasts, then providing testosterone, you know, doesn't make the breasts go away, right? You still need the double mastectomy. So why could not that person, a 14 year old, not -- her and her parents not consent to that?

A So I'm having trouble with your construction, particularly related to the age.

But I would say that I think that parents and their adolescent children who are less than 18 potentially are capable of consenting to top surgery. And it would depend,

then, on the specific clinical circumstance.
It's hard for me to answer your abstract formulation.
Q You provided an example in your declaration on -- I guess as an example of how the medical community often relies on
10:10:22 low-quality evidence. And your example was that a doctor might prescribe, you know, 20 minutes of exercise and a low-calorie diet as a way to treat obesity. And I guess your point was there were no randomized controlled studies showing that 20 minutes of exercise and a good diet, you know, is always
10:10:47 going to treat obesity.
But in that example, the risks of following that protocol are pretty low, aren't they?
A **Yes.**
Q Yeah. And would you agree that it might make sense to
10:11:04 follow minimal low-quality evidence for low risks for high reward endeavors, such as exercising for 20 minutes, but that we might want higher quality of evidence or more robust mound of it before relying on it for something where the risks were quite high?
10:11:24 **A That assumes that we cannot make decisions until some speculative future in which that evidence is available. Unfortunately, clinicians have to make decisions based on the evidence that is currently available to them.**
Q Okay.
10:11:48 MR. BOWDRE: May have just a moment to confer with

counsel?
THE COURT: Yes.
MR. BOWDRE: Thank you, Dr. Antommaria.
THE WITNESS: Thank you.
10:12:02 MR. POWERS: No further questions.
THE COURT: All right. May the witness be excused?
Sir, you can step down. Thank you.
THE WITNESS: Thank you, sir.
THE COURT: All right. In the interim -- do you have
10:12:15 something you want to say?
MR. DAVIS: No, Judge. I wanted to see how you wanted to proceed.
THE COURT: All right. Well, I thought -- I know we have several parties seeking leave to file briefs, including
10:12:27 several states and several professional organizations. I just wanted to see if the parties wanted to address that very quickly, whether there are any objections or not.
MR. LACOUR: I will go first, if that's all right, Your Honor.
10:12:41 THE COURT: That's fine.
MR. LACOUR: Would you like me to approach the podium?
THE COURT: Yes, please.
MR. LACOUR: Your Honor, we think that the brief from states should come in. It was filed in a timely manner, indeed
10:12:57 before Alabama's brief was even on file, which gave plaintiffs

time to assess those arguments before their brief was filed.
We do not -- for similar reasons, we do not think that the brief from the AAP should come in. They did not file it until we were actually here about the beginning of opening
10:13:20 statements. So there was not time to look it over.
I will be candid. I have not even had time to read it myself. I think some people on the team have, but there has been a lot to do in a very short amount of time.
And so I think for that reason the Court would -- we would
10:13:38 oppose that brief coming in at this moment.
THE COURT: All right. What about original plaintiffs?
MR. DOSS: Your Honor, we think both sets of amicus briefs are another data point that Your Honor could consider in
10:13:55 looking at all of the evidence and thinking through all the arguments.
We have no opposition to the several states, their amicus brief, provided that the amicus brief of the professional organizations is also allowed to be filed in.
10:14:10 This has been a long week. I think, if I remember correctly, the states' brief was filed on Tuesday, the professional organizations' brief was filed on Wednesday. I don't think the timing makes any difference one way or the other.
10:14:25 But to the extent Your Honor is wishing to consider any

amicus brief, I would submit that both should be considered, Your Honor.
THE COURT: Well, I certainly will consider them all, you know, on final merits. The issue is whether we consider
10:14:41 them now. Obviously, if I do consider them now, we are looking at this deadline.
Does anyone have any thoughts on that, just the practicality of me trying to take that in and consider it with all this evidence under a time crunch? I think that's worth
10:14:56 addressing by both sides.
MR. DOSS: As I read the states' brief, Your Honor, it expresses general criticism as to what it -- what they refer to as consensus-based medicine. I don't really see the states' amicus brief is presenting really any legal argument, as best I
10:15:18 could tell. The only legal citations were two citations to dissenting opinions from the U.S. Supreme Court. It seemed to me more of a policy statement rather than really much of evidence or legal argument.
As to the professional organizations, their amicus brief,
10:15:37 I think we have gotten a sense of what those positions are over the past day and a half from Dr. Ladinsky and Dr. Hawkins. If consideration of any brief is going to delay consideration of the merits for present purposes, I would say -- I would submit defer consideration of those amicus briefs until later. We're
10:16:03 just trying to get the preliminary relief at this point.

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was similar between TW and CW (p=0,7284) and different in the comparison TWvsCM (p=0,0325). In TW group, the median of blood glucose was 84 mg / dL, HBA1c 5.1%, total cholesterol 146 mg / dL, HDLc 43 mg / dL, LDLc 89 mg / dL and triglycerides 81.5 mg / dL. In the comparison with other groups, there was no difference from the statistical point of view. It is necessary to emphasize the HDLc of TW (43 mg/dL) which was exactly the same of CM (p>0,999) and lower than CW (60 mg/dL)(p=0,0720). Systolic Blood Pressure (SBP)(mmHg) of TW (126±13) was higher than that of CW (95±11;p<0.001) and equal to that of CM (115±9;p=0.1489). Regards Diastolic Blood Pressure (DBP) (mmHg), the medians of TW, CW and CM were 80, 60 and 80, respectively, and in the comparison TWxCW p = 0.0070 and TWxCM p> 0.9999. **Discussion:** Youth TW (16.3 ± 1.4 yo) taking an average estradiol dose of 1.5 ± 1.0 mg/day, with an average AGHT duration of 12.3 ± 9.9 months matched to controls on age and BMI did have higher HDL than CW and TW participants were more insulin resistant than CM. About SBP of that youth TW (107 ± 12), it was lower than CW 113 ± 7 (p>0,05) and CM 116 ± 8 (p<0,001). Other previous study showed that after 6 months of estradiol use, in doses ranging from 2 to 8 mg daily glucose enhanced 6 mg/dL (from 86 to 92) as well as TC from 170 to 178 mg/dL, HDLc from 50 to 54 mg/dL, TGL from 102 to 115 mg/dL, and LDL did not change (93), while a systematic review and meta-analysis showed increased only in TG levels. SBP and DBP increased on average of 7,2 mmHg and 5,7 mmHg, respectively. **Conclusion:** Metabolic findings observed after the first few months of TW GAHT appear to remain at long term, except for HDLc. SBP and DBP appear to increase in the long term, after a drop initially observed.

Reproductive Endocrinology

TRANSGENDER CARE

Case Report: Invasive Endometrial Cancer in a Trans Man and Risk of Testosterone Therapy

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Background: Only one case of uterine cancer in a trans man on testosterone is noted in literature prior to this case. No clinical evidence nor guidelines exist regarding testosterone therapy for this subset of patients.
Clinical Case: A 41-year-old trans man was seen by Gynecology for vaginal bleeding, with work-up revealing thickened endometrium and biopsy with endometrial adenocarcinoma. Testosterone therapy was held, and patient underwent total hysterectomy with BSO and bilateral pelvic/aortic lymph node dissection. Pathology demonstrated stage IIIA invasive adenocarcinoma, endometrium type with focal squamous differentiation, low grade. The tumor extended into the endocervical stroma with small metastasis to one ovary. He received adjunct pelvic radiation and sandwich chemotherapy with carboplatin and taxol. Concurrently, he was referred to Endocrinology for management of hormone replacement therapy (HRT). He originally started weekly testosterone injections and anastrozole at an outside facility in 2016 and underwent bilateral mastectomy in 2017. Testosterone was held perioperatively and during chemoradiation, for a total duration of 9 months. The patient experienced worsening gender dysphoria during this time. Discussion was held on goal to restart HRT in the setting of a theoretical risk of testosterone conversion to estradiol with increased risk of cancer recurrence; thus, patient initially chose to delay re-initiation of HRT. Following the completion of chemotherapy, he started on low-dose (30mg) weekly IM testosterone with plans for continued monitoring of testosterone and estradiol levels.
Conclusion: Research is needed in monitoring the effects of testosterone therapy on reproductive organs in patients assigned female at birth, and whether anastrozole therapy has protective effects for estrogen-driven cancers. Further, guidance is needed on monitoring of uterine lining in trans men and whether this should be standard of practice.

Reproductive Endocrinology

TRANSGENDER CARE

Development of Hip Bone Geometry in Transgender Adolescents Resembles the Experienced Gender if GnRHa Treatment Is Started in Early, but Not Late, Puberty

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Bone geometry can be described in terms of periosteal and endocortical growth and is partly determined by sex steroids. Periosteal and endocortical apposition are thought to be regulated by testosterone and estrogen, respectively. Gender-affirming hormone (GAH) treatment with sex steroids in transgender people might affect bone geometry. However, in adult transgender people no change in bone geometry during GAH was observed. In this study, we investigated changes in bone geometry among transgender adolescents using a gonadotropin-releasing hormone agonist (GnRHa) and GAH prior to achieving peak bone mass. Transgender adolescents treated with GnRHa and subsequent GAH at our center before the age of 18 years were eligible for inclusion. Participants were grouped based on their Tanner stage at the start of GnRHa treatment and divided into early, mid, and late puberty groups. Hip Strength Analysis software calculating subperiosteal width (SPW) and endocortical diameter (ED) was applied to dual-energy X-ray absorptiometry scans performed at start of GnRHa and GAH treatments, and after ≥ 2 years of GAH treatment. Mixed model analyses were performed to study differences over time. Data were visually compared with reference values of the general population retrieved from the literature. A total of 322 participants were included, of whom 106 trans women and 216 trans men. In both trans women and trans men participants resembled the reference curve for SPW and ED of the experienced gender, but only when GnRHa was started during early puberty. Those who started during mid- and late puberty remained

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within the reference curve of the gender assigned at birth. A possible explanation might be sought in the phenomenon of programming, which conceptualizes that stimuli during critical windows of development can have major consequences throughout one's lifespan. Therefore, this study adds insights into sex-specific bone geometry development during puberty of transgender adolescents treated with GnRHa, as well as the general population.

Reproductive Endocrinology

TRANSGENDER CARE

Effect on Kidney Function During Gender Affirming Hormonal Treatment in Transgender Individuals

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Background: Accurate interpretation of laboratory values with sex-specific reference ranges presents a challenge in transgender individuals on gender affirming hormone therapy (GAHT). Creatinine (Cr), the most common marker used for kidney function, varies significantly with body mass and composition. Both Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equations account for sex in estimating glomerular filtration rate. GAHT can effect Cr values in 2 potential ways: 1) by causing changes in muscle mass and body fat redistribution as early as 3 months after GAHT initiation and 2) by direct effects of sex hormones on kidney function. Previous studies have shown Cr values approaching affirmed gender identity as early as 6 months when on GAHT without mention of sex steroid levels. In this study we sought to describe the changes in serum Cr after initiation of GAHT in an effort to better understand expected changes and interpretation of lab data in TG individuals.
Methods: A retrospective chart analysis on all adult TG patients initiated on GAHT at our institution from January 2011 to 2020 was completed. We reviewed demographics, baseline health information, body mass index, and lab values including Cr, sex hormone levels, A1C, and fasting blood glucose. Lab values were obtained prior to GAHT, at the start of GAHT, at 3, 6, and 12 months after GAHT. Matched pair testing was conducted with sex steroid levels and Cr values in transgender men (TM) on testosterone and transgender women (TW) on estradiol in order to compare the median pre GAHT Cr to median Cr levels at 3, 6, and 12 months.
Results: 84 TW with a median age of 30 and 24 TM with a median age of 23 were included for analysis. TW and TM had a low rate of existing kidney disease (4.9%, 0%), diabetes mellitus (4.8%, 0%), and hypertension (10.8%, 4.5%) respectively. TW on GAHT achieved a goal estradiol level (≥100 pg/ml) at a rate of 37.3%, 51.7%, and 71.1% and suppressed testosterone to a goal level (<60ng/ml) at a rate of 44.4%, 54.7%, and 76.5% at 3, 6, and 12 months respectively. There was no significant change in Cr values at 3 months, but significantly decreased on average by -0.07 (p<0.001) at 6 months, and by -0.09 (p<0.001) at 12 months. TM on GAHT achieved a goal testosterone level (≥240 ng/dl) at a rate of
64.3%, 80.0%, and 72.3% at 3, 6, and 12 months respectively. Cr values increased significantly on average by 0.14 (p=0.036) at 3 months, by 0.21 (p=0.004) at 6 months, and by 0.15 (p=0.003) at 12 months.
Conclusions: In TW on GAHT, clinicians can consider using affirmed gender Cr reference ranges as early as 6 months. Similarly in TM on GAHT, affirmed gender Cr reference ranges can be used as early at 3 months. It remains to be seen whether changes in Cr levels reflect changes in sex steroid levels or sex steroid direct effects. Additionally, research is needed to determine if change in Cr levels reflect true changes in GFR.

Reproductive Endocrinology

TRANSGENDER CARE

First Evidence of Cardiopulmonary Adaptation to Physical Effort in Transgender Women After Long-Term Hormone Therapy: A Cross-Sectional Study

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Introduction: Cisgender men (CM) and women (CM) present different acute cardiopulmonary (CP) adaptation to effort. The smallest arteriovenous difference in oxygen (O2) and cardiac output (CO) in CW determine a lower maximum VO2 (VO2max) than CM. CP capacity adaptation to effort of TW undergoing gender-affirming hormone therapy (GAHT) was not yet reported. **Objective:** To evaluate CP capacity of TW in long-term GAHT. **Methods:** A cross-sectional study was carried out with 8 TW (average age of 34.0 ±4.8 yo), 8 CM and 8 CW matched on age, body mass index and activity level. All TW were non-gonadectomized subjects and were in estrogen [transdermal estradiol (n=2), oral estradiol (n=3) and conjugated estrogen (n=3)], plus cyproterone acetate (n=8) therapy in an average time of 15.6 ±8.7 years. Body composition was assessed by InBody 720, and participants' level of physical activity by IPAQ (International Physical Activity Questionnaire) short form. Total testosterone (ng/dL) levels of TW, CW and CM were 83,5 (12,0;637,0), 20,5 (12,0;41,0) and 480,5 (264,0;843,0), respectively. Hemoglobin levels of TW, CW and CM were 14,2 (13,5;14,9), 14,35 (12,8;14,7) and 15,35 (14,0;18,2), respectively. Everyone performed a CP exercise testing on a treadmill with an incremental effort. **Results:** Mean VO2max (L/min) in the group of TW was 2648±575.5, of CW 2128±394.0 and of CM 3235±554.0 (TWvsCW p=0.1311; TWvsCM p=0.0806; CWvsCM p=0.009). Free fat mass (FFM) of TW was 55.56±6.88 kg, CW 38.98±4,09 kg, and CM 64,98±6,29 kg (TWvsCW p<0,0001; TWvsCM p=0,024; CWvsCM p<0,0001). Analysis of VO2max/FFM (L/min/kg), TW´s rate was 46.6±6.2, CW´s was 54.6±8.4 and CM´s was

Patient Information for Informed Consent
FEMINIZING MEDICATIONS FOR TRANSGENDER CLIENTS
Minors and Parents/Guardians
University of Alabama at Birmingham Pediatric Endocrinology
Multidisciplinary Gender Health Team

Before using medications to transition and feminize, you and your parents or guardians need to know the possible advantages, disadvantages and risks of these medications. We have listed them here for you. It's important that you understand all of this information before you begin taking these medications.

Please read the following with your parent or guardian. Once your questions or concerns are addressed, and you have decided to proceed with the medication(s), both you and your parent or guardian will need to sign this information and consent form.

We are happy to answer any questions you have.

What are the different medications that can feminize my appearance?

Part of transition for many transgender people involves taking hormones. For hormone treatment to be most effective, transgender girls and women take not only estrogens (female hormones), but also medicines to block their body from producing or utilizing testosterone (male hormones).

Different forms of the hormone estrogen are used to feminize appearance in transgender females. Estrogen can be given as an injection, weekly or every other week, as a pill, daily or twice a day, or as a patch, which is changed every three or four days.

Medications that block the production or effects of testosterone are called androgen blockers. Androgen is another term for male sex hormones. Spironolactone is the androgen blocker that is most commonly used in the United States. Other medicines are sometimes used, but because spironolactone is relatively safe, inexpensive, and effective to block testosterone, it is the primary androgen blocker used for transgender women.

Every medication has risks, benefits, and side effects that are important to understand before starting. The effects and side effects of medicines used for transition need to be monitored with laboratory studies and regular visits to your provider to make sure that there are no negative effects on your body.

Both the medicines that you take, as well as the process of transitioning can affect your mood. While trans women are relieved and happy with the changes that occur, it is important that you are under the care of a gender-qualified therapist while undergoing transition. The therapist can work with you, your family and friends and your school staff.

Estrogen can cause blood clots. We must be careful that you are not at risk to develop a blood clot. Who should not take estrogen?

Estrogen should not be used by anyone who has a history of

- an estrogen-dependent cancer
- a disorder that makes them more likely to get blood clots that could travel to the lungs (unless they are also taking blood thinners and are followed by a specialist)

Estrogen should be used with caution and only after a full discussion of risks by anyone who

- has a strong family history of breast cancer or other cancers that grow quicker when estrogens are present
- has uncontrolled diabetes
- has heart disease
- has chronic hepatitis or other liver disease
- has uncontrolled high cholesterol
- has migraines or seizure
- is obese
- smokes cigarettes

Both you and your parent or guardian should initial and date each statement on this form to show that you and your parent or guardian understand the benefits, risks, and changes that may occur from taking these medications.

Effects of Feminizing Medications

_____ _____I know that estrogen or anti-androgens – or both – may be prescribed to feminize my appearance.

_____ _____I know it can take several months or longer for the effects to become noticeable. I know that no one can predict how fast – or how much – change will happen.

_____ _____I know that if I am taking estrogen I will develop breasts.

- I know it takes several years for breasts to get to their full size.
- I know the breasts will remain, even if I stop taking estrogen.
- I know I might have a milky discharge from my nipples (called galactorrhea). If I do, I know I should check it out with my healthcare provider because it could be caused by the estrogen or by something else.
- I know that while we do not know the exact risk the risk, my risk of breast cancer may be increased to as high as if I had been born female
- I know that I should take care of my breasts like every other woman. This includes annual breast exams from my health provider, and when I am older, regular mammograms.

_____ _____I know that the following changes are usually not permanent — they are likely to go away if I stop taking the medicines.

- I know my body hair will become less noticeable and will grow more slowly. But it won't stop completely, even if I take the medicines for years.
- I know I will probably have less fat on my abdomen and more on my buttocks, hips, and thighs. It will be redistributed to a more female shape — changing from "apple" shape to "pear" shape.
- I know that if I have the predisposition to have male pattern baldness it may start later than it would have, but may not stop completely.
- If I stop taking hormones I may lose my hair faster than if I hadn't taken hormones.
- I know I may lose muscle and strength in my upper body.
- I know that my skin may become softer.

_____ _____I know that my body will make less testosterone (an androgen, or male hormone). This may affect my sex life in different ways and future ability to cause a pregnancy:

- I know my sperm may no longer get to full maturity. This could make me less able to cause a pregnancy. I also know that there is a small risk that I might never produce mature sperm again. But I know that it's also possible that my sperm could still mature even while I am taking hormones. So, I know that I might get someone pregnant if we have vaginal intercourse and we don't use birth control.
- The options for sperm banking have been explained to me.
- I know that my testicles may shrink down to half their size. Even so, I know that they are part of my body and that I need to take care of them unless I have surgery to remove them. This means that I will need regular checkups for them.
- I know that I won't have as much semen when I ejaculate.
- I know it is likely that I won't have erections upon waking as often as before, and it is likely that I will have fewer spontaneous erections.
- I know I may not be able to achieve or maintain an erection for penetrative sex.
- I know that I may want to masturbate less or have sex less, and may find it harder to ejaculate when I do.
- I know this treatment may (but is not assured to) make me permanently unable to make a woman pregnant.

_____ _____I know that some parts of my body will not change much by using these medicines.

- I know the hair of my beard and mustache may grow more slowly than before. It may become less noticeable, but it will not go away unless I have treatments like electrolysis.
- I know the pitch of my voice will not rise, and my speech patterns will not become more like a woman's.
- I know my Adam's apple (called the laryngeal prominence) will not shrink.
- Although these medicines can't make these changes happen, there are other treatments that may be helpful.

_____ _____ I know that there may be mood changes with these medicines. I agree to continue therapy with a qualified therapist.

_____ _____I know if I have any concerns about these issues, you can make referrals for me to help me explore other treatment options.

Risks of Feminizing Medications

_____ _____I know that the side effects and safety of these medicines are not completely known. There may be long-term risks that are not yet known.

_____ _____I know not to take more medicine than I am prescribed. I know it increases health risks. I know that taking more than I am prescribed won't make changes happen more quickly or more significantly.

_____ _____ I know these medicines may damage the liver and may lead to lead to liver disease. I know I should be checked for possible liver damage as long as I take them.

_____ _____I know these medicines cause changes that other people will notice. Some transgender people have experienced discrimination because of this. I know my clinician can help me find advocacy and support resources.

Risks of Estrogen

_____ _____I know that taking estrogen increases the risk of blood clots or problems with blood vessels that can result in

- chronic problems with veins in the legs
- heart attack
- pulmonary embolism – blood clot to the lungs – which may cause permanent lung damage or death
- stroke, which may cause permanent brain damage or death

_____ _____I know that the risk of blood clots is much worse if I smoke cigarettes. I know the danger is so high that I should stop smoking completely if I start taking estrogen. I know that I can ask my clinician for advice about how to stop smoking.

_____ _____ I know taking estrogen can increase the deposits of fat around my internal organs. This can increase my risk for diabetes and heart disease.

_____ _____I know taking estrogen can raise my blood pressure. I know that if it goes up, my clinician can work with me to try to control it with diet, lifestyle changes, and/or medication.

_____ _____I know that taking estrogen increases my risk of getting gallstones. I know I should talk with my clinician if I get severe or long-lasting pain in my abdomen.

_____ _____I know that estrogen can cause nausea and vomiting. I know I should talk with my clinician if I have long-lasting nausea or vomiting.

_____ _____I know that estrogen can cause migraines or make them worse if I already have them. I know I should talk with my clinician if I have headaches or migraines often or if the pain is unusually severe.

_____ _____I know that it is not yet known if taking estrogen increases the risk of prolactinomas. These are non-cancerous tumors of the pituitary gland. I know they are not

usually life threatening, but they can damage vision and cause headaches if they are not treated properly. I know that changes in vision, headaches that are worse when I wake up in the morning, and milky discharge from my nipples can be signs of a prolactinoma, and I should talk to my health care provider if I develop these symptoms. There is a blood test that can check for this.

_____ _____I know that I am more likely to have dangerous side effects if

- I smoke.
- I am overweight.
- I have a personal or family history of blood clots.
- I have a personal or family history of heart disease and stroke.
- My family has a history of breast cancer.

Risks of Androgen Antagonists (Spironolactone)

_____ _____I know that spironolactone affects the balance of water and salts in the kidneys. This may

- Increase the amount of urine I produce, making it necessary to urinate more frequently.
- Increase thirst.
- Rarely, cause high levels of potassium in the blood, which can cause changes in heart rhythms that may be life-threatening.
- Reduce blood pressure.

_____ _____I know some androgen antagonists make it more difficult to evaluate test results for cancer of the prostate. This can make it more difficult to check up on prostate problems. I know that if I am over 50, I should discuss appropriate prostate cancer screening with my care provider. I know that even if I have genital sex reassignment surgery the prostate is not usually removed.

Prevention of Medical Complications

_____ _____I agree to take feminizing medications as prescribed. And I agree to tell my care provider if I have any problems or am unhappy with the treatment.

_____ _____I know that the dose and type of medication that's prescribed for me may not be the same as someone else's.

_____ _____I know I need periodic physical exams and blood tests to check for any side effects.

_____ _____I know that in addition to periodic checks from my provider, I must also treat my body with respect. This means that paying attention and talking to my provider if I develop any symptoms that might be side effects from medicines. This also means keeping my partners and myself safe, when and if I choose to have sex with others, by using condoms or methods to keep me safe from sexually transmitted infections (STIs).

_____ _____I know that feminization medications can interact with other drugs and prescribed and over the counter medicines. These include alcohol, diet supplements, herbs, other hormones, and street drugs. This kind of interaction can cause dangerous complications. I know that I need to prevent complications because they can be life threatening. That's why I need to be honest with my provider about whatever else I take. I also know that I will continue to get medical care here no matter what I share about what I take.

_____ _____I know that it can be risky for anyone with certain conditions to take these medicines. I agree to be evaluated if my clinician thinks I may have one of them. Then we will decide if it's a good idea for me to start or continue using them.

_____ _____I know that I should stop taking estrogen two weeks before any surgery or when I may be immobile for a long time (for example, if I break my leg and am in a cast). This will lower the risk of getting blood clots. I know I can start taking it again a week after I'm back to normal or when my clinician says it's okay.

_____ _____ I know that even if I have to stop my estrogens, I may still be able to take the testosterone blockers that I am on, to help prevent the effects of my testicles producing testosterone again.

_____ _____I know that using these medicines to feminize is an off-label use. I know this means it is not approved by the Food and Drug Administration (FDA). I know that the medicine and dose that is recommended for me is based on the judgment and experience of my health care provider and the best information that is currently available in the medical literature.

_____ _____I know that I can choose to stop taking these medicines at any time. I know that if I decide to do that, I should do it with the help of my clinician. This will help me make sure there are no negative reactions. I also know my clinician may suggest that I cut the dose or stop taking it at all if certain conditions develop. This may happen if the side effects are severe or there are health risks that can't be controlled.

Alternatives

There are alternatives to using feminizing medicines to help people appear more feminine. Some transgender people choose to not take hormones or have surgery and may only socially transition. If you are interested in alternatives, talk with your health care provider about your options.

Our signatures below confirm that

- My clinician has talked with me and my parents or guardian about
  - the benefits and risks of taking feminizing medication
  - the possible or likely consequences of hormone therapy
  - potential alternative treatments
- I understand the risks that may be involved.
- I know that the information in this form includes the known effects and risks. I also know that there may be unknown long-term effects of risks.
- I have had enough opportunity to discuss treatment options with my clinician.
- All of my questions have been answered to my satisfaction.
- I believe I know enough to give informed consent to take, refuse, or postpone therapy with feminizing medications.

Based on all this information

_____ I want to begin taking estrogen.

_____ I want to begin taking androgen antagonists (e.g., spironolactone).

_____ I do not wish to begin taking feminizing medication at this time.

Patient Signature	Date
Signature of Parent or Guardian	Date
Prescribing clinician signature	Date

Your health is important to us. If you have any questions or concerns please call us at (205) 638 9107. We are happy to help you.

Client Information for Informed Consent

TESTOSTERONE FOR TRANSGENDER CLIENTS
Minors and Parents/Guardians
University of Alabama at Birmingham Pediatric Endocrinology
Multidisciplinary Gender Health Team

Before using testosterone to transition and masculinize your body, you and your parents or guardians need to know the possible advantages, disadvantages and risks of these medications. We have listed them here for you. It's important that you understand all of this information before you begin taking these medications.

Please read the following with your parent or guardian. Once your questions or concerns are addressed, and you have decided to proceed with the medication(s), both you and your parent or guardian will need to sign this information and consent form.

We are happy to answer any questions you have.

What is testosterone?

It is the sex hormone that makes certain features appear typically male. It builds muscle and causes the development of facial hair and a deeper voice.

How is testosterone taken?

It is usually injected every one to four weeks. It is not used as a pill because the body may not absorb it properly and may cause potentially fatal liver problems. Some people use skin creams and patches, but they tend to be more expensive and aren't recommended for initiating puberty or for use in teenagers and young adults.

The doses used for injection differ from product to product and from patient to patient. They may range from 50 to 400mg. The injections are given in a large muscle to slow the release of the hormone. You may experience unwanted swings in hormone levels. You may control the swings by changing how often the dose is given and how much of a dose is given.

Every medication has risks, benefits, and side effects that are important to understand before starting. The effects and side effects of medicines used for transition need to be monitored with laboratory studies and regular visits to your provider to make sure that there are no negative effects on your body.

The medicines that you take, as well as the process of transitioning can affect your mood. While trans men are usually relieved and happy with the changes that occur, it is important that you are under the care of a gender-qualified therapist while undergoing transition. The therapist can work with you, your family and friends and your school staff.

Warning — Who should not take testosterone?

It should *not* be used by anyone who is pregnant or has uncontrolled coronary artery disease as it could increase your risk for a fatal heart attack:

It should be used with caution and only after a full discussion of risks by anyone who

- Has acne
- Has a family history of heart disease or breast cancer
- Has had a blood clot
- Has high levels of cholesterol
- Has liver disease
- Has a high red-blood-cell count
- Is obese
- Smokes cigarettes

Periodic blood tests to check on the effects of the hormone will be needed. Routine breast exams and pelvic exams with Pap tests should be continued, when applicable.

Summary of Testosterone Benefits and Risks

BENEFITS	RISKS

• Appearing more like a man
  ○ Bigger clitoris
  ○ Coarser skin
  ○ Lower voice
  ○ More body hair
  ○ More facial hair
  ○ More muscle mass
  ○ More strength
  ○ No more menstrual periods
• More physical energy
• More sex drive
• Protection against bone thinning (osteoporosis)
	• Acne (may permanently scar)
	• Blood clots (thrombophlebitis), risk significantly increased by smoking
	• Emotional changes, for example, more aggression
	• Headache
	• High blood pressure (hypertension)
	• Increased red-blood-cell count
	• Infertility
	• Inflamed liver
	• Interaction with drugs for diabetes and blood thinning — for example Coumadin and Warfarin
	• Male pattern baldness
	• More abdominal fat — redistributed to a male shape
	• More risk of heart disease
	• Swelling of hands, feet, and legs
	• Weight gain

Both you and your parent or guardian should initial and date each statement on this form to show that you and your parent or guardian understand the benefits, risks, and changes that may occur from taking this medications.

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Masculinizing

_____I know that testosterone may be prescribed to make me appear less like a woman and more like a man.

_____ I know it can take several months or longer for the effects to become noticeable. I know that no one can predict how fast – or how much – change will happen. I know that the changes may not be complete for two to five years after I start.

_____I know that the following changes are likely and permanent even if I stop taking testosterone:

- Bigger clitoris — typically about half an inch to a little more than an inch
- Deeper voice
- Gradual growth of mustache and beard
- Hair loss at the temples and crown of the head — possibility of being completely bald
- More, thicker, and coarser hairs on abdomen, arms, back, chest, and legs

_____ I know that the following changes are usually not permanent — they are likely to go away if I stop taking testosterone:

- Acne (although there may be permanent scars)
- Menstrual periods typically stop one to six months after starting
- More abdominal fat – redistributed to a male shape: decreased on buttocks, hips, and thighs; increased in abdomen – changing from "pear shape" to "apple shape"
- More muscle mass and strength
- More sex drive
- Vaginal dryness

_____I know that the effects of testosterone on fertility are unknown. I have been told that I may or may not be able to get pregnant even if I stop taking testosterone. I know that I might still get pregnant even after testosterone stops my menstrual periods. I know about my birth control options (if applicable). And I know that I can't take testosterone if I am pregnant and that I must take a pregnancy test prior to starting testosterone therapy.

_____ I know that some aspects of my body will not be changed:

- Losing some fat may make my breasts appear slightly smaller, but they will not shrink very much.
- My voice will deepen, but other aspects of the way I speak may not sound more masculine.
- Although testosterone can't make these changes happen, there are other treatments that may be helpful.

_____I know that there may be mood changes with these medicines. I agree to continue therapy with a qualified therapist.

_____I know if I have any concerns about these issues, you can make referrals for me to help me explore other treatment options.

Risks of Testosterone

_____I know the medical effects and the safety of testosterone are not completely known. There may be long-term risks that are not yet known.

_____I know not to take more testosterone than prescribed. Taking too much:

- Will increase health risks
- Won't make changes happen more quickly or more significantly
- Can cause my body to convert extra testosterone into estrogen, and that can slow down or stop my appearing more masculine

_____I know that testosterone can cause changes that increase my risk of heart disease. These changes include having:

- Less good cholesterol (HDL) that may protect against heart disease and more bad cholesterol (LDL) that may increase the risk of heart disease
- Higher blood pressure
- More deposits of fat around my internal organs

_____I know that my risk of heart disease is higher if people in my family have had heart disease, if I am overweight, or if I smoke.

_____I know that I should have periodic heart-health checkups for as long as I take testosterone. This means I must watch my weight and cholesterol levels and have them checked by my clinician.

_____I know testosterone can damage the liver and possibly lead to liver disease and I should be checked for possible liver damage for as long as I take testosterone.

_____I know testosterone can increase my red blood cells and hemoglobin. This increase is usually only to what is normal for a man and shouldn't cause any health risks. However, there is a small possibility that higher levels of red blood cells and hemoglobin may increase my risk of life-threatening problems such as stroke or heart attack. That's why I know I need to have periodic blood checks for as long as I take testosterone.

_____I know that taking testosterone can increase my risk for diabetes. It may decrease my body's response to insulin, cause weight gain, and increase deposits of fat around my internal organs. Therefore, I should have periodic checks of my blood glucose for as long as I take testosterone.

_____I know my body can turn testosterone into estrogen and that no one knows if that could increase the risk of cancers of the breast, the ovaries, or the uterus.

_____I know taking testosterone can thin the tissue of my cervix and the walls of my vagina. This can lead to tears or abrasions during vaginal sex or play with a male or female partner. These tears increase my risk of getting a sexually transmitted infection, including HIV. I know I should speak frankly with my primary care provider about my sex life to learn the best ways to prevent and check for infections.

_____I know that testosterone can give me headaches or migraines. I know that it's best to talk with my clinician if I get them a lot or if the pain is unusually severe.

_____I know that testosterone can cause emotional changes. For example, I could become more irritable, frustrated, or angry. I know that my clinician can help me find resources to explore and cope with these changes.

_____I know that testosterone causes changes that other people will notice. Some transgender people have experienced harassment, discrimination, and violence because of this. Others have lost the support of loved ones. I know my clinician can help me find advocacy and support resources.

Prevention of Medical Complications

_____I agree to take testosterone as prescribed. I agree to not purchase testosterone or other hormones without my physician's knowledge, and I agree to tell my clinician if I have any problems or am unhappy with the treatment.

_____I know that the dose and type of medication that's prescribed for me may not be the same as someone else's.

_____I understand that the medications prescribed are for my use only and I will not supply these medications to others.

_____I know I need periodic physical exams and blood tests to check for any side effects.

_____I know testosterone can interact with other drugs and medicines. These include alcohol, diet supplements, herbs, other hormones, and street drugs. This kind of interaction can cause complications. I know that I need to prevent complications because they can be life-threatening. That's why I need to be honest with my clinician about whatever else I take. I also know that I will continue to get medical care here no matter what I share about what I take.

_____ _____ I know that it can be risky for anyone with certain conditions to take testosterone. I agree to be evaluated if my clinician thinks I may have one of them. Then we will decide if it's a good idea to start or continue using testosterone.

_____ _____ I know that using testosterone to masculinize is an off-label use. This means it is not approved by the Food and Drug Administration (FDA). I know that the medicine and dose that is recommended for me is based on the judgment and experience of my health care provider and the best information that is currently available in the medical literature.

_____ _____ I understand that my insurance company may not cover the costs of this treatment. If so, I accept responsibility for any charges associated with this treatment. Costs of treatment can be obtained by contacting The Pediatric Endocrinology office at 205 638 9107.

_____I know that I can choose to stop taking testosterone at any time. I know that if I decide to do that, I should do it with the help of my clinician. This will help me make sure there are no negative reactions. I also know my clinician may suggest that I cut the dose or stop taking it at all if certain conditions develop. This may happen if the side effects are severe or there are health risks that can't be controlled.

Alternatives

There are alternatives to using testosterone to help people appear more masculine. Some transgender people choose to not take hormones or have surgery and may only socially transition. If you are interested in alternatives, talk with your health care provider about your options.

Our signatures below confirm that:

- My clinician has talked with me and my parents or guardians about
  - The benefits and risks of taking testosterone
  - The possible or likely consequences of hormone therapy
  - Potential alternative treatments
- I understand the risks that may be involved.
- I know that the information in this form includes the known effects and risks. I also know that there may be unknown long-term effects of risks.
- I have had enough opportunity to discuss treatment options with my clinician.
- All of my questions have been answered to my satisfaction.
- I believe I know enough to give informed consent to take, refuse, or postpone testosterone therapy.

Based on all this information:

_____I want to begin taking testosterone.

_____I do not wish to begin taking testosterone at this time.

_____ _____
Patient Signature Date

_____ _____
Signature of Parent or Guardian Date

_____ _____
Prescribing Clinician Signature Date

Your health is important to us. If you have any questions or concerns please call us at (205) 638 9107. We are always happy to help you.

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standard high-risk and very high-risk disease, respectively, had high-risk Decipher scores, and 22.0%, 16.1% and 11.4%, respectively, had low Decipher scores, supporting use of the proposed three-tiered subclassification. As the Decipher test was designed to inform which patients are likely to develop metastases following radical prostatectomy[7], that risk grouping based on the same outcomes correlates with Decipher scores is reassuring in this context.

Subclassification of high-risk prostate cancer could prove useful as a tool to guide further investigation. However, risk categories such as those established by the NCCN are recognized to be heterogeneous, and their utility lies in their ability to provide initial, clear guidance on management decisions. More evidence is necessary to show that this further subclassification warrants differential clinical management. For prognostication purposes, multiple nomograms and risk assessment tools, such as the University of California San Francisco (UCSF)-Cancer of the Prostate Risk Assessment (CAPRA) score and Memorial Sloan Kettering Cancer Center's prostate cancer nomograms, are available that can currently provide substantially more granular, individualized prognostic information than subclassification.

Efforts to integrate genomics and clinicopathological risk might ultimately have important clinical applications. For example, Cooperberg et al.[8] showed that Decipher score and Cancer of the Prostate Risk assessment postsurgical (CAPRA-S) score are both independently associated with cancer-specific survival after radical prostatectomy. Furthermore, combining CAPRA-S and Decipher scores improved risk stratification compared with using each separately[8]. Importantly, Spratt and colleagues[9] recently devised a system that integrates existing genomic and clinical tools to improve risk stratification, showing that combining their clinical–genomic risk grouping has a higher concordance index than using NCCN clinical risk group alone to predict metastasis (0.84 versus 0.73) and results in 30% of patients being reclassified using the genomic–clinical groupings compared with the three-tier (low/intermediate/high) NCCN classification. A recent consensus statement highlighted some of the potential and challenges of adding biomarkers to prostate cancer care, particularly the need for biomarkers to add value above an existing multivariable model incorporating all available information, the need to show clinically utility and the importance of matching the disease state and population in which a biomarker was tested to that in which it is applied[10].

Validating and testing schema incorporating both clinical and genomic data, integrating them with advances in pathology and critically assessing clinical decisions based on these data will be critical aims going forward. This endeavour has the potential for a real benefit in patients with high-risk disease, in whom administering the appropriate therapy at the right time is crucial.

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Competing interests
The authors declare no competing interests.

RELATED LINKS
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TRANSGENDER MEDICINE

Advancing knowledge of transgender medical intervention effects

Joshua D. Safer

In a recently published study, surveys of transgender individuals on hormone therapy provide insight into the self-reported effects of medication where previous thinking was only speculative. Still, controlled studies are required to avoid overinterpreting the clinical significance of specific findings in the context of what might be expected in the general population.

Refers to van Dijk, D. et al. Explorative prospective evaluation of short-term subjective effects of hormonal treatment in trans people — results from the European Network for the Investigation of Gender Incongruence. *J. Sex. Med.* **16**, 1297–1309 (2019).

When assessing the risks and benefits of transgender hormone therapy, the evidence base to guide decision-making is thin. Although transgender hormone treatment seems to be generally safe when prescribed under medical supervision[1,2], the data that exist are mostly from medical record review of convenience samples with dozens or hundreds of patients. Studies include broad surveys of passively collected laboratory tests[3] along with reassuring retrospective chart reviews looking at specific laboratory tests, such as prolactin[4] and oestradiol[5] levels, for which concerns have been raised in guidelines owing to possible adverse effects of therapy[6,7]. The nature of these studies has been to show minimal harm rather than to show benefit. Thus, a recently published article by van Dijk et al.[8] is a welcome addition to the literature in an area with nearly no rigorous study. In addition to the risks of hormonal treatment, patients query their physicians about the specific effects and the time course of effects of hormone therapy. Providers with large transgender

patient panels have long suspected many consequences of hormone treatment, but reliably collected data of patient-reported outcomes (both harms and benefits) do not exist. Rigorously measured objective data would be ideal but even systematic polls from convenience samples are lacking.

The paper by van Dijk et al.[8] describes self-reported scores in multiple subjective categories, including physical, cognitive, emotional and sexual symptoms, providing the integrated opinions of a sizable cohort of individuals (TABLE 1). The investigators were able to query nearly 200 trans men along with nearly 200 trans women during their first year of hormone treatment, with most queried at baseline, 3 months, 6 months and 12 months. Survey questions ranged from non-specific items, such as fatigue, to items that would be predicted to be associated with hormonal therapy, such as clitoral changes or hair changes. Specifically, in transgender men, the authors report increased scores for hot flashes, balding, sexual desire, acne, voice instability, transient weight gain and transient clitoral pain, and decreased scores for emotional instability, fear and menses. In transgender women, the authors report increased scores for hot flashes, emotional instability, night sweats, fatigue, weight gain, olfactory sense, brittle nails, mood swings and breast tenderness, and decreased scores for sexual desire.

The authors also attempted to separate symptom reports by route of hormone administration. In transgender men, sexual desire increased in those using testosterone ester injection products compared with transdermal testosterone administration and very long acting testosterone injection products.

The data presented by van Dijk et al. are provocative, somewhat intuitive, but not conclusive. First, the symptom scores in the paper are arbitrary and might or might not represent clinically significant changes even for items with apparent statistical significance. For example, whether the report of brittle nails in transgender women over time represents a true difference is unclear. This problem is especially apparent for end points in which transgender men and transgender women reported similar changes, like hot flashes and weight gain, despite their different medication regimens. Second, even if the symptom scores are clinically significant, the investigation includes a very large number of end points with near certainty of a type I error. That is, by chance, some end points are likely to achieve statistical significance although no real change has occurred. Notably, the same team reported differing results in an earlier paper[3]; for example, hot flashes had not been noted in transgender men in that report. The authors attribute this discrepancy to the difference in sample size and time course of the studies. However, the number of end points suggests that the differences between studies might be random. In this scenario, symptom scores that the authors attribute to transient effects of treatment might also reflect regression to the mean when multiple queries are made. Third, the scoring is made with baseline values as a reference. Thus, it is easy to envision that the changes reported are not treatment-related changes but secular changes that might be observed in any population participating in surveys and thinking about these symptoms on a continual basis. For those categories in which scores diverged between transgender men and transgender women, secular change concerns are better addressed than for other end points. The investigators observed increased sexual desire in transgender men (who had increased testosterone levels as a result of treatment) and decreased sexual desire in transgender women (who had decreased testosterone levels as a result of treatment). Finally, the attempts of the investigators to ascertain differences between routes of administration are confounded by the lack of control for the dose received by the patient. Patients receiving more active ingredient might have different results to those receiving a lower dose.

The report by van Dijk and colleagues[8] is a major move forward in elucidating areas of transgender medical care where nearly no data exist. Carefully collated information from an uncontrolled convenience sample is a major improvement over expert opinion. However, in areas where differences were noted, studies that control for secular change and that provide better context for the interpretation of the clinical relevance of the findings are needed before the data can be considered sufficiently conclusive to inform change in practice.

Table 1 | **Self-reported changes in transgender patients in the first year of hormone therapy**[8]

Symptom	Trans men	Trans women
Acne	↑↑	–
Balding	↑↑	↓↓
Breast tenderness	–	↑↑
Brittle nails	–	↑↑
Clitoral pain	↑	–
Emotional instability	↓↓	↑↑
Fatigue	–	↑↑
Fear	↓↓	–
Hot flashes	↑↑	↑↑
Menses	↓↓	–
Mood swings	–	↑↑
Night sweats	↑	↑↑
Olfactory sense	–	↑↑
Sexual desire	↑↑	↓↓
Sleeping difficulty	–	↓
Voice instability	↑↑	–
Weight	↑	↑↑

Only those changes that achieved statistical significance are shown. ↑↑, persistently increased; ↑, transiently increased; ↓↓, persistently decreased; ↓, transiently decreased.

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Competing interests

J.D.S. served on an advisory panel for Endo Pharmaceuticals in June 2018.

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Original article

Psychological Functioning in Transgender Adolescents Before and After Gender-Affirmative Care Compared With Cisgender General Population Peers

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A B S T R A C T

Purpose: Transgender adolescents are at risk for internalizing and externalizing problems, along with high suicidality rates, and poor peer relations. The present study compared transgender adolescents before and after gender-affirmative care with a sample of nonclinical age-equivalent cisgender adolescents from the general population on psychological well-being and aimed to investigate the possible effect of transgender care involving puberty suppression.
Methods: In this cross-sectional study, emotional and behavioral problems were assessed by the Youth Self-Report in a sample of 272 adolescents referred to a specialized gender identity clinic who did not yet receive any affirmative medical treatment and compared with 178 transgender adolescents receiving affirmative care consisting of puberty suppression and compared with 651 Dutch high school cisgender adolescents from the general population.
Results: Before medical treatment, clinic-referred adolescents showed more internalizing problems and reported increased self-harm/suicidality and poorer peer relations compared with their age-equivalent peers. Transgender adolescents receiving puberty suppression had fewer emotional and behavioral problems than the group that had just been referred to transgender care and had similar or fewer problems than their same-age cisgender peers on the Youth Self-Report domains.
Conclusions: Transgender adolescents show poorer psychological well-being before treatment but show similar or better psychological functioning compared with cisgender peers from the general population after the start of specialized transgender care involving puberty suppression.

IMPLICATIONS AND CONTRIBUTION

This study found increased behavioral and emotional problems among adolescents referred to a specialized gender identity clinic compared with their cisgender peers from the general population. After the start of gender-affirming treatment, the transgender adolescents showed similar or better psychological functioning compared with their cisgender peers from the general population.

In recent years, a sharp increase has been seen in media attention, clinical referrals, and number of publications on adolescents with gender dysphoria (GD), the DSM-5 term used to describe the incongruence between one's birth-assigned gender and the experienced gender [1,2]. A number of these studies report on psychological functioning and show that feelings of GD are frequently associated with psychological difficulties [3].

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Adolescents referred to specialized gender identity clinics have prevalence rates of depression ranging from 12% to 58% and for anxiety 16% to 24% [3–8]. In these studies, histories of suicidal thoughts and self-harming behaviors were reported by 34%–51% and 12%–39% of youth, respectively, in the various studies [3–8]. In addition, comparison studies of transgender youth with lesbian, gay, and bisexual adolescents revealed comparable rates of psychological difficulties [9]. Several studies have used the standardized self-report and parental measures of the Youth Self-Report (YSR) and the Child Behavior Checklist [10,11] and found more behavioral and emotional problems in transgender youth compared with the normative samples of these measures [12,13]. In general, comparisons made with normative samples drawn from the general population show similar findings, with a predominance of internalizing problems over externalizing problems (for an overview, see [14]). Summarizing the YSR and Child Behavior Checklist results, transgender adolescents show psychological problems comparable to clinical norm populations, with some cross-national variation in levels of psychological problems between North America and Europe [15,16].

A framework for understanding GD and the associated mental health disparities is offered by the minority stress model that posits that sexual minorities experience chronic stressors related to the stigmatization of their identities [17,18]. Psychological functioning is better when there is more acceptance of GD by the youth and their environment, including better peer relations [12,16]. In addition, other more general risk factors might be related, and other models of explanations have been proposed [14]. In addition, the onset of puberty and the developing body might endorse an intensification of psychological distress [19].

Transgender care for adolescents with GD is often offered in a step-wise model. During the first phase, the nature of the adolescent's gender identity and general psychosocial functioning are explored, and medical interventions are not yet provided [19]. During the second phase, adolescents with GD receive puberty suppression by means of reversible gonadotropin-releasing hormone analogs to "create time" to enable further exploration of the decision for gender-affirming treatments without the accompanying distress caused by the physical changes of puberty [19]. Thereafter, gender-affirming hormones (GAHs) can be provided, androgens in assigned girls at birth and estrogens in assigned boys at birth to induce the development of secondary sex characteristics of the experienced gender [19–21]. The present article will refer to assigned boys or girls when assigned gender at birth is boy or girl, respectively, which may be incongruent from the experienced gender in the group of adolescents with GD.

The first follow-up studies evaluating the use of puberty suppression in relation to psychological well-being in adolescents with GD come from the Netherlands and showed that behavioral and emotional problems and depressive symptoms decreased and general functioning significantly improved during treatment [22,23]. A study from the United Kingdom showed that psychological support and puberty suppression were associated with an improved global psychosocial functioning in adolescents with GD with a combination of psychological support and puberty suppression, attributing to a greater improvement than psychological support only [24]. These psychological evaluation studies were performed using self-reported psychosocial functioning (internalizing and externalizing problems, suicidality, and peer relations) in comparison with normative standardization samples. The YSR normative sample was recruited over 20 years ago, and a more recent recruited sample from the general population is lacking [11]. The present study is the first to compare transgender adolescents receiving gender-affirmative treatment by means of puberty suppression with recently collected nonclinical cisgender peers from the general population, exploring psychological functioning and the role of specialized transgender care.

Methods

Participants and procedure

The samples in this study consisted of consecutive referrals to the Center of Expertise on Gender Dysphoria of the VU University Medical Center (VUmc) in Amsterdam, the Netherlands, between 2012 and 2015, and a control group of cisgender adolescents recruited in 2015 in the general population. During this period, 504 adolescents were seen in our gender identity service. Fifty-three participants did not complete the assessment process and did, therefore, not participate in this study. The reason for dropout was failure to complete the questionnaire or alternation of symptoms of GD. Of the adolescents diagnosed with GD, 179 were about to start GAH treatment. One participant did not complete the questionnaire and was thus excluded.

Therefore, in this cross-sectional study, the three groups that were compared consisted of (1) adolescents who just started the assessment process (n = 272; mean age = 14.5 years; 116 assigned boys at birth and 156 assigned girls at birth), (2) adolescents diagnosed with GD who were on puberty suppression and about to start GAH treatment (n = 178; mean age = 16.8 years; 68 assigned boys at birth and 110 assigned girls at birth), and (3) cisgender adolescents recruited from the general population (n = 651; mean age = 15.4 years; 346 assigned boys at birth and 305 assigned girls at birth). Adolescents who just started the diagnostic procedure were assessed during their first sessions at the VUmc. Adolescents diagnosed with GD were assessed before the start of GAH. During both assessments, parents and children completed several questionnaires [20].

Data from the comparison group of cisgender adolescents from the general population were recruited by means of the help of different secondary schools in different provinces in the Netherlands. After consent of the parents, the adolescents completed a paper-pencil survey during regular class times.

Measures

Key demographic variables that were collected included the adolescents' birth-assigned gender, age, ethnicity, level of education, and parent's marital status. The demographic characteristics of the three groups are shown in Table 1.

The Dutch version of the YSR was used to assess internalizing and externalizing problem behavior, self-harm/suicidality, and poor peer relations [11]. The YSR consists of a total of 118 items, rated on a 0- to 2-point scale: "never," "sometimes," or "often," asking adolescents about their emotional and behavioral problems during the previous 6 months. The YSR is well established with regard to reliability and validity and has acceptable reliability and adequate criterion and construct validity [11]. The YSR has one item specifically pertaining to GD: "wish to be of the opposite sex" (Item 110). In line with previous studies, this item was scored as 0 to avoid increased associations with psychological challenges and GD [25]. For internalizing and

Table 1
General characteristics for transgender adolescents and the general population sample

Variable	General population (n = 651)	Transgender at referral (n = 272)	Transgender using puberty suppression (n = 178)
Age (in years)			
Mean (SD)	15.39 (1.36)	14.47 (2.18)	16.75 (1.24)
Ethnicity, n (%)			
Dutch	580 (89.1)	185 (68)	131 (73.6)
Non-Dutch	67 (10.3)	30 (11)	16 (9)
Unknown	4 (.6)	57 (21)	31 (17.4)
Level of education, n (%)			
VMBO	99 (15.2)	203 (74.3)	126 (70.6)
HAVO	274 (42.1)	29 (10.8)	29 (16.4)
VWO	278 (42.7)	40 (14.9)	23 (13)
Parent's marital status, n (%)			
Both parents	520 (79.9)	153 (56.3)	103 (57.9)
Other	129 (19.8)	116 (42.6)	74 (41.6)
Unknown	2 (.3)	3 (1.1)	1 (.6)

HAVO = higher general continued education; SD = standard deviation; VMBO = prevocational education; VWO = preparatory scholarly education.

externalizing problems, mean scale scores and clinical range percentages (>90th percentile in nonreferred samples) were calculated. To assess peer relations, and following the procedure as done in previous studies [25], a Peer Relations scale was created from three YSR items: "I don't get along with other kids" (Item 25), "I get teased a lot" (Item 38), and "I am not liked by other kids" (Item 48). Self-harm/suicidality was examined by two YSR items, namely, "I deliberately try to hurt or kill myself" (Item 18) and "I think about killing myself" (Item 91) as metrics of suicidality.

Analyses

First, multivariate general linear modeling (GLM) analysis was used to analyze between-group differences for internalizing, externalizing, suicidality, and peer relations together. Second, a multivariate GLM analysis with assigned gender at birth and a gender by group interaction as additional predictors was used to identify possible gender differences. These analyses were followed by univariate GLM analyses with Bonferroni correction to correct for multiple comparisons. Third, multivariate GLM analyses with group and assigned gender at birth as predictors and age, ethnicity, level of education, and parent's marital status as covariates were performed. Fourth, Cohen's *d* was used to measure the effect sizes between the groups [26]. Finally, clinical range percentages were calculated for internalizing and externalizing.

Results

Mean scores for internalizing, externalizing, suicidality, and peer relations

Table 2 shows the mean scores for internalizing, externalizing, suicidality, and peer relations per sample. On average, the scores of the transgender adolescents who have just been referred on internalizing, suicidality, and peer relations were higher than the scores of the transgender adolescents using puberty suppression and the cisgender comparison group, respectively. A multivariate GLM analysis with group as a fixed factor and the internalizing, externalizing, suicidality, and poor peer relations as the dependent measures showed an overall difference using Pillai's trace ($F = 707.61$, $df = 4$; $p < .001$). Subsequent analyses for the internalizing, externalizing, suicidality, and poor peer relations indicated that groups differed from each other on internalizing, suicidality, and poor peer relations (all three univariate p values $< .001$) but not on externalizing ($p = .709$).

Post hoc analyses

Post hoc analyses showed that transgender adolescents who just have been referred had significantly higher scores on internalizing, suicidality, and peer relations compared with the cisgender comparison group and transgender adolescents using puberty suppression. In addition, the transgender adolescents using puberty suppression scored significantly lower on internalizing problems but higher on peer relations compared with the comparison group. No differences were found between adolescents using puberty suppression and the comparison group on self-harm/suicidality (Table 2 provides all effect sizes).

Gender differences

When we added assigned gender at birth as a predictor, we confirmed the main effect of group ($F = 686.47$, $df = 4$; $p < .001$), and the previously mentioned univariate group effects for internalizing, suicidality, and peer relations were also confirmed (all $p < .001$). In addition, we found a main effect for gender ($F = 14.22$, $df = 4$; $p < .001$) and a group by gender interaction effect ($F = 9.52$, $df = 8$; $p < .001$). Subsequent univariate analysis found an effect for gender and an interaction effect on internalizing and peer relations. Within-group post hoc *t* tests revealed that the interaction arose on internalizing because in the cisgender comparison group, assigned girls at birth had higher mean scores than assigned boys at birth, whereas in both the transgender groups, no differences were found in internalizing scores between assigned girls and assigned boys at birth. On the peer relations, the interaction arose because in both transgender groups, assigned boys at birth had higher scores, whereas in the cisgender comparison group, assigned girls at birth had higher scores. Table 3 provides mean scores by assigned gender at birth. In addition, as for the demographic variables age, ethnicity, level of education, and parent's marital status statistical group differences were found, all analyses were repeated with these variables as covariates and showed similar findings.

Table 2
Mean scores on the Youth Self-Report for internalizing, externalizing, peer relations, and suicidality problems for transgender adolescents and the general population sample

Measures[c]	General population (n = 651)		Transgender at referral (n = 272)		Transgender using puberty suppression (n = 178)		Statistical analysis[a]		Effect sizes Cohen's d[b]		
	Mean	SD	Mean	SD	Mean	SD	F[d]	p values	GP versus T0[e]	GP versus T1[e]	T0 versus T1[e]
Internalizing	9.71	7.73	11.67	8.38	7.76	6.68	14.16	<.001	−.24	.30	.52
Externalizing	10.25	6.10	10.19	6.33	9.82	5.79	.34	.709	.01	.07	.06
Peer relations	.41	.81	1.08	1.31	.70	1.06	12.58	<.001	−.62	−.31	.32
Suicidality	.19	.60	.41	.78	.17	.52	44.26	<.001	−.32	.04	.36

SD = standard deviation.
[a] Additional post hoc analyses comparing the transgender group at referral, the transgender group using puberty blockers, and the general population sample, demonstrated that on internalizing, peer relations, and suicidality, the adolescents at referral had significantly higher scores than the adolescents using suppression and the adolescents from the general population. In addition, the adolescents using puberty suppression scored significantly lower on internalizing but significantly higher on peer relations compared with the general population sample.
[b] Effect sizes Cohen's *d*: .80 or higher is a large effect size, .50–.79 a medium effect size, .20–.49 small, and effect sizes <.20 are negligible [26].
[c] Internalizing problems = disturbances of emotions (e.g., depression, anxiety; absolute range: 0–62); externalizing problems = behavioral excess or disturbances of conduct (e.g., aggression, hyperactivity; absolute range: 0–64); peer relations = problems with relations with peers (absolute range: 0–6); suicidality = thinking about or attempting suicide (absolute range: 0–4) [11].
[d] *df* = 2.
[e] GP = sample of cisgender adolescents from the general population; T0 = sample of transgender adolescents referred to transgender affirmative care who did not receive any medical treatment; T1 = transgender adolescents receiving affirmative care consisting of puberty suppression.

Finally, four (internalizing, externalizing, poor peer relations, and self-harm/suicidality) between-group analyses for each assigned gender at birth were performed using Bonferroni correction. These analyses showed that of the four between group comparisons for assigned boys at birth at referral with cisgender boys, significant higher scores were found for internalizing ($d = -.66$), peer relations ($d = -.92$), and self-harm/suicidality ($d = -.63$) for the assigned boys who just started the assessment. Assigned girls at birth who just started the assessment only scored significantly higher than the cisgender girls on peer relations ($d = -.36$). The three other scales were not significantly different.

In the transgender adolescent sample using puberty suppression, the assigned boys at birth scored only higher on peer relations ($d = -.53$) but not on the three other scales compared with the cisgender boys. For the assigned girls at birth using puberty suppression compared with the cisgender girls, the scores on internalizing were found to be significantly lower ($d = .63$). No other significant differences were found.

Of the four scale comparisons for assigned boys at birth at referral with the assigned boys at birth using puberty suppression, significant lower scores were found for those using puberty suppression on internalizing ($d = .54$), peer relations ($d = .41$), and self-harm/suicidality ($d = .37$). For the comparisons between the assigned girls at referral with the assigned girls using puberty suppression, significant lower scores were found for those using puberty suppression on internalizing ($d = .50$) and self-harm/suicidality ($d = .35$).

Clinical range percentages

Of the transgender adolescents just referred to the clinic, 31.3% had clinical range scores for internalizing problems (assigned boys at birth: 35.3% and assigned girls at birth: 28.2%), and 17.3% (assigned boys at birth: 6.0% and assigned girls at birth: 25.6%) had those for externalizing compared with 22.9% (assigned boys at birth: 13.0% and assigned girls at birth: 34.1%) and 13.8% (assigned boys at birth: 11.3% and assigned girls at birth: 16.7%) of the cisgender comparison sample. For the transgender adolescents using puberty suppression, the percentages were 16.3% for internalizing (assigned boys at birth: 16.2% and assigned girls at birth: 16.4%) and 14.0% for externalizing (assigned boys at birth: 8.8% and assigned girls at birth:17.3%).

Table 3
Mean scores on the Youth Self-Report by gender assigned at birth for internalizing, externalizing, peer relations, and suicidality for transgender adolescents and the general population sample

Measures[a]	General population					Transgender at referral					Transgender using puberty suppression				
	Assigned boys (n = 346)		Assigned girls (n = 305)		Effect sizes Cohen's d[b]	Assigned boys (n = 116)		Assigned girls (n = 156)		Effect sizes Cohen's d[b]	Assigned boys (n = 68)		Assigned girls (n = 110)		Effect sizes Cohen's d[b]
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Internalizing	7.21	5.89	12.54	8.55	−.73	11.74	7.74	11.62	8.84	.01	7.79	6.76	7.74	6.66	.01
Externalizing	10.90	5.91	9.50	6.24	.23	9.69	5.52	10.56	6.86	−.14	10.32	6.26	9.51	5.31	.14
Peer relations	.38	.77	.45	.85	−.09	1.45	1.46	.81	1.11	.49	.91	1.18	.57	.95	.32
Suicidality	.12	.44	.27	.73	−.25	.39	.73	.42	.81	−.04	.16	.48	.18	.54	−.04

SD = standard deviation.
[a] Internalizing problems = disturbances of emotions (e.g., depression, anxiety; absolute range: 0–62); externalizing problems = behavioral excess or disturbances of conduct (e.g., aggression, hyperactivity; absolute range: 0–64); peer relations = problems with relations with peers (absolute range: 0–6); suicidality = thinking about or attempting suicide (absolute range: 0–4) [11].
[b] Within group effect size differences; Cohen's *d*: .80 or higher is a large effect size, .50–.79 a medium effect size, .20–.49 small, and <.20 is negligible [26].

Endorsement of self-harm/suicidality

In the sample of transgender adolescents at referral, 74 (27.2%) endorsed the metric of suicidality. In the sample of transgender adolescents using puberty suppression, this was n = 22 (12.4%). In the cisgender comparison group, the percentage was 11.9% (n = 77).

Discussion

Our study revealed that adolescents referred for gender-affirmative care have increased behavioral and emotional problems, especially internalizing problems, reported increased self-harm/suicidality, and poorer peer relations compared with cisgender adolescents from the general population. This finding, including the clinical range percentage for internalizing problems, is in line with the current literature that in general, transgender adolescents are at risk for mental health problems [3–8]. However, our study also showed that transgender adolescents receiving gender-affirmative care involving puberty suppressing treatment not only have less emotional and behavior problems than transgender adolescents who have just been referred to gender-affirmative care but also reported similar rates of mental health problems as their nonclinical cisgender peers on internalizing problems (with a lower clinical range percentage) and self-harm/suicidality but not on peer relation problems. This second finding of less internalizing problems and self-harm/suicidality is also in line with previous follow-up studies on transgender adolescents [22,23], providing further evidence that transgender adolescents could benefit from gender-affirmative care.

With regard to gender differences, we found that in both the transgender samples, assigned boys at birth scored higher on internalizing than assigned girls at birth, which is contrary to general population adolescents' mean scores but in line with previous findings [12]. For externalizing, and also in contrast with general population mean scores, assigned girls at birth who have just been referred but not assigned girls at birth on puberty suppression scored somewhat higher than assigned boys at birth with GD. These findings are partly in line with the hypothesis that the sex-typical pattern of more internalizing problems in girls and more externalizing problems in boys in the cisgender population might be inversed in transgender people [12]. This hypothesis deserves more research.

A clinical implication of these findings is the need for worldwide availability of gender-affirmative care, including puberty suppression for transgender adolescents to alleviate mental health problems of transgender adolescents. It should be acknowledged that the care provided in the present study also involved the offering of appropriate mental health care. Thus, transgender care providers need to actively screen for mental health problems and offer this care. In addition, clinicians should receive special training to provide this care, for example, to become more experienced in disentangling psychological problems stemming from bullying related to GD or having other origins. Our study found that transgender adolescents using puberty suppression consider their peer relations better than adolescents at referral but still reported more challenges with peers than the cisgender adolescents. As it has been established in different studies that stigmatization and peer victimization seem to be common for transgender people [27], and psychological problems are correlated with peer support [28], clinicians should also take the importance of peer support during the transition into account.

Although the treatment with puberty suppression for adolescents with GD is now available in an increasing number of countries, the small amount of scientific evidence of the medical safety and efficacy and the psychological efficacy comes from a limited number of studies, mostly performed in the Netherlands [22]. It should, therefore, additionally be stressed that the gender-affirmative treatment described in the Dutch protocol is a highly protocolled treatment with regard to eligibility criteria and psychological support, including affirmative psychoeducation of GD for youth and parents or caregivers and the continued discussion of psychosexual development with themes such as school and friendships but also dating and romantic relationships [29]. This does imply that the findings of our study might not apply to all transgender adolescents, as, for example, in other health care systems, psychological support is incomparable to the psychological support received following the Dutch protocol [29]. More research is needed to see whether our findings of effective affirmative care involving puberty suppression improving the mental health of transgender adolescents is generalizable to other countries.

In addition, the results of this study should be seen in the light of three limitations. First, this study did not make use of a random nonclinical national probability sample. However, although the mean scores in this study of the general population comparison sample were consistent with the findings of the YSR standardization sample used in other studies in the Dutch population [11,22], the generalizability of our findings might not be corroborated. Second, although the YSR is a well-validated questionnaire for behavioral and emotional challenges [11], it cannot be equated with a diagnosis of any mental health condition made by clinical assessment. Third and most important, although those individuals with and without a GD diagnosis after assessment did not differ in internalizing, externalizing, peer relations, and suicidality scores at baseline in the group that has just been referred to the clinic, the cross-sectional design of this study with different participants in the groups before and after puberty suppression may potentially limit the results with participants being different on characteristics not measured and controlled for. The present study can, therefore, not provide evidence about the direct benefits of puberty suppression over time and long-term mental health outcomes. Conclusions about long-term benefits of puberty suppression should thus be made with extreme caution needing prospective long-term follow-up studies with a repeated measure design with individuals being followed over time to confirm the current findings.

Future studies should, therefore, not only investigate the benefit of gender-affirmative care in other health care settings together with a matched nonclinical general population sample but should also make comparisons to transgender adolescents receiving GAH treatment and gender-affirming surgery to investigate the impact of these treatments on long-term mental health. As this study did not ask specifically for the increasingly recognized nonbinary identities [30], future studies should also cover if nonbinary transgender adolescents might equally benefit from this type of gender-affirmative care. Despite the previously mentioned limitations, this first study comparing a group of transgender adolescents just referred for gender-affirmative care, a group of transgender adolescents receiving treatment with puberty suppression, and a group of cisgender adolescents

from the general population showed that when affirmative care involving puberty suppression is provided, transgender adolescents may have comparable mental health levels to their cisgender peers. This type of gender-affirmative care seems thus extremely important for this group.

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RESEARCH ARTICLE

Short-term outcomes of pubertal suppression in a selected cohort of 12 to 15 year old young people with persistent gender dysphoria in the UK

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Abstract

Background

In adolescents with severe and persistent gender dysphoria (GD), gonadotropin releasing hormone analogues (GnRHa) are used from early/middle puberty with the aim of delaying irreversible and unwanted pubertal body changes. Evidence of outcomes of pubertal suppression in GD is limited.

Methods

We undertook an uncontrolled prospective observational study of GnRHa as monotherapy in 44 12–15 year olds with persistent and severe GD. Prespecified analyses were limited to key outcomes: bone mineral content (BMC) and bone mineral density (BMD); Child Behaviour CheckList (CBCL) total t-score; Youth Self-Report (YSR) total t-score; CBCL and YSR self-harm indices; at 12, 24 and 36 months. Semistructured interviews were conducted on GnRHa.

Results

44 patients had data at 12 months follow-up, 24 at 24 months and 14 at 36 months. All had normal karyotype and endocrinology consistent with birth-registered sex. All achieved suppression of gonadotropins by 6 months. At the end of the study one ceased GnRHa and 43 (98%) elected to start cross-sex hormones.

There was no change from baseline in spine BMD at 12 months nor in hip BMD at 24 and 36 months, but at 24 months lumbar spine BMC and BMD were higher than at baseline (BMC +6.0 (95% CI: 4.0, 7.9); BMD +0.05 (0.03, 0.07)). There were no changes from baseline to 12 or 24 months in CBCL or YSR total t-scores or for CBCL or YSR self-harm indices, nor for CBCL total t-score or self-harm index at 36 months. Most participants reported

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positive or a mixture of positive and negative life changes on GnRHa. Anticipated adverse events were common.

Conclusions

Overall patient experience of changes on GnRHa treatment was positive. We identified no changes in psychological function. Changes in BMD were consistent with suppression of growth. Larger and longer-term prospective studies using a range of designs are needed to more fully quantify the benefits and harms of pubertal suppression in GD.

Introduction

Gender dysphoria (GD) describes the experience of incongruence between an individual's experienced gender and the sex they were assigned at birth. GD [1] in children and young people, also known as Gender Incongruence [2] and previously known as Gender Identity Disorder (GID), is associated with considerable distress or impairment in social, school or other important areas of functioning [3,4]. Interventions include psychosocial support, therapy and medical or surgical interventions to align the body with the identified gender [3,5]. Terminology in this field can be challenging [6]. Here we use birth-registered sex to refer to the sex assigned at birth by clinicians based upon external genitalia [6]. Gender identity refers to a young person's personal sense of their gender. We use the terms 'continuation' and 'discontinuation' to refer to GD across childhood and adolescence.

GD in adolescence is highly likely to continue into adult life where gender dysphoria persists after the onset of puberty [3]. Those with earlier onset or more intense GD and those in whom the development of secondary sexual characteristics in puberty is associated with increasing gender dysphoria or psychological distress are more likely to have persistent GD [3,7]. In adolescents with severe and persistent GD, international [8] and national [9–11] guidelines recommend the use of treatments to suppress the rise in sex hormones (oestradiol or testosterone) in young people during puberty. Gonadotropin releasing hormone analogues (GnRHa) are synthetic peptides that work by stimulating gonadotropin release in a tonic fashion which desensitises the gonadotropin receptors, resulting in reversible suppression of sex hormone production.

In GD, GnRHa can be used from the early/middle stages of puberty with the aim of delaying irreversible and unwanted pubertal body changes and giving young people the opportunity to explore their gender identity during a period when puberty is not advancing [3]. This period also allows clinicians more time to assess the stability of young people's gender identity [6]. Despite this treatment being given in mid-puberty it is also called early puberty suppression, where 'early' refers to earlier than the historic practice of suppression after completion of puberty.

Pubertal suppression is currently practised in the majority of international centres across Europe, the Americas and Australasia, as evidenced by a recently published survey of 25 international centres by the European Society of Paediatric Endocrinology (ESPE) [12]. Pubertal suppression with GnRHa as monotherapy is a time-limited strategy, due to the potential for side effects with long-term use. In the UK, for those commencing under age 15 years, use of GnRHa alone ceases after 16 years when young people face a decision to return to the sex hormones produced by their body or begin cross-sex hormones [5]. There are limited data on the outcomes of pubertal suppression in the treatment of young people with GD [3,13]. A recent

systematic review included data on the physical and mental health outcomes of pubertal suppression using GnRHa in over 500 young people [4]. Longer-term follow-up data on pubertal suppression in GD are limited to individuals from four cohorts [14–19].

In 2011 a study was begun to evaluate the proximal outcomes of mid-pubertal suppression using GnRHa in young people with persistent GD (see http://gids.nhs.uk/our-early-intervention-study). Use in the UK began after mid-pubertal suppression had been incorporated into international guidelines [20] and had become available in the USA [21,22], the Netherlands [15], Australia [23] and a number of European countries. The Gender Identity Development Service (GIDS) at the Tavistock and Portman NHS Foundation Trust, London, is a national service for children and young people with GD, drawing from England, Wales and Ireland. Mid-pubertal suppression was offered by the GIDS from 2011 initially only within an ethically approved uncontrolled observational research study with prospective data collection, where all participants received GnRHa. We anticipated that we would recruit 10–15 young people per year for 3 years and follow them up to the end of monotherapy with GnRHa. At the time, a randomised controlled study was not considered feasible due to very small numbers and inability to retain participants in the control arm, as the control treatment would have resulted in progression into near complete puberty and an increasing number of UK families were accessing mid-pubertal suppression internationally. Allocation blinding was also not considered feasible in young people using a product requiring monthly injections.

Here we describe the short-term outcomes of 44 young people with GD from this research cohort, recruited aged 12–15 years and followed to the end of GnRHa monotherapy after age 16 years. This paper describes their medical, psychological and social outcomes during the GnRHa treatment pathway up to the point of decisions about whether or not to undertake further physical treatment. The aims of the study as defined at inception in 2011 were:

1. To evaluate the benefits and risks for physical and mental health and wellbeing of mid-pubertal suppression in adolescents with GD
2. To add to the evidence base regarding the efficacy of GnRHa treatment for young people with GD
3. To evaluate continuation and discontinuation of GD and the continued wish for gender reassignment within this group.

Methods

We undertook an uncontrolled prospective observational study of GnRHa monotherapy in a highly selected group of young people with persistent and severe GD.

Participants

The cohort consisted of 44 sequentially eligible young people, aged 12 to 15 years, who were recruited between April 2011 and April 2014 and who commenced GnRHa treatment between June 2011 and April 2015. They were all recruited from patients referred to the GIDS.

Eligibility criteria were chosen to match those used for a Netherlands cohort [24], namely that the young person:

A. is aged 12–15 years

B. Psychological criteria

1. has been seen by the GIDS for at least 6 months and attended at least 4 interviews for assessment and therapeutic exploration of their gender identity development.

2. psychological stability sufficient to withstand the stresses of medical treatment for GID.
3. fulfils the following criteria relating to GID:
   a. Throughout childhood (defined as over 5 years) the adolescent has demonstrated an intense pattern of cross-gendered behaviours and cross-gender identity.
   b. The adolescent has gender dysphoria that is significantly increased with the onset of puberty. Following assessment the clinician(s) working with the young person deem that there is a high likelihood of the young person experiencing severe psychological distress consequent on experiencing full pubertal development before pubertal suppression is implemented.
4. The young person and their parents/guardians are actively requesting pubertal suppression.
5. is able to give informed consent.

C. Physical/medical criteria

1. is in established puberty:
   - For birth-registered males Tanner (genital and pubic hair (PH)) stage 3 and above.
   - For birth-registered females Tanner (breast and PH) stage 2 and above.
   The rationale for the sex difference was that the pubertal growth spurt which early intervention aims to avoid occurs typically two years earlier in females (Tanner stage 2–3) than in males (Tanner stage 3–4), thus earlier intervention is required in females.
2. has normal endocrine function and karyotype consistent with birth registered sex.

Note that the presence of mildly elevated androgens in birth registered females consistent with polycystic ovarian syndrome is not an exclusion criterion.

Exclusion criteria:

1. Inability to participate with full investigatory protocol e.g. needle phobia, failure to attend for tests and scans.
2. Body mass index (BMI) <2nd centile for age and birth-registered sex [20].
3. Serious psychiatric conditions (e.g. psychosis, bipolar condition, anorexia nervosa, severe body-dysmorphic disorder unrelated to GD).
4. Inability to give informed consent according to the Fraser/Gillick guidelines.
5. Low spine or hip bone mineral density (BMD) on DXA scan: more than 2 SD below expected BMD for age and birth-registered sex. In exceptional circumstances a low BMD was acceptable if:
   i. it was felt to be clinically appropriate by the treating clinicians, who felt that on the balance of risks, pubertal suppression was justified despite the later risk of osteoporosis
   ii. the young person and parents understood the risks of GnRHa treatment for bone density (i.e. potential risks of later osteoporosis)
   iii. The young person and parents consented to more frequent monitoring of BMD (repeat DXA scans 6 months after starting GnRHa and yearly thereafter while on GnRHa) despite the small DXA radiation dose

iv. The young person and parents consented to stopping treatment if raw BMD fell whilst on GnRHa.

The treatment

The treatment under study was suppression of puberty using the GnRHa *triptorelin* together with psychosocial support and therapy, from study entry until the end of the GnRHa monotherapy pathway at age 16 years or older. GnRHa monotherapy ceased when young people either started cross-sex hormones (and continued on GnRHa) or stopped GnRHa. Treatment duration was therefore from 1 to 4 or 5 years depending on age at study entry. Consenting young people were given triptorelin 3.75mg by intramuscular injection every 28 days during the treatment period. Two participants who found monthly injections difficult were moved to a ten-weekly preparation of 11.25mg of triptorelin. The aim of treatment was to suppress gonadotropins and sex hormones to near pre-pubertal levels [13]. Continued regular attendance for psychological support and therapy throughout the study was a precondition of GnRHa prescription. In addition local psychological services provided support for co-occuring difficulties for participants as required.

Procedures and pathway

All young people and families attending the GIDS during the study period were provided with an information leaflet about research underway within the unit. Those wishing to find out more about the study discussed it with their GIDS clinicians and those deemed likely to be eligible were given detailed written study information. Those wanting to participate were invited to a medical clinic at UCLH for an initial discussion. At the first medical clinic, young people and families were seen by a senior paediatric endocrinology clinician together with a senior GIDS clinician, who discussed with the family the then current state of knowledge and rationale for treatment, eligibility criteria and potential risks and benefits of participation. Risks included the anticipated side-effects of GnRHa treatment including symptoms resulting from the withdrawal of sex steroids (headaches, hot flushes), fatigue, loss of libido and low mood, the potential that treatment could influence the continuation of their GD and the potential for unknown risks. It was emphasised that young people needed to continue with both regular medical and psychosocial follow-up during the study and that treatment would cease if they did not comply with the treatment or monitoring requirements. A full medical history was elicited and the clinicians also reviewed a summary of the psychological history and assessment from the GIDS. In this visit information sheets were re-provided if families had lost them or forgotten details of the study. If young people and families remained interested in participation, medical investigations were organised and families were invited for a repeat discussion and a formal evaluation of eligibility at a second medical clinic visit approximately 3 months later. Families were asked to think about the issues raised in the meeting and to discuss with their GIDS clinicians if necessary, in order to discuss further at the second visit.

At the second medical clinic visit, the same clinicians repeated the discussion of risks and benefits and explored understanding with the young person and family. A chaperoned medical examination was undertaken including pubertal assessment and the results of medical investigations were reviewed. Endocrine and GIDS clinicians jointly reviewed eligibility and offered participation in the study to those deemed eligible.

The implications of treatment for fertility were discussed at the first and second medical visits and all young people were urged to consider storing gametes before starting GnRHa. Access to storage depended on regional availability within the NHS. Note that counselling on fertility

continued across the study, and clinicians periodically checked with young people who had decided against storage whether they wished to revisit their decision.

Informed consent was obtained in writing from both the young person and a parent or carer holding parental responsibility. The ability of the young person and parents to give informed consent was assessed jointly by the senior adolescent endocrine and GIDS clinicians, informed by written notes from the GIDS team. The consent forms were read with the young person and the parent by the clinicians to be sure they fully understood the information on the forms before signing.

48 young people and families attended the medical clinics for discussion of participation in the trial, of whom 44 wished to participate. Eight young people (7 birth assigned males) were not eligible for participation at the second medical visit as they were not yet sufficiently advanced in puberty. They were followed up every 3–6 months and entered the study subsequently when sufficiently advanced in puberty (median waiting time 7 months).

The date of signing the consent form was taken as the start of study treatment, although it frequently took one to three months for GnRHa treatment to start due to administrative requirements. Participants were followed up in the endocrine clinic, 3–6 monthly in the first 18 months and 12-monthly thereafter, till the end of the treatment pathway, defined as the date on or after the 16th birthday when a decision was made to either cease GnRHa or start cross-sex hormones. The final participant completed the pathway in February 2019.

Outcomes

The following data were collected:

A. Baseline explanatory variables

1. Sex and gender: Young people were classified by their sex assigned at birth (birth-registered sex) and self-identified gender.
2. Ethnicity: Ethnicity was obtained from clinic records. For analysis, ethnicity was grouped as white, South Asian, black or mixed.
3. Puberty: Pubertal status at baseline was classified using information on genital/breast and pubic hair Tanner stages as appropriate. This was summarized into a single pubertal stage, with the breast/genital stage taking precedence if there was discrepancy between breast/genital and public hair stage.
4. Clinical data: These consisted of a) identification of normal phenotype on physical examination for birth-registered sex; b) venepuncture assessment of endocrinology (gonadotropins, prolactin, oestrogen or testosterone, adrenal androgens, thyroid function; and a short synacthen test in birth-registered females only), karyotype, full blood count, renal and liver function, calcium and vitamin D; and c) imaging including wrist bone age and (in birth-registered females only) pelvic ultrasound scan. Medical assessment at baseline and follow-up was consistent with Endocrine Society guidelines [8,20].

B. Study outcomes

Study outcomes concerned domains including response to treatment, bone health, safety indicators and adverse events, psychological function; participant experience and satisfaction; and decisions regarding treatment following GnRHa. Outcome data were collected at routine clinic visits to GIDS or medical clinics at UCLH and timings therefore varied. For the purposes of these analyses, data for each participant were assigned to baseline (before treatment) and to the closest of the following outcome periods: 12, 24, 36 and 48 months on treatment. For safety and response to pubertal suppression outcomes, data were also examined at 6 months.

1. Response to pubertal suppression

Gonadotropins (LH, FSH), testosterone (in birth-registered males) and oestrogen (birth-registered females) were measured after venepuncture. Height, weight and blood pressure were recorded by trained clinic staff. BMI z-score for age and birth-registered sex was calculated [25]. Menarcheal status and presence/absence of menstrual periods was obtained by report from birth-registered females.

2. Bone health

Bone mineral content (BMC) and bone mineral density (BMD) in the lumbar (L1 to L4) spine and hip (total hip) were measured by dual energy X-ray absorptiometry (DEXA) scans using a Hologic Discovery QDR series model 010–1549 (Hologic Inc, Bedford, MA, USA). BMD z-scores for age and birth-registered sex appropriate to this machine were calculated [26]. BMD z-scores for spine and hip were further adjusted for height (height-adjusted z-scores) using published formulae [27].

3. Safety indicators and adverse events

Blood samples were collected by venepuncture for liver and renal function, full blood count, calcium and vitamin D, prolactin, adrenal androgens and thyroid function. Participants were routinely questioned about adverse events at medical clinic visits, including anticipated events such as headaches, hot flushes or fatigue plus any other unanticipated events.

4. Psychological function

Psychological outcomes included a clinical outcome routinely collected after GIDS appointments and a range of outcomes assessed using questionnaires. A standardised set of psychological questionnaires used in the GIDS clinic was completed at the time young people were deemed potentially eligible and referred to the medical clinic. Questionnaires were completed at home by the young person and parent between GIDS clinical meetings, and a research assistant followed up families to ensure their completion. Questionnaires were repeated approximately every 12 months on treatment.

i. General psychological functioning

The Child Behaviour Checklist (CBCL) (parent report) and Youth Self Report (YSR) (self-report) are general measures of psychological functioning and part of the Achenbach System of Empirically Based Assessment (ASEBA; www.aseba.org). The CBCL consists of 113 questions and is validated for children aged 6–18 years in international population samples [28]. The YSR consists of 112 questions and is validated in international populations of young people aged 11–18 years [29]. Questions in both are scored on a three-point Likert scale (0 = absent, 1 = occurs sometimes, 2 = occurs often), with the time frame for item responses being the past six months. Scoring for both instruments provides a total problems score, an internalizing problems score (items which assess anxious/depressed, withdrawn-depressed, and somatic complaints) and an externalizing score (focusing on rule-breaking and aggressive behaviours). Each questionnaire was scored with Assessment Data Manager Software using ASEBA standard norms and t-scores were generated based on reference data for birth-registered sex and broad age-ranges (here 12–18 years). Higher scores indicate greater morbidity. To account for normative change within our age-range, we used international reference data [29] to transform YSR raw scores into z-scores for year of age. As reference data from the UK were not available, reference data from both Australia and the Netherlands were used.

ii. Self-harm index

Self-harm actions and thoughts were assessed through two questions in each of the CBCL (parent report) and YSR (self-report): Item 18 (I deliberately try to hurt or kill myself) and Item 91 (I think about killing myself). Possible responses for each question were 0 = not true, 1 = somewhat or sometimes true, or 2 = very true or often true. We followed previous studies in calculating a self-harm index score to avoid multiple statistical comparisons across

correlated categorical-response variables. The index was calculated as the sum of the two items in each scale to create an index from 0 to 4 for each of the CBCL and YSR [30–32], a higher score indicating greater self-harm thoughts and behaviour.

iii. Health related quality of life (HRQoL)

This was assessed through separate young person and parent Kidscreen-52 questionnaires, each consisting of 52 items which assess HRQoL across ten dimensions: physical well-being; psychological well-being; moods and emotions; self-perception; autonomy; relations with parents and home life; social support and peers; school environment; social acceptance (bullying); and financial resources. All items use five-point Likert-style scales to assess either the frequency (never-seldom-sometimes-often-always) of certain behaviours/feelings or the intensity of an attitude (not at all–slightly-moderately-very-extremely). The measure was developed for young people aged 8–18 years, with the recall period of one week. The questionnaires provide scores in the form of continuous t-scores for the ten subscales derived from a multinational European sample [33]. Lower scores indicate lower HRQoL, i.e. greater morbidity.

iv. Body image

The Body Image Scale (BIS) is a self-report measure of 30 items used to assess body image satisfaction or dissatisfaction validated for age 12+. The instrument considers 30 body features which the respondent is asked to rate in terms of satisfaction on a five-point scale (1 = very satisfied, 2 = satisfied, 3 = neutral, 4 = dissatisfied, and 5 = very dissatisfied). The BIS provides a total score in the form of a continuous score for the total scale as well as for three subscales assessing primary sexual characteristics, secondary sexual characteristics and 'neutral' characteristics (i.e. non-sexual characteristics, e.g. nose) [34]. Higher scores represent higher degrees of body dissatisfaction.

v. Gender dysphoria

The Utrecht Gender Dysphoria Scale (UGDS) is a self-report measure used to assess the intensity of GD validated for age 12+. It comprises of 12 statements with agreement on a five-point scale (1 = agree completely, 2 = agree somewhat, 3 = neutral, 4 = disagree somewhat, and 5 = disagree completely). There are separate versions for birth-registered males and females. Items are summed to give a single total score, with higher scores indicating greater GD.

vi. Clinical outcomes

The Children's Global Assessment Scale (CGAS) is a rating of functioning in children and young people aged 6–17 years, extensively used as a routine clinical measure in child and adolescent mental health services in the UK. Treating clinicians assign young people a single score between 1 and 100, based on a clinician's assessment of a range of aspects related to a child's psychological and social functioning, with the time period being the previous month. Higher scores indicate better functioning, with categories ranging from 'extremely impaired' (1–10) to 'doing very well' (91–100) [35].

5. Participant experience and satisfaction with GnRHa

Young people were invited to participate in semi-structured qualitative interviews at 6–15 months and 15–24 months after starting GnRHa. Interviews were conducted in person or by telephone with a research assistant. If young people were unavailable, questions were posted to be completed and returned. The interview consisted of 12 questions related to changes young people had experienced in ten domains since starting on GnRHa: life overall, memory, focus, sense of direction, mood, energy levels, relationships with friends, relationships with family, gender role and sexuality. For each domain, young people were asked first about the general direction of change in that domain (whether changes were positive, neutral, negative or mixed positive and negative) and then asked for examples of changes experienced and why they assigned the chosen change rating. At the end of the interview two further questions were asked about change in any other experiences (i.e. allowing open ended responses) and whether

young people wished to continue on GnRHa treatment. Note there was no interview conducted before young people started GnRHa. Interviews were recorded in contemporaneous written notes by the researcher. The questionnaire is provided in the S1 Appendix.

6. Further treatment decisions

Decisions made at the end of the GnRHa pathway were recorded in terms of which if any further treatment for GD young people chose.

Note that other measures of gender dysphoria (Gender Identity Interview; Recalled Childhood Gender Identity Scale) were specified in our original protocol, however they were discontinued during the study as: a) they were historical instruments with poor construct validity and the binary references to male and female roles were challenging for some participants; and b) repeated questioning about gender dysphoria resulted in some distress to respondents. Our protocol had originally included the ASEBA Teacher Report Form (TRF), however we were unable to obtain data from teachers so this outcome was dropped. The Social Responsiveness Scale (SRS) was a baseline only assessment of autistic traits; these data will be analysed in the future.

Analysis plan

Analyses were conducted according to the Statistical Analysis and Dissemination Plan, lodged with the ethics committee that approved the study before the analysis started (see S2 Appendix: Statistical Analysis Plan). The analysis plan was designed to report data on all outcomes but to minimise the likelihood of chance findings due to the large number of outcomes and small sample size. Sample sizes necessarily varied across follow-up as young people were recruited at different ages (12–15 years) but left the study soon after their 16th birthday. All 44 participants had data at 12 months follow-up. As participants necessarily left the study soon after their 16th birthday, numbers reduced after 12 months follow-up as participants could no longer remain in the study. Note this does not represent drop-out. There were 24 left at 24 months, 14 at 36 months and 4 at 48 months. In view of this, outcome reporting was restricted to change from baseline to 12, 24 and 36 months. We made no attempt to account for missing data due to the small sample size and the likelihood of the data missing not at random.

We restricted analyses to primarily descriptive statistics, with formal statistical testing of change across the study restricted to six pre-specified outcomes, i.e.:

1. Overall psychological functioning
   a. parent report: CBCL total t-score
   b. young person self-report: YSR total t-score
2. Self-harm index
   a. parent report: CBCL self-harm index
   b. young person self-report: YSR self-harm index
3. Bone health
   a. BMD and BMC for lumbar spine
   b. BMD and BMC for hip

Assessment of change was through paired t-tests for normally distributed data and the Wilcoxon matched-pairs sign-rank test for non-normal data. The number of formal statistical tests conducted in the study was 16; with overall significance at $p = 0.05$ and a Bonferroni correction, the appropriate threshold for statistical significance is about $p = 0.003$.

In our results and conclusions we refer to change in outcomes only for those that were formally tested. Reporting for other continuous outcomes was restricted to mean and 95% confidence intervals (95%CI) or median and interquartile range (IQR). For categorical outcomes, simple proportions were reported. We reported laboratory tests as normal or abnormal based upon laboratory reference data for age, with the exception of gonadotropins. We did not report data where the sample size was less than 8.

Analysis of potential predictors of outcome was confined a priori to two factors, birth-registered sex and pubertal stage at baseline. Three pre-specified continuous outcomes were examined at 12 months, namely:

1. BMD for lumbar spine
2. YSR total t-score
3. CGAS score

Associations were examined using linear regression of follow-up score on baseline score, adding each baseline factor separately to the model and considering the interaction of predictor with baseline score. All analyses were conducted using Stata 16 (Statacorp, College Station TX).

Responses to the semi-structured interview questionnaires were analysed simply for thematic content in terms of the direction and amount of change that young people experienced in each domain. This involved coding responses about experiences since starting GnRHa into categories; i.e. either positive/improving, negative/deteriorating, both positive and negative, no change or not known. The question on change in sexuality was coded as yes change, no change or not known. Wishes to continue with GnRHa were coded as yes, no or don't know.

To compare our findings with the literature, we drew upon recent reviews [3,4,6,13] and updated a recent review [4] from 1 June 2017 to 31 December 2019 using the same search terms in Medline (see S1 Appendix).

Ethics

Ethical approval for the study was obtained from the National Research Ethics Service (NRES: reference 10/H0713/79) in February 2011. Study consent allowed the use of routinely collected clinical data (medical and psychological) as part of clinical treatment for the study. Study procedures including consent were reviewed by the UK Health Research Authority.

Data sharing. These are highly sensitive data from a small group of vulnerable young people treated in a single service and the risk of identification and disclosure is high. Research ethics permissions at the time the study was undertaken did not include permission to share data. After discussions with the Health Research Authority, UK, an anonymised dataset modified to remove sensitive data and minimise disclosure risk of personal information has been deposited with the UK Data Service.

Results

Participants received psychosocial assessment and support within the GIDS before entering the study for a median of 2.0 years (IQR 1.4 to 3.2; range 0.7 to 6.6). The median time between first medical assessment at UCLH and starting treatment was 3.9 months (IQR 3.0 to 8.4; range 1.6 to 25.7). Median time in the study was 31 months (IQR 20 to 42, range 12 to 59).

Baseline characteristics of the participants by birth-registered sex are shown in Table 1. Median age at consent was 13.6 years (IQR 12.8 to 14.6, range 12.0 to 15.3). A total of 25 (57%) were birth-registered as male and 19 (43%) as female. At study entry, birth-registered males

Table 1. Participant characteristics at baseline.

		Total sample	Birth-registered sex	
		n = 44	male	female
			n = 25	n = 19
Age at consent (years)	Median (IQR)	13.6 (12.8, 14.6)	13.4 (12.7, 14.1)	13.9 (13.5, 14.7)
Ethnic group n (%)	white	39 (89)	24 (96)	15 (79)
	South Asian	1 (2)	1 (4)	0
	black	2 (5)	0	2 (11)
	Mixed ethnicity	2 (5)	0	2 (11)
Pubertal status n (%)	Stage 2	0	0	0
	Stage 3	19 (43)	17 (68)	2 (10)
	Stage 4	16 (36)	5 (20)	11 (58)
	Stage 5	9 (21)	3 (12)	6 (32)
Menarcheal status n (%)	Premenarcheal	-	-	4 (21)
	Post-menarcheal	-	-	15 (79)
Time in study (months)	Median (IQR)	31 (20, 42)	37 (24, 43)	29 (17, 36)
Age at end of pathway (years)	Median (IQR)	16.1 (16.0, 16.4)	16.1 (16.0, 16.5)	16.1 (16.0, 16.3)

At baseline, all participants had normal endocrinology, karyotype, imaging and clinical phenotype on physical examination for birth-registered sex and normal full blood count and liver and renal function. No participants had evidence of disorders of sexual differentiation. Eight participants (18%) had vitamin D insufficiency at baseline and were given vitamin D supplements.

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were predominantly in stage 3 puberty (68%) whilst birth-registered females were predominantly in stages 4 (58%) or 5 (32%) with 79% (15/19) post-menarcheal. 89% of participants were of white ethnicity. Birth-registered females were on average 6 months older than birth-registered males at study entry.

Response to treatment

All participants achieved adequate suppression of gonadotropins and sex hormones by 6 months (mean LH 0.5IU/L; mean FSH 1.4IU/L) and maintained it throughout the study (see Table 2). Liver function, basic haematology and biochemistry were normal in all participants at 3–6 months. All post-menarcheal birth-registered females reported amenorrhoea in the 3 months after starting GnRHa treatment and remained so throughout treatment. No participants reported progression in pubertal development. Height and weight were normal at baseline. Height growth continued through the study but more slowly than expected for age, thus

Table 2. Growth and gonadotropin levels at baseline, 12, 24 and 36 months.

Growth		Baseline		12 months		24 months		36 months	
		n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)
Height	z-score	44	0.4 (0.1, 0.7)	44	0.2 (-0.1, 0.4)	24	0.0 (-0.4, 0.4)	14	0.0 (-0.5, 0.5)
Weight	z-score	44	0.8 (0.4, 1.3)	44	0.8 (0.3, 1.3)	24	0.6 (-0.1 1.3)	14	1.0 (0.1, 1.9)
BMI	z-score	44	0.7 (0.2, 1.1)	44	0.7 (0.2, 1.2)	24	0.6 (-0.1, 1.3)	14	1.1 (0.3, 1.9)
Gonadotropins									
LH	IU/L	42*	4.2 (2.8, 5.6)	44	0.60 (0.42, 0.68)	17	0.40 (0.22, 0.60)	7	0.30 (0.14, 0.46)
FSH	IU/L	42*	3.9 (3.2, 4.5)	44	1.3 (1.0, 1.7)	17	1.0 (0.6, 1.5)	7	1.4 (0.7, 2.2)

*In two participants data recorded as normal at baseline were not available.

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height z-score fell over time (Table 2). Weight and BMI z-scores were stable from baseline to 24 months but increased at 36 months.

Three participants had brief periods off GnRHa prior to their 16th birthday. In one, treatment was withdrawn by clinicians due to non-attendance at clinics and restarted 4 months later. Another requested a period off GnRHa to think further about treatment in view of other things happening in their life; they restarted 4 months later. A third, birth-registered male, stopped GnRHa for 9 months to attempt to store sperm, contrary to their earlier decision not to, and restarted afterwards.

Median age at the end of the GnRHa pathway was 16.1 years (Table 1). A quarter of participants made their decision more than six months later, either because they wished to delay due to school exams or other events or because clinicians felt they were not yet ready to make the decision. One young person decided to stop GnRHa and not start cross-sex hormones, due to continued uncertainty and some concerns about side-effects of cross-sex hormones. The remaining 43 (98%) elected to start cross-sex hormones.

Bone mineral density. BMD was available on 44 participants at baseline, 43 at 12 months, 24 at 24 months and 12 at 36 months (Table 3). Numbers were lower for hip than for spine as some hip scans were not done for technical reasons. The table shows mean values at baseline and 12, 24 and 36 months, along with mean baseline values corresponding to the paired samples at each time point. There was no change from baseline in spine or hip at 12 months nor in hip at 24 and 36 months, but at 24 months lumbar spine BMC and BMD were higher than at baseline, as was lumbar BMC at 36 months. Lumbar and hip BMD age-adjusted z-scores were in the normal range at baseline but point-estimates fell at 12 and 24 months but not at 36 months. Point-estimates for height-adjusted z-scores for lumbar and hip BMD also fell at 12 and 24 months but not at 36 months.

Psychological outcomes. For the standardised questionnaires, baseline assessments were conducted at a median of 0.5 (IQR 0.4, 0.8) years before starting treatment, and were available for all 44 participants by self-report and 43 by parental report. Data on the CBCL, YSR, Kidscreen-52, BIS and CGAS were normally distributed whilst those for UGDS and the CBCL and YSR self-harm indices were skewed.

The first psychological follow-up was at a median of 13 (IQR 12, 14) months after start of treatment, with ASEBA data available for 41 participants (parent and self-report). ASEBA data at 24 months (median 25 (21, 28)) were available on 20 young people by parent report and 15 by self-report, and at 36 months (median 36 (29, 39)) on 11 by parent report and 6 by self-report.

Formal testing was undertaken only for key ASEBA outcomes (Table 4). For the CBCL total t-scores, there was no change from baseline to 12, 24 or 36 months. Similarly for the YSR total t-score, there was no change from baseline to 12 or 24 months; YSR data at 36 months (n = 6) were not analysed. There were no significant changes in parent-report CBCL self-harm index scores from baseline to 12, 24 or 36 months, nor for self-report YSR self-harm index scores.

Other psychological outcomes are described in Table 5. Point-estimates of scores on the Kidscreen-52, BIS, UGDS and CGAS showed little change over time."

The pre-specified outcomes of BMD at lumbar spine, YSR total t-score and CGAS score at 12 months, adjusted separately for birth-registered sex and baseline pubertal status, along with the baseline level of the outcome, are shown in Table 6. None of the outcomes were associated with birth-registered sex or pubertal status, and there were no important interactions.

Participant experience, satisfaction and side effects. 41 participants completed interviews at 6–15 months (median 9) and 29 at 15–24 months (median 21); 3 missed both. Fig 1 shows proportions with positive or negative changes for life overall, mood and friendships, with summary data for all questions shown in S1 Appendix (S1 and S2 Tables).

Table 3. Bone mineral density outcomes at baseline, 12, 24 and 36 months.

		Baseline		12 months					24 months				
				Baseline for those followed up	Follow-up	Change	p		Baseline for those followed up	Follow-up	Change	p	
		n	Mean (95% CI)	n	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)		n	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	
Lumbar	BMC	44	39.5 (35.9, 43.1)	42	39.6 (35.8, 43.4)	41.2 (38.2, 44.2)	1.6 (0.2, 3.1)	0.03	24	34.1 (30.3, 37.9)	40.1 (36.7, 43.5)	6.0 (4.0, 7.9)	<0.0001
	BMD	44	0.76 (0.71, 0.80)	43	0.76 (0.71, 0.80)	0.77 (0.72, 0.81)	0.01 (-0.00, 0.03)	0.17	24	0.68 (0.63, 0.74)	0.73 (0.68, 0.78)	0.05 (0.03, 0.07)	0.0001
Hip	BMC	43	25.2 (23.2, 27.1)	39	25.5 (23.4, 27.6)	26.1 (24.4, 27.9)	0.7 (-0.2, 1.5)	0.13	22	23.9 (21.2, 26.6)	26.3 (24.1, 28.6)	2.4 (0.7, 4.1)	0.008
	BMD	43	0.80 (0.75, 0.86)	39	0.81 (0.75, 0.87)	0.82 (0.78, 0.86)	0.01 (-0.02, 0.05)	0.6	22	0.76 (0.68, 0.85)	0.79 (0.74, 0.84)	0.03 (-0.04, 0.10)	0.4
BMD z-scores	Spine	44	-0.3 (-0.7, 0.0)	43	-0.3 (-0.7, 0.1)	-1.0 (-1.3, -0.7)			24	-0.5 (-1.1, 0.0)	-1.5 (-2.1, -0.8)		
	HAZ spine	44	-0.5(-0.8, -0.1)	43	-0.4 (-0.8, -0.1)	-1.0 (-1.3, -0.6)			24	-0.7 (-1.2, -0.1)	-1.3 (-1.9, -0.7)		
	Hip	43	-0.5 (-0.9, -0.1)	39	-0.5 (-0.9, -0.1)	-1.0 (-1.3, -0.6)			21	-0.5 (-1.1, 0.1)	-1.4 (-2.0, -0.9)		
	HAZ hip	43	-0.7 (-1.0, -0.3)	39	-0.6 (-1.0, -0.2)	-0.9 (-1.3, -0.5)			21	-0.5 (-1.1, 0.1)	-1.2 (-1.7, -0.6)		

		36 months				
		Baseline for those followed up	Follow-up	Change	p	
		n	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	
Lumbar	BMC	12	37.05 (31.0, 43.1)	42.4 (37.4, 47.4)	5.3 (2.8, 7.8)	0.0007
	BMD	12	0.72 (0.65, 0.80)	0.76 (0.70, 0.82)	0.03 (.00, 0.07)	0.05
Hip	BMC	12	26.1 (22.1, 30.0)	26.8 (21.2, 32.3)	0.7 (-3.8, 5.2)	0.7
	BMD	12	(0.82, 0.73, 0.91)	0.81 (0.74, 0.88)	-0.009 (-0.05, 0.03)	0.6
BMD z-scores	Spine	12	-0.2 (-1.0, 0.6)	-1.5 (-2.2, -0.8)		
	HAZ spine	12	-0.4 (-1.2, 0.3)	-1.3 (-2.2, -0.5)		
	Hip	12	-0.3 (-1.3, 0.6)	-1.1 (-1.8, -0.5)		
	HAZ hip	12	-0.5 (-1.5, 0.5)	-1.0 (-1.8, -0.2)		

BMD: bone mineral density; BMC bone mineral content; HAZ height adjusted z-score.
BMD z-scores were not formally tested–see Methods.

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Most participants reported positive or a mix of positive-negative changes in their life at both time points. At 6–15 months 46% reported only positive changes, including feeling happier, relieved, less facial hair or stopping periods. A further 37% reported both positive and negative changes such as feeling happier but also experiencing hot flushes and headaches. In addition 12% reported overall negative changes namely hot flushes, tiredness, and feeling more emotional, while 5% reported no change. At 15–24 months, 55% reported solely positive changes such as feeling happier, no longer experiencing side effects and feeling more

Table 4. ASEBA outcomes at baseline, 12, 24 and 36 months.

				12 months					24 months				
			Baseline		Baseline for those followed up	Follow-up	Change	p		Baseline for those followed up	Follow-up	Change	p
		n	mean (95% CI)	n	mean (95% CI)	mean (95% CI)	mean (95% CI)		n	mean (95% CI)	mean (95% CI)	mean (95% CI)	
Parent report CBCL	Total problems t-score	43	61.6(58.4, 64.7)	41	61.5(58.2, 64.7)	61.8(58.4, 65.1)	0.3(-2.0, 2.6)	0.8	20	61.2(56.5, 65.8)	60.2(54.6, 65.8)	-1.0(-4.0, 2.1)	0.5
	Externalising problems t-score	43	55.8(52.4, 59.3)	41	55.7(52.1, 59.3)	55.4(51.8, 59.0)			20	55.4(49.9, 60.9)	55.2(48.9, 61.5)		
	Internalising problems t-score	43	62.1(58.7, 65.5)	41	61.8(58.3, 65.3)	62.9(59.5, 66.3)			20	60.4(55.7, 65.1)	60.1(54.6, 65.6)		
Self-report YSR	Total problems t-score	44	57.9(55.0, 60.8)	41	57.6(54.5, 60.6)	58.4(54.6, 62.2)	0.8(-3.1, 4.8)	0.7	15	55.1(50.9, 59.2)	56.5(50.6, 62.5)	1.5(-3.4, 6.3)	0.5
	Total problems z-score (ref: Netherlands)	44	1.01(0.67, 1.36)	41	0.97(0.62, 1.33)	0.99(0.55, 1.42)			15	0.66(0.17,1.15)	0.65(-0.05, 1.36)		
	Total problems z-score (ref: Australia)	44	0.72(0.37, 1.06)	41	0.68(0.32, 1.03)	0.68(0.24, 1.12)			15	0.39(-0.11,0.89)	0.37(-0.32, 1.07)		
	Externalising problems t-score	44	52.3(49.2, 55.5)	41	52.3(49.2, 55.4)	52.5(48.7, 56.3)			15	53.1(48.5, 57.6)	52.3(45.3, 59.4)		
	Internalising problems t-score	44	58.0(54.9, 61.2)	41	57.7(54.3, 61.0)	60.1(55.9, 64.3)			15	53.9(49.9, 58.0)	55.9(50.8, 61.1)		
Self-harm scores													
Parent report CBCL	Median (IQR)	43	0(0, 1)	40	0(0, 1)	0(0, 1)		0.3	20	0(0, 1)	0(0, 1)		>0.9
Self-report YSR	Median (IQR)	43	0(0, 1)	39	0(0, 1)	0(0, 2)		0.4	15	0(0, 0)	0(0, 0)		0.3

		36 months				
			Baseline for those followed up	Follow-up	Change	p
		n	mean (95% CI)	mean (95% CI)	mean (95% CI)	
Parent report CBCL	Total problems t-score	11	62.4(55.1, 69.6)	61.1(52.3, 69.9)	-1.3(-6.6, 4.0)	0.6
	Externalising problems t-score	11	56.8(48.0, 65.6)	56.2(48.3, 64.1)		
	Internalising problems t-score	11	60.4(53.5, 67.2)	62.5(53.6, 71.5)		
Self-harm scores						
Parent report CBCL	Median (IQR)	11	0(0, 1)	0(0, 1)		0.8

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comfortable with puberty suspended. A further 17% reported both positive and negative changes including less body hair but continued growth in height, or having clearer skin but also experiencing more hunger, weight gain and tiredness. 17% reported largely negative changes such as mood swings, tiredness and hot flushes whilst 10% reported no change.

Reports of change in mood were mixed. At 6–15 months, the majority reported mood to be improved (49%), mixed changes (such as both feeling happier but experiencing some mood swings; 15%) or no change (7%), however 24% reported negative changes in mood such as

Table 5. Other psychological outcomes at baseline, 12, 24 and 36 months.

		Baseline		12 months		24 months		36 months	
		n	mean (95% CI)	n	mean (95% CI)	n	mean (95% CI)	n	mean (95% CI)
Kidscreen-52 HRQOL									
Parent report CBCL t-scores	Physical wellbeing	42	44.9(41.4, 48.5)	36	40.4(37.5, 43.3)	14	40.5(36.8, 44.2)		
	Psychological Wellbeing	41	39.8(36.7, 42.8)	36	39.0(35.4, 42.6)	14	42.4(36.9, 48)		
	Moods and Emotions	41	40.6(37.6, 43.6)	36	41.2(37.3, 45.1)	14	42.5(36.3, 48.7)		
	Self-perception	42	34.6(32.6, 36.5)	36	34.8(32.0, 37.5)	14	34.8(31.3, 38.2)		
	Autonomy	42	46.2(43.2, 49.2)	36	48.2(45.0, 51.4)	14	46.7(41, 52.4)		
	Parent relations and home life	42	48.1(44.5, 51.6)	35	46.7(42.9, 50.5)	14	49.5(44.1, 54.9)		
	Social support and peers	39	48.0(44.7, 51.4)	36	51.9(48.4, 55.3)	13	51.4(45.6, 57.2)		
	School environment	42	38.2(35.0, 41.4)	35	39.4(35.3, 43.4)	13	43.7(36, 51.3)		
	Social acceptance	39	44.7(40.7, 48.7)	32	42.3(38.1, 46.4)	13	43.5(35.9, 51.2)		
	Financial resources	42	37.9(33.9, 41.9)	36	35.8(31.5, 40.2)	14	36.3(26.4, 46.3)		
Self-report t-scores	Physical wellbeing	42	45.1(41.8, 48.5)	36	41.5(38.0, 45.0)	13	43.9(38.9, 48.9)		
	Psychological Wellbeing	42	43.0(39.6, 46.4)	36	41.1(37.0, 45.2)	14	51(45.8, 56.2)		
	Moods and Emotions	42	46.3(42.7, 49.9)	36	43.9(40.4, 47.3)	14	50.1(45.5, 54.7)		
	Self-perception	42	38.8(36.7, 40.9)	36	37.9(35.1, 40.6)	14	43.1(39.9, 46.2)		
	Autonomy	42	46.6(43.6, 49.6)	36	46.7(42.9, 50.5)	13	51.9(47.4, 56.4)		
	Parent relations and home life	42	49.7(46.2, 53.2)	36	48.7(45.2, 52.3)	14	58.4(53.3, 63.5)		
	Social support and peers	37	45.6(42.5, 48.7)	35	48.1(44.6, 51.6)	14	49.7(44.3,55.1)		
	School environment	41	45.9(42.3, 49.4)	36	44.7(39.7, 49.7)	14	49(43.6, 54.3)		
	Social acceptance	41	47.4(43.5, 51.3)	33	45.5(40.9, 50.1)	13	53.6(46.3, 60.8)		
	Financial resources	42	42.2(38.1, 46.3)	34	43.2(38.2, 48.1)	14	46.3(39.1, 53.5)		
Body image scale	Overall score	42	3.1(2.8, 3.3)	40	3.2(3.0, 3.4)	16	3(2.7, 3.2)	8	3.1(2.4, 3.7)
	Primary characteristics score	42	4.5(4.2, 4.7)	39	4.3(4.2, 4.5)	16	4.5(4.3, 4.7)	8	4.2(3.9, 4.5)
	Secondary characteristics score	41	2.9(2.6, 3.1)	40	3(2.8, 3.3)	16	2.9(2.5, 3.2)	8	2.9(2, 3.8)
	Neutral characteristics score	42	2.5(2.203, 2.707)	40	2.7(2.5, 3.0)	-	-		
Utrecht Gender dysphoria score	Median (IQR)	41	4.8(4.6, 5.0)	40	4.7(4.6, 5.0)	18	4.7(4.3, 5.0)		
Clinical outcome									
CGAS global score	Mean (95% CI)	42	62.9(59.6, 66.2)	35	64.1(59.9, 68.3)	18	65.7(59.6, 71.8)	12	66.0(58.1, 73.9)

Note: Change in outcomes in this Table were not formally tested.

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Table 6. Associations between birth-registered sex and baseline pubertal status and outcomes at 12 months.

		Outcomes at 12 months adjusted for baseline								
		BMD at lumbar spine			YSR total t-score			GCAS score		
		n	Coefficient (95% CI)	p	n	Coefficient (95% CI)	p	n	Coefficient (95% CI)	p
Birth-registered sex										
Main effect (baseline value of outcome)		43	0.86 (0.75, 0.97)	<0.0001	41	0.43 (0.05, 0.82)	0.03	33	0.74 (0.42, 1.06)	<0.0001
Birth-registered sex	Male (ref)		0			0			0	
	Female		-0.02 (-0.05, 0.01)	0.2		2.1 (-5.2, 9.4)	0.6		-3.2 (-10.0, 3.5)	0.3
Pubertal status										
Main effect (baseline value of outcome)		43	0.85 (0.72, 0.97)	<0.0001	41	0.43 (0.01, 0.84)	0.04	33	0.69 (0.37, 1.00)	<0.0001
Pubertal stage at baseline	3		0.008 (-0.03, 0.04)	0.7		0.2 (-8.3, 8.7)	0.9		1.6 (-5.5, 8.8)	0.6
	4 (ref)		0			0			0	
	5		-0.009 (-0.05, 0.03)	0.7		0.4 (-9.9, 10.8)	0.9		-7.9 (-17.6, 1.8)	0.11

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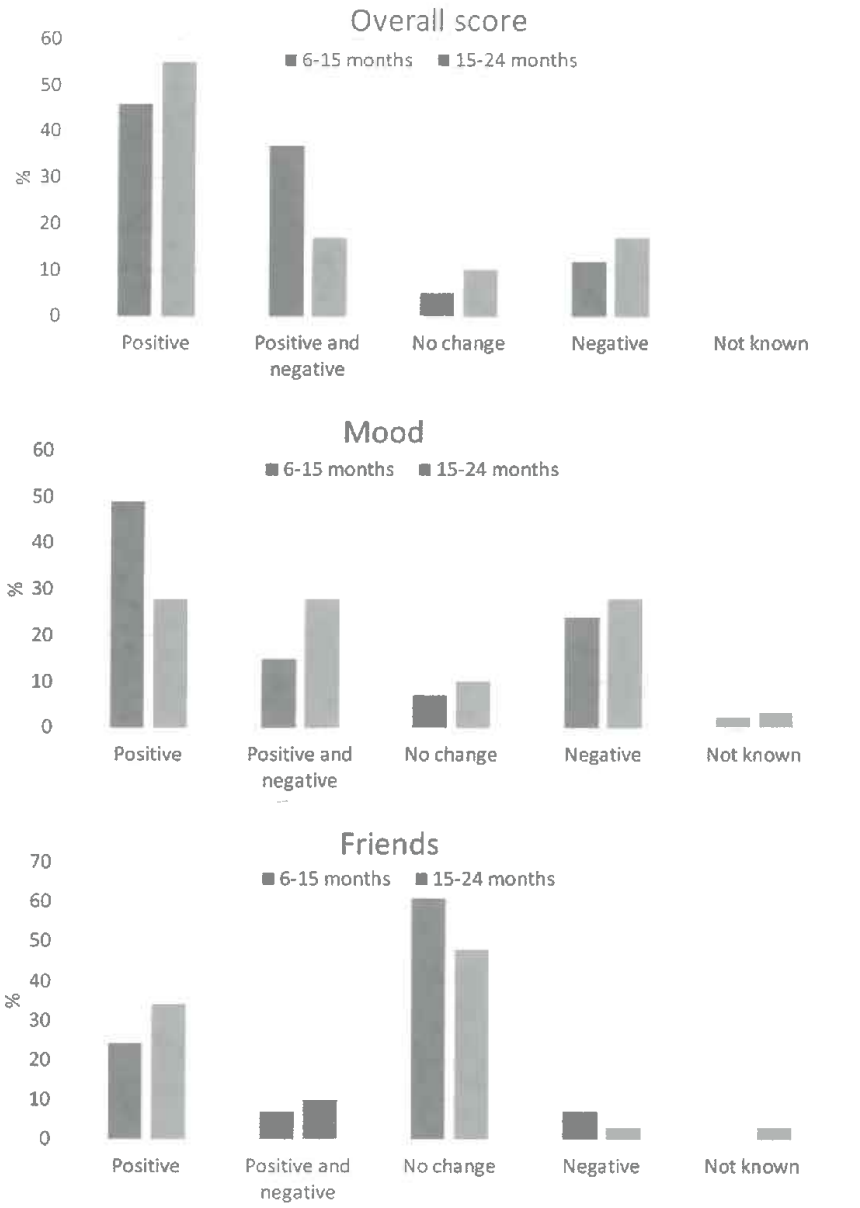

Fig 1. Ratings of change in life overall, mood and friendships at 6–15 months (n = 41) and 15–24 months (n = 29).

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experiencing more mood swings or feeling low. Findings at 15–24 months were similar. The most common negative change was reduced energy levels, reported by 29% at 6-15m and 38% at 15-24m.

Young people's reports of change in family and peer relationships were predominantly positive or neutral at both time points. Positive changes included feeling closer to the family,

Table 7. Adverse events reported across the study.

Participants	0-6m	7-12m	13-24m	25+m
	n = 44	n = 44	n = 36	n = 24
	n (%)	*n (%)*	*n (%)*	*n (%)*
Mild headaches or hot flushes	11 (25%)	10 (23%)	8 (22%)	4 (17%)
Moderate or severe headaches and hot flushes	2 (5%)	4 (9%)	1 (3%)	0
Fatigue—mild	2 (5%)	3 (7%)	3 (8%)	1 (4%)
Fatigue–moderate or severe	0	0	0	0
Mood swings	1 (2%)	0	0	0
Weight gain	1 (2%)	0	1 (3%)	0
Sleep problems	1 (2%)	0	1 (3%)	0
Other events	0	0	0	0
Total events recorded*	18	17	14	5

* individuals may have more than 1 event.

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feeling more accepted and having fewer arguments. Those reporting both positive and negative change reported feeling closer to some family members but not others. At 6–15 months, negative family changes were largely from family members not accepting their trans status or having more arguments. But by 15–24 months only one young person reported this. Improved relationships with peers related to feeling more sociable or confident and widening their circle of friends; negative changes related to bullying or disagreements at school. Again, at 15–24 months only one young person reported negative change, related to feelings of not trusting friends.

At 6–15 months, changes in gender role were reported by 66% as positive, including feeling more feminine/masculine, living in their preferred gender identity in more (or all) areas of life and feeling more secure in their gender identity, with no negative change reported. At 15–24 months, most reported no change although 41% reported positive changes including experimenting more with physical appearance and changing their details on legal documents.

All young people affirmed at each interview that they wished to continue with GnRHa treatment. Note that this was also the case when asked routinely at medical clinics (excepting those who briefly ceased GnRHa as noted above).

Adverse events. Adverse events are shown in Table 7. All adverse events were minor and anticipated, i.e. they were previously described in study participant information and/or noted in the triptorelin medication package inserts. Anticipated adverse events were common in the first two years, particularly mild headaches or hot flushes which were reported in 25% at 0-6m, 23% at 7-12m and 22% at 13-24m. Moderate or severe headaches and/or hot flushes were uncommon. Birth-registered females with distressing headaches or hot flushes were offered 'add-back' oestrogen therapy, and two accepted treatment briefly with very small doses of oestradiol, which was effective in reducing symptoms. Mild fatigue was reported by 5–8% over the first two years and no participants reported moderate or severe fatigue. Sleep problems, mood swings and weight gain were reported by very small numbers and in each case symptoms were mild. Adverse events were less common after 12 months of treatment.

Discussion

We report the short and medium-term outcomes of a prospective cohort of 44 young people with persistent and severe GD treated with GnRHa resulting in pubertal suppression from mid-puberty for 1–4 years. Young people were considered for recruitment after lengthy

assessment, spending an average of 2 years and up to 6 years within the GIDS psychological service before being referred to the endocrine clinic for assessment to enter the study. Medical assessment found no endocrine abnormalities at baseline. GnRHa treatment started in the majority of participants in later stages of puberty, with 57% in puberty stages 4 and 5 and 79% of birth-registered females being post-menarcheal. After starting GnRHa all quickly achieved and maintained suppression of pubertal hormones and none experienced pubertal progression. At the end of the study, 43 (98%) chose to start cross-sex hormones whilst one young person chose to stop GnRHa and continue with puberty consistent with their birth-registered sex.

As anticipated, pubertal suppression reduced growth that was dependent on puberty hormones, i.e. height and BMD. Height growth continued for those not yet at final height, but more slowly than for their peers so height z-score fell. Similarly for bone strength, BMD and BMC increased in the lumbar spine indicating greater bone strength, but more slowly than in peers so BMD z-score fell. These anticipated changes had been discussed with all participants before recruitment to the study. Young people experienced little change in mean weight or BMI z-score in the first two years. The rise in weight and BMI z-score at 36 months may represent a trend towards greater adiposity in those on GnRHa for a prolonged period, or reflect a higher baseline in this group.

Information on side-effects was available through routine reporting in medical clinics and in the participant experience interviews. Anticipated side effects of treatment were common, particularly mild symptoms directly related to suppression of sex hormones. Severe symptoms were uncommon. Fatigue or low energy was reported rarely in medical clinic assessments but frequently at interview (38% at 15-24m). The relationship of symptoms such as headaches, fatigue and sleep disturbance to GnRHa treatment is unclear as they are all very common in early adolescence [36,37], although a conservative perspective would regard them as side-effects of treatment.

Young people experienced little change in psychological functioning across the study. We found no differences between baseline and later outcomes for overall psychological distress as rated by parents and young people, nor for self-harm. Outcomes that were not formally tested also showed little change.

Participant experience of treatment as reported in interviews was positive for the majority, particularly relating to feeling happier, feeling more comfortable, better relationships with family and peers and positive changes in gender role. Smaller numbers reported having mixed positive and negative changes. A minority (12% at 6–15 months and 17% at 15–24 months) reported only negative changes, which were largely related to anticipated side effects. None wanted to stop treatment due to side effects or negative changes. We are not aware of comparative patient experience data from other cohorts.

The median age at consent in our study was very similar to that in the earliest published outcome study of mid-pubertal suppression using GnRHa treatment in Dutch young people (13.6 years) [24]. Similarly to this Dutch cohort, all but one of our participants elected to start cross-sex hormones after completing the GnRHa pathway. However they spent an average of 31 months on GnRHa compared with 23 months in the Dutch cohort [24]. In our study, the successful suppression of puberty and cessation of menses with GnRHa, the impact on height growth [4,16,38] and BMD [4,16] and the normality of liver and renal function through treatment were each consistent with previous reports [4,16].

Our findings that BMD increased over time in the lumbar spine but more slowly than in same age peers, resulting in a fall in z-score, are similar to others [4,14,39,40]. The fall in height-adjusted BMD z-score was consistent with but larger than the fall in height z-score. We found that birth-registered sex and pubertal status at baseline were not associated with later BMD. There is evidence that accretion of bone mass resumes and that BMD increases with the

start of cross-sex hormone therapy [4,14,39,41]. Future research needs to examine longer-term change in BMD in young people treated with mid-pubertal suppression.

We reported a range of adverse events previously described to be associated with pubertal suppression [42], with the exception of mild sleep disturbance although this is a known association with triptorelin use. As anticipated, the withdrawal of sex hormones produces symptoms such as headaches and lack of energy, although in the great majority (11 of 13 at 0–6 months; 10 of 14 at 7–12 months; 8 of 9 at 13–24 months) the symptoms were minor. Symptoms diminished over time as has previously been noted [4], and no young people chose to cease treatment due to the side-effects.

Our finding that 1 participant ceased pubertal suppression and did not commence cross-sex hormones is somewhat similar to the experience of one US cohort and a second Dutch cohort; Kuper et al. described that 2 of approximately 57 young people aged 10–15 years who commenced pubertal suppression treatment stopped this treatment without commencing cross-sex hormones [17]. Brik et al. reported that in a cohort of 137 young people who began GnRHa between 10 and 18 years and were followed until eligible to commence cross-sex hormones, 5 (3.6%) ceased treatment and did not later commence cross-sex hormones [19].

Three longitudinal studies from the Netherlands and the USA have examined psychological function over time in cohorts of young people treated with GnRHa and then cross-sex hormones [17,18,24], although the two US cohorts were of limited size. Our study adopted the same psychological outcome measures as the Dutch cohort, to facilitate comparison [24]. Mean baseline YSR scores in our cohort were similar to those previously reported in 141 young people aged 12–18 years from the London GIDS [43], and baseline CBCL and YSR scores were close to those at baseline from the original Dutch cohort [24]. A number of other studies have shown that young people with GD have higher scores on the CBCL or YSR than same-age population peers, and that they are similar to young people referred to clinical services for a range of mental health problems [44–46]. Population-based studies in America support higher baseline levels of mental health problems amongst young people with GD, with the prevalence of self-harm notably higher than for male or female peers [47,48]. Young people in our study had baseline YSR scores 0.7–1.0 SD higher than norms for age in comparable countries [29,46].

We found no evidence of change in psychological function with GnRHa treatment as indicated by parent report (CBCL) or self-report (YSR) of overall problems, internalising or externalising problems or self-harm. This is in contrast to the Dutch study which reported improved psychological function across total problems, externalising and internalising scores for both CBCL and YSR and small improvements in CGAS [24]. It also contrasts with a previous study from the UK GIDS of change in psychological function with GnRHa treatment in 101 older adolescents with GD (beginning > 15.5 years) which reported moderate improvements in CGAS score over 12 months of GnRHa treatment [49]. CGAS scores in this previous study increased from 61 to 67 with GnRHa treatment, similar to those (63 at baseline, 66 at 24 months) in our study. Follow-up of the Kuper et al. cohort found non-significant changes in depression and anxiety scores in those (n = 25) who had only pubertal suppression treatment, although improvements were seen in the whole sample combining these with those receiving cross-sex hormones [17]. A second US cohort reported that in 23 young people who had received pubertal suppression (using GnRHa or anti-androgens in birth-registered males and either GnRHa or medroxyprogesterone in birth-registered females), there was a reduction in depression scores in birth-registered males but not females.

A recent large US survey found that those who received pubertal suppression in early or mid adolescence had lower odds of lifetime suicidal ideation when studied in adulthood compared with those who did not, regardless of whether they later received cross-sex hormones

and after adjustment for a range of confounding factors [50]. This implies an enduring benefit of pubertal suppression on psychological function, however the cross-sectional design and retrospective exposure classification means the findings require replication. Data are also available from other conditions in which GnRHa is used to suppress puberty during adolescence. A trial of GnRHa suppression of puberty during early adolescence in young people born small-for-gestational-age (SGA) who were also treated with human growth hormone (GH) reported that those treated with GnRHa had similar cognitive and psychological function in adult life to those treated only with GH [51].

The differences between our findings and the previous GIDS study re change in psychological function may relate simply to sample size. But why our findings differ from those of the Dutch study is unclear. They may relate to the timing of assessments; we assessed young people multiple times whereas in the Dutch study the second assessment was shortly before starting cross-sex hormone treatment. Alternatively, there may have been baseline differences in the two cohorts. Whilst some aspects of psychological function were similar, as noted above, the baseline CGAS scores were notably higher in the Dutch group (indicating better function). A previous international comparison study has found that young people aged 12–18 years with GD from the UK have higher scores indicating greater problems on the CBCL and YSR than those from the Netherlands, Belgium and Switzerland [52].

Psychological distress and self-harm are known to increase across early adolescence. Normative data show rising YSR total problems scores with age from age 11 to 16 years in non-clinical samples from a range of countries [29]. Self-harm rates in the general population in the UK and elsewhere increase markedly with age from early to mid-adolescence, being very low in 10 year olds and peaking around age 16–17 years [53–56]. Our finding that psychological function and self-harm did not change significantly during the study is consistent with two main alternative explanations. The first is that there was no change, and that GnRHa treatment brought no measurable benefit nor harm to psychological function in these young people with GD. This is consonant with the action of GnRHa, which only stops further pubertal development and does not change the body to be more congruent with a young person's gender identity. The second possibility is that the lack of change in an outcome that normally worsens in early adolescence may reflect a beneficial change in trajectory for that outcome, i.e. that GnRHa treatment reduced this normative worsening of problems. In the absence of a control group, we cannot distinguish between these possibilities. We aimed to use normative reference data to examine this issue. However age- and gender-standardised t-scores for ASEBA and other outcomes cannot answer this question as they cover a very broad age range (e.g. 12–18 years). We had anticipated that z-scores on the YSR available by calendar year for two comparable countries (Netherlands; Australia) might be informative however confidence intervals were too wide to draw reliable inferences.

Gender dysphoria and body image changed little across the study. This is consistent with some previous reports [24] and was anticipated, given that GnRHa does not change the body in the desired direction, but only temporarily prevents further masculinization or feminization. Other studies suggest that changes in body image or satisfaction in GD are largely confined to gender affirming treatments such as cross-sex hormones or surgery [57]. We found that birth-registered sex and baseline pubertal status were not associated with later psychological functioning on GnRHa, consistent with previous reports [24,49].

These data correct reports from a recent letter by Biggs [58] which used preliminary data from our study which were uncleaned and incomplete data used for internal reporting. In addition there were many statistical comparisons which inflated the risk of type 1 error. Our statistical analysis plan restricted testing all outcomes for differences by sex due to the type 1

error risk. Contrary to Biggs's letter, we found no evidence of reductions over time in any psychological outcomes, and no material differences by sex.

Strengths and limitations

Our study provides comprehensive data on this cohort during follow-up, with an anonymised dataset containing standardised scores deposited to allow other researchers to replicate our findings where data-sharing allows. The study size and uncontrolled design were key limitations. The small sample size limited our ability to identify small changes in outcomes. This was an uncontrolled observational study and thus cannot infer causality. Further, many of the outcomes studied here, including psychological function, self-harm and BMD, undergo normative changes by age and developmental stage during puberty that could confound any observed effect of GnRHa treatment in an uncontrolled study. The analysis plan aimed to take these issues into account as far as possible, however this particularly limits the potential for the study to show benefits or harms from treatment. However, some conclusions can be drawn. It is unlikely that the reported adverse events such as headaches do not relate directly to GnRHa treatment. Equally, given that there were no changes in psychological function and differences in point estimates were minimal for nearly all outcomes, it is unlikely that the treatment resulted in psychological harm. Observational studies are important sources of data on harms of treatment [59–61].

Our data are subject to a number of other limitations. This was an unfunded study undertaken within a clinical service and we were dependent on the clinical service for data collection. There were varying sample sizes for differing tests as some participants did not attend certain investigations and some follow-up medical tests were processed locally to patients; these data are reported as normal or otherwise. Missing items on psychological questionnaires resulted in some unusable data. Some young people found repeated completion of questionnaires about gender issues intrusive and refused to complete them at later follow-ups, as has been reported in other studies [62]. This questionnaire fatigue also affected parent responses. Scoring of psychological questionnaire data was rechecked at the completion of the study however this was not possible in very small numbers of participants in whom only scale scores rather than individual item data were preserved during data migration in hospital clinical information systems. In sensitivity analyses, repeat analysis of ASEBA psychological outcomes restricted to those with rescored data showed highly similar findings to the full sample (see S3 Table in S1 Appendix).

A more detailed qualitative evaluation of participant experience was not possible due to lack of interviewer time, and reporting of interview data was restricted to perceptions of positive or negative change and the giving of examples.

Implications and conclusions

Treatment of young people with persistent and severe GD aged 12–15 years with GnRHa was efficacious in suppressing pubertal progression. Anticipated effects of withdrawal of sex hormones on symptoms were common and there were no unexpected adverse events. BMD increased with treatment in the lumbar spine and was stable at the hip, and BMD z-score fell consistent with delay of puberty. Overall participant experience of changes on GnRHa treatment was positive. We identified no changes in psychological function, quality of life or degree of gender dysphoria.

The great majority of this cohort went on to start cross-sex hormones, as was hypothesized given the severity and continuation of their GD. However one young person did not, providing some evidence that development of gender identity continues on GnRHa treatment and

confirming the importance of continuing supportive psychological therapy to allow further exploration of gender identity and a range of future pathways whilst on GnRHa.

This cohort will be followed up longer term to examine physical and mental health outcomes into early adulthood. However larger and longer-term prospective studies using a range of designs are needed to more fully quantify the harms and benefits of pubertal suppression in GD and better understand factors influencing outcomes [3]. These are beginning to be funded in a number of countries [63].(https://logicstudy.uk) Given that pubertal suppression may be both a treatment in its own right and also an intermediate step in a longer treatment pathway, it is essential for such studies to examine benefits and harms across the longer pathway including pubertal suppression and initiation of cross-sex hormones.

Supporting information

S1 Appendix.
(DOCX)

S2 Appendix. Statistical analysis plan.
(DOCX)

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