EXHIBIT 61

Endocrine Treatment of Gender-Dysphoric/ Gender-Incongruent Persons: An Endocrine Society* Clinical Practice Guideline

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*Cosponsoring Associations: American Association of Clinical Endocrinologists, American Society of Andrology, European Society for Pediatric Endocrinology, European Society of Endocrinology, Pediatric Endocrine Society, and World Professional Association for Transgender Health.

Objective: To update the "Endocrine Treatment of Transsexual Persons: An Endocrine Society Clinical Practice Guideline," published by the Endocrine Society in 2009.

Participants: The participants include an Endocrine Society–appointed task force of nine experts, a methodologist, and a medical writer.

Evidence: This evidence-based guideline was developed using the Grading of Recommendations, Assessment, Development, and Evaluation approach to describe the strength of recommendations and the quality of evidence. The task force commissioned two systematic reviews and used the best available evidence from other published systematic reviews and individual studies.

Consensus Process: Group meetings, conference calls, and e-mail communications enabled consensus. Endocrine Society committees, members and cosponsoring organizations reviewed and commented on preliminary drafts of the guidelines.

Conclusion: Gender affirmation is multidisciplinary treatment in which endocrinologists play an important role. Gender-dysphoric/gender-incongruent persons seek and/or are referred to endocrinologists to develop the physical characteristics of the affirmed gender. They require a safe and effective hormone regimen that will (1) suppress endogenous sex hormone secretion determined by the person's genetic/gonadal sex and (2) maintain sex hormone levels within the normal range for the person's affirmed gender. Hormone treatment is not recommended for prepubertal gender-dysphoric/gender-incongruent persons. Those clinicians who recommend gender-affirming endocrine treatments—appropriately trained diagnosing clinicians (required), a mental health provider for adolescents (required) and mental health

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Abbreviations: BMD, bone mineral density; DSD, disorder/difference of sex development; DSM, Diagnostic and Statistical Manual of Mental Disorders; GD, gender dysphoria; GnRH, gonadotropin-releasing hormone; ICD, International Statistical Classification of Diseases and Related Health Problems; MHP, mental health professional; VTE, venous thromboembolism.

professional for adults (recommended)—should be knowledgeable about the diagnostic criteria and criteria for gender-affirming treatment, have sufficient training and experience in assessing psychopathology, and be willing to participate in the ongoing care throughout the endocrine transition. We recommend treating gender-dysphoric/gender-incongruent adolescents who have entered puberty at Tanner Stage G2/B2 by suppression with gonadotropin-releasing hormone agonists. Clinicians may add gender-affirming hormones after a multidisciplinary team has confirmed the persistence of gender dysphoria/gender incongruence and sufficient mental capacity to give informed consent to this partially irreversible treatment. Most adolescents have this capacity by age 16 years old. We recognize that there may be compelling reasons to initiate sex hormone treatment prior to age 16 years, although there is minimal published experience treating prior to 13.5 to 14 years of age. For the care of peripubertal youths and older adolescents, we recommend that an expert multidisciplinary team comprised of medical professionals and mental health professionals manage this treatment. The treating physician must confirm the criteria for treatment used by the referring mental health practitioner and collaborate with them in decisions about gender-affirming surgery in older adolescents. For adult gender-dysphoric/gender-incongruent persons, the treating clinicians (collectively) should have expertise in transgender-specific diagnostic criteria, mental health, primary care, hormone treatment, and surgery, as needed by the patient. We suggest maintaining physiologic levels of gender-appropriate hormones and monitoring for known risks and complications. When high doses of sex steroids are required to suppress endogenous sex steroids and/or in advanced age, clinicians may consider surgically removing natal gonads along with reducing sex steroid treatment. Clinicians should monitor both transgender males (female to male) and transgender females (male to female) for reproductive organ cancer risk when surgical removal is incomplete. Additionally, clinicians should persistently monitor adverse effects of sex steroids. For gender-affirming surgeries in adults, the treating physician must collaborate with and confirm the criteria for treatment used by the referring physician. Clinicians should avoid harming individuals (via hormone treatment) who have conditions other than gender dysphoria/gender incongruence and who may not benefit from the physical changes associated with this treatment. (***J Clin Endocrinol Metab*** **102: 3869–3903, 2017)**

Summary of Recommendations

1.0 Evaluation of youth and adults

1.1. We advise that only trained mental health professionals (MHPs) who meet the following criteria should diagnose gender dysphoria (GD)/gender incongruence in adults: (1) competence in using the Diagnostic and Statistical Manual of Mental Disorders (DSM) and/or the International Statistical Classification of Diseases and Related Health Problems (ICD) for diagnostic purposes, (2) the ability to diagnose GD/gender incongruence and make a distinction between GD/gender incongruence and conditions that have similar features (*e.g.*, body dysmorphic disorder), (3) training in diagnosing psychiatric conditions, (4) the ability to undertake or refer for appropriate treatment, (5) the ability to psychosocially assess the person's understanding, mental health, and social conditions that can impact gender-affirming hormone therapy, and (6) a practice of regularly attending relevant professional meetings. (Ungraded Good Practice Statement)

1.2. We advise that only MHPs who meet the following criteria should diagnose GD/gender incongruence in children and adolescents: (1) training in child and adolescent developmental psychology and psychopathology, (2) competence in using the DSM and/or the ICD for diagnostic purposes, (3) the ability to make a distinction between GD/gender incongruence and conditions that have similar features (*e.g.*, body dysmorphic disorder), (4) training in diagnosing psychiatric conditions, (5) the ability to undertake or refer for appropriate treatment, (6) the ability to psychosocially assess the person's understanding and social conditions that can impact gender-affirming hormone therapy, (7) a practice of regularly attending relevant professional meetings, and (8) knowledge of the criteria for puberty blocking and gender-affirming hormone treatment in adolescents. (Ungraded Good Practice Statement)

1.3. We advise that decisions regarding the social transition of prepubertal youths with GD/gender incongruence are made with the assistance of an MHP or another experienced professional. (Ungraded Good Practice Statement).

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1.4. We recommend against puberty blocking and gender-affirming hormone treatment in prepubertal children with GD/gender incongruence. (1 |⊕◯◯◯)

1.5. We recommend that clinicians inform and counsel all individuals seeking gender-affirming medical treatment regarding options for fertility preservation prior to initiating puberty suppression in adolescents and prior to treating with hormonal therapy of the affirmed gender in both adolescents and adults. (1 |⊕⊕⊖◯)

2.0 Treatment of adolescents

2.1. We suggest that adolescents who meet diagnostic criteria for GD/gender incongruence, fulfill criteria for treatment, and are requesting treatment should initially undergo treatment to suppress pubertal development. (2 |⊖⊕◯◯)

2.2. We suggest that clinicians begin pubertal hormone suppression after girls and boys first exhibit physical changes of puberty. (2 |⊕⊕◯◯)

2.3. We recommend that, where indicated, GnRH analogues are used to suppress pubertal hormones. (1 |⊕⊖◯◯)

2.4. In adolescents who request sex hormone treatment (given this is a partly irreversible treatment), we recommend initiating treatment using a gradually increasing dose schedule after a multidisciplinary team of medical and MHPs has confirmed the persistence of GD/gender incongruence and sufficient mental capacity to give informed consent, which most adolescents have by age 16 years. (1 |⊕⊕◯◯).

2.5. We recognize that there may be compelling reasons to initiate sex hormone treatment prior to the age of 16 years in some adolescents with GD/gender incongruence, even though there are minimal published studies of gender-affirming hormone treatments administered before age 13.5 to 14 years. As with the care of adolescents ≥16 years of age, we recommend that an expert multidisciplinary team of medical and MHPs manage this treatment. (1 |⊕◯◯◯)

2.6. We suggest monitoring clinical pubertal development every 3 to 6 months and laboratory parameters every 6 to 12 months during sex hormone treatment. (2 |⊕⊕◯◯)

3.0 Hormonal therapy for transgender adults

3.1. We recommend that clinicians confirm the diagnostic criteria of GD/gender incongruence and the criteria for the endocrine phase of gender transition before beginning treatment. (1 |⊕⊕⊕◯)

3.2. We recommend that clinicians evaluate and address medical conditions that can be exacerbated by hormone depletion and treatment with sex hormones of the affirmed gender before beginning treatment. (1 |⊕⊕⊖◯)

3.3. We suggest that clinicians measure hormone levels during treatment to ensure that endogenous sex steroids are suppressed and administered sex steroids are maintained in the normal physiologic range for the affirmed gender. (2 |⊕⊕◯◯)

3.4. We suggest that endocrinologists provide education to transgender individuals undergoing treatment about the onset and time course of physical changes induced by sex hormone treatment. (2 |⊖◯◯◯)

4.0 Adverse outcome prevention and long-term care

4.1. We suggest regular clinical evaluation for physical changes and potential adverse changes in response to sex steroid hormones and laboratory monitoring of sex steroid hormone levels every 3 months during the first year of hormone therapy for transgender males and females and then once or twice yearly. (2 |⊖⊖◯◯)

4.2. We suggest periodically monitoring prolactin levels in transgender females treated with estrogens. (2 |⊕⊕◯◯)

4.3. We suggest that clinicians evaluate transgender persons treated with hormones for cardiovascular risk factors using fasting lipid profiles, diabetes screening, and/or other diagnostic tools. (2 |⊖⊖◯◯)

4.4. We recommend that clinicians obtain bone mineral density (BMD) measurements when risk factors for osteoporosis exist, specifically in those who stop sex hormone therapy after gonadectomy. (1 |⊕⊕◯◯)

4.5. We suggest that transgender females with no known increased risk of breast cancer follow breast-screening guidelines recommended for non-transgender females. (2 |⊕⊕◯◯)

4.6. We suggest that transgender females treated with estrogens follow individualized screening according to personal risk for prostatic disease and prostate cancer. (2 |⊕◯◯◯)

4.7. We advise that clinicians determine the medical necessity of including a total hysterectomy and oophorectomy as part of gender-affirming surgery. (Ungraded Good Practice Statement)

5.0 Surgery for sex reassignment and gender confirmation

5.1. We recommend that a patient pursue genital gender-affirming surgery only after the MHP and the clinician responsible for endocrine transition therapy both agree that surgery is medically necessary and would benefit the patient's overall health and/or well-being. (1 ⊕⊖○○)

5.2. We advise that clinicians approve genital gender-affirming surgery only after completion of at least 1 year of consistent and compliant hormone treatment, unless hormone therapy is not desired or medically contraindicated. (Ungraded Good Practice Statement)

5.3. We advise that the clinician responsible for endocrine treatment and the primary care provider ensure appropriate medical clearance of transgender individuals for genital gender-affirming surgery and collaborate with the surgeon regarding hormone use during and after surgery. (Ungraded Good Practice Statement)

5.4. We recommend that clinicians refer hormone-treated transgender individuals for genital surgery when: (1) the individual has had a satisfactory social role change, (2) the individual is satisfied about the hormonal effects, and (3) the individual desires definitive surgical changes. (1 ⊕⊖○○)

5.5. We suggest that clinicians delay gender-affirming genital surgery involving gonadectomy and/or hysterectomy until the patient is at least 18 years old or legal age of majority in his or her country. (2 ⊕⊖○○).

5.6. We suggest that clinicians determine the timing of breast surgery for transgender males based upon the physical and mental health status of the individual. There is insufficient evidence to recommend a specific age requirement. (2 ⊕⊖○○)

Changes Since the Previous Guideline

Both the current guideline and the one published in 2009 contain similar sections. Listed here are the sections contained in the current guideline and the corresponding number of recommendations: Introduction, Evaluation of Youth and Adults (5), Treatment of Adolescents (6), Hormonal Therapy for Transgender Adults (4), Adverse Outcomes Prevention and Long-term Care (7), and Surgery for Sex Reassignment and Gender Confirmation (6). The current introduction updates the diagnostic classification of "gender dysphoria/gender incongruence." It also reviews the development of "gender identity" and summarizes its natural development. The section on clinical evaluation of both youth and adults, defines in detail the professional qualifications required of those who diagnose and treat both adolescents and adults. We advise that decisions regarding the social transition of prepubertal youth are made with the assistance of a mental health professional or similarly experienced professional. We recommend against puberty blocking followed by gender-affirming hormone treatment of prepubertal children. Clinicians should inform pubertal children, adolescents, and adults seeking gender-confirming treatment of their options for fertility preservation. Prior to treatment, clinicians should evaluate the presence of medical conditions that may be worsened by hormone depletion and/or treatment. A multidisciplinary team, preferably composed of medical and mental health professionals, should monitor treatments. Clinicians evaluating transgender adults for endocrine treatment should confirm the diagnosis of persistent gender dysphoria/gender incongruence. Physicians should educate transgender persons regarding the time course of steroid-induced physical changes. Treatment should include periodic monitoring of hormone levels and metabolic parameters, as well as assessments of bone density and the impact upon prostate, gonads, and uterus. We also make recommendations for transgender persons who plan genital gender-affirming surgery.

Method of Development of Evidence-Based Clinical Practice Guidelines

The Clinical Guidelines Subcommittee (CGS) of the Endocrine Society deemed the diagnosis and treatment of individuals with GD/gender incongruence a priority area for revision and appointed a task force to formulate evidence-based recommendations. The task force followed the approach recommended by the Grading of Recommendations, Assessment, Development, and Evaluation group, an international group with expertise in the development and implementation of evidence-based guidelines (1). A detailed description of the grading scheme has been published elsewhere (2). The task force used the best available research evidence to develop the recommendations. The task force also used consistent language and graphical descriptions of both the strength of a recommendation and the quality of evidence. In terms of the strength of the recommendation, strong recommendations use the phrase "we recommend" and the number 1, and weak recommendations use the phrase "we suggest" and the number 2. Cross-filled circles indicate the quality of the evidence, such that ⊕○○○ denotes very low–quality evidence; ⊕⊕○○, low quality; ⊕⊕⊕○, moderate quality; and ⊕⊕⊕⊕, high quality. The task force has confidence that persons who receive care according to the strong recommendations will derive, on average, more benefit than harm. Weak recommendations require more careful consideration of the person's circumstances, values, and preferences to determine the best course of action. Linked to each recommendation is a description of the evidence and the

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values that the task force considered in making the recommendation. In some instances, there are remarks in which the task force offers technical suggestions for testing conditions, dosing, and monitoring. These technical comments reflect the best available evidence applied to a typical person being treated. Often this evidence comes from the unsystematic observations of the task force and their preferences; therefore, one should consider these remarks as suggestions.

In this guideline, the task force made several statements to emphasize the importance of shared decision-making, general preventive care measures, and basic principles of the treatment of transgender persons. They labeled these "Ungraded Good Practice Statement." Direct evidence for these statements was either unavailable or not systematically appraised and considered out of the scope of this guideline. The intention of these statements is to draw attention to these principles.

The Endocrine Society maintains a rigorous conflict-of-interest review process for developing clinical practice guidelines. All task force members must declare any potential conflicts of interest by completing a conflict-of-interest form. The CGS reviews all conflicts of interest before the Society's Council approves the members to participate on the task force and periodically during the development of the guideline. All others participating in the guideline's development must also disclose any conflicts of interest in the matter under study, and most of these participants must be without any conflicts of interest. The CGS and the task force have reviewed all disclosures for this guideline and resolved or managed all identified conflicts of interest.

Conflicts of interest are defined as remuneration in any amount from commercial interests; grants; research support; consulting fees; salary; ownership interests [*e.g.*, stocks and stock options (excluding diversified mutual funds)]; honoraria and other payments for participation in speakers' bureaus, advisory boards, or boards of directors; and all other financial benefits. Completed forms are available through the Endocrine Society office.

The Endocrine Society provided the funding for this guideline; the task force received no funding or remuneration from commercial or other entities.

Commissioned Systematic Review

The task force commissioned two systematic reviews to support this guideline. The first one aimed to summarize the available evidence on the effect of sex steroid use in transgender individuals on lipids and cardiovascular outcomes. The review identified 29 eligible studies at moderate risk of bias. In transgender males (female to male), sex steroid therapy was associated with a statistically significant increase in serum triglycerides and low-density lipoprotein cholesterol levels. High-density lipoprotein cholesterol levels decreased significantly across all follow-up time periods. In transgender females (male to female), serum triglycerides were significantly higher without any changes in other parameters. Few myocardial infarction, stroke, venous thromboembolism (VTE), and death events were reported. These events were more frequent in transgender females. However, the quality of the evidence was low. The second review summarized the available evidence regarding the effect of sex steroids on bone health in transgender individuals and identified 13 studies. In transgender males, there was no statistically significant difference in the lumbar spine, femoral neck, or total hip BMD at 12 and 24 months compared with baseline values before initiating masculinizing hormone therapy. In transgender females, there was a statistically significant increase in lumbar spine BMD at 12 months and 24 months compared with baseline values before initiation of feminizing hormone therapy. There was minimal information on fracture rates. The quality of evidence was also low.

Introduction

Throughout recorded history (in the absence of an endocrine disorder) some men and women have experienced confusion and anguish resulting from rigid, forced conformity to sexual dimorphism. In modern history, there have been numerous ongoing biological, psychological, cultural, political, and sociological debates over various aspects of gender variance. The 20th century marked the emergence of a social awakening for men and women with the belief that they are "trapped" in the wrong body (3). Magnus Hirschfeld and Harry Benjamin, among others, pioneered the medical responses to those who sought relief from and a resolution to their profound discomfort. Although the term transsexual became widely known after Benjamin wrote "The Transsexual Phenomenon" (4), it was Hirschfeld who coined the term "transsexual" in 1923 to describe people who want to live a life that corresponds with their experienced gender vs their designated gender (5). Magnus Hirschfeld (6) and others (4, 7) have described other types of trans phenomena besides transsexualism. These early researchers proposed that the gender identity of these people was located somewhere along a unidimensional continuum. This continuum ranged from all male through "something in between" to all female. Yet such a classification does not take into account that people may have gender identities outside this continuum. For instance, some experience themselves as having both a male and female gender identity, whereas others completely renounce any gender classification (8, 9). There are also reports of individuals experiencing a continuous and rapid involuntary alternation between a male and female identity (10) or men who do not experience themselves as men but do not want to live as women (11, 12). In some countries, (*e.g.*, Nepal, Bangladesh, and Australia), these nonmale or nonfemale genders are officially recognized (13). Specific treatment protocols, however, have not yet been developed for these groups.

Instead of the term transsexualism, the current classification system of the American Psychiatric Association uses the term gender dysphoria in its diagnosis of persons who are not satisfied with their designated gender (14). The current version of the World Health Organization's ICD-10 still uses the term transsexualism when diagnosing adolescents and adults. However, for the ICD-11, the World Health Organization has proposed using the term "gender incongruence" (15).

Treating persons with GD/gender incongruence (15) was previously limited to relatively ineffective elixirs or creams. However, more effective endocrinology-based treatments became possible with the availability of testosterone in 1935 and diethylstilbestrol in 1938. Reports of individuals with GD/gender incongruence who were treated with hormones and gender-affirming surgery appeared in the press during the second half of the 20th century. The Harry Benjamin International Gender Dysphoria Association was founded in September 1979 and is now called the World Professional Association for Transgender Health (WPATH). WPATH published its first Standards of Care in 1979. These standards have since been regularly updated, providing guidance for treating persons with GD/gender incongruence (16).

Prior to 1975, few peer-reviewed articles were published concerning endocrine treatment of transgender persons. Since then, more than two thousand articles about various aspects of transgender care have appeared.

It is the purpose of this guideline to make detailed recommendations and suggestions, based on existing medical literature and clinical experience, that will enable treating physicians to maximize benefit and minimize risk when caring for individuals diagnosed with GD/gender incongruence.

In the future, we need more rigorous evaluations of the effectiveness and safety of endocrine and surgical protocols. Specifically, endocrine treatment protocols for GD/gender incongruence should include the careful assessment of the following: (1) the effects of prolonged delay of puberty in adolescents on bone health, gonadal function, and the brain (including effects on cognitive, emotional, social, and sexual development); (2) the effects of treatment in adults on sex hormone levels; (3) the requirement for and the effects of progestins and other agents used to suppress endogenous sex steroids during treatment; and (4) the risks and benefits of gender-affirming hormone treatment in older transgender people.

To successfully establish and enact these protocols, a commitment of mental health and endocrine investigators is required to collaborate in long-term, large-scale studies across countries that use the same diagnostic and inclusion criteria, medications, assay methods, and response assessment tools (*e.g.*, the European Network for the Investigation of Gender Incongruence) (17, 18).

Terminology and its use vary and continue to evolve. Table 1 contains the definitions of terms as they are used throughout this guideline.

Biological Determinants of Gender Identity Development

One's self-awareness as male or female changes gradually during infant life and childhood. This process of cognitive and affective learning evolves with interactions with parents, peers, and environment. A fairly accurate timetable exists outlining the steps in this process (19). Normative psychological literature, however, does not address if and when gender identity becomes crystallized and what factors contribute to the development of a gender identity that is not congruent with the gender of rearing. Results of studies from a variety of biomedical disciplines—genetic, endocrine, and neuroanatomic—support the concept that gender identity and/or gender expression (20) likely reflect a complex interplay of biological, environmental, and cultural factors (21, 22).

With respect to endocrine considerations, studies have failed to find differences in circulating levels of sex steroids between transgender and nontransgender individuals (23). However, studies in individuals with a disorder/difference of sex development (DSD) have informed our understanding of the role that hormones may play in gender identity outcome, even though most persons with GD/gender incongruence do not have a DSD. For example, although most 46,XX adult individuals with virilizing congenital adrenal hyperplasia caused by mutations in *CYP21A2* reported a female gender identity, the prevalence of GD/gender incongruence was much greater in this group than in the general population without a DSD. This supports the concept that there is a role for prenatal/postnatal androgens in gender development (24–26), although some studies indicate that prenatal androgens are more likely to affect gender behavior and sexual orientation rather than gender identity *per se* (27, 28).

Researchers have made similar observations regarding the potential role of androgens in the development of gender identity in other individuals with DSD. For example, a review of two groups of 46,XY persons, each with androgen synthesis deficiencies and female raised, reported transgender male (female-to-male) gender role changes in 56% to 63% and 39% to 64% of patients, respectively (29). Also, in 46,XY female-raised individuals with cloacal

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Table 1. Definitions of Terms Used in This Guideline

Biological sex, biological male or female: These terms refer to physical aspects of maleness and femaleness. As these may not be in line with each other (e.g., a person with XY chromosomes may have female-appearing genitalia), the terms biological sex and biological male or female are imprecise and should be avoided.
Cisgender: This means not transgender. An alternative way to describe individuals who are not transgender is "non-transgender people."
Gender-affirming (hormone) treatment: See "gender reassignment"
Gender dysphoria: This is the distress and unease experienced if gender identity and designated gender are not completely congruent (see Table 2). In 2013, the American Psychiatric Association released the fifth edition of the DSM-5, which replaced "gender identity disorder" with "gender dysphoria" and changed the criteria for diagnosis.
Gender expression: This refers to external manifestations of gender, expressed through one's name, pronouns, clothing, haircut, behavior, voice, or body characteristics. Typically, transgender people seek to make their gender expression align with their gender identity, rather than their designated gender.
Gender identity/experienced gender: This refers to one's internal, deeply held sense of gender. For transgender people, their gender identity does not match their sex designated at birth. Most people have a gender identity of man or woman (or boy or girl). For some people, their gender identity does not fit neatly into one of those two choices. Unlike gender expression (see below), gender identity is not visible to others.
Gender identity disorder: This is the term used for GD/gender incongruence in previous versions of DSM (see "gender dysphoria"). The ICD-10 still uses the term for diagnosing child diagnoses, but the upcoming ICD-11 has proposed using "gender incongruence of childhood."
Gender incongruence: This is an umbrella term used when the gender identity and/or gender expression differs from what is typically associated with the designated gender. Gender incongruence is also the proposed name of the gender identity–related diagnoses in ICD-11. Not all individuals with gender incongruence have gender dysphoria or seek treatment.
Gender variance: See "gender incongruence"
Gender reassignment: This refers to the treatment procedure for those who want to adapt their bodies to the experienced gender by means of hormones and/or surgery. This is also called gender-confirming or gender-affirming treatment.
Gender-reassignment surgery (gender-confirming/gender-affirming surgery): These terms refer only to the surgical part of gender-confirming/gender-affirming treatment.
Gender role: This refers to behaviors, attitudes, and personality traits that a society (in a given culture and historical period) designates as masculine or feminine and/or that society associates with or considers typical of the social role of men or women.
Sex designated at birth: This refers to sex assigned at birth, usually based on genital anatomy.
Sex: This refers to attributes that characterize biological maleness or femaleness. The best known attributes include the sex-determining genes, the sex chromosomes, the H-Y antigen, the gonads, sex hormones, internal and external genitalia, and secondary sex characteristics.
Sexual orientation: This term describes an individual's enduring physical and emotional attraction to another person. Gender identity and sexual orientation are not the same. Irrespective of their gender identity, transgender people may be attracted to women (gynephilic), attracted to men (androphilic), bisexual, asexual, or queer.
Transgender: This is an umbrella term for people whose gender identity and/or gender expression differs from what is typically associated with their sex designated at birth. Not all transgender individuals seek treatment.
Transgender male (also: trans man, female-to-male, transgender male): This refers to individuals assigned female at birth but who identify and live as men.
Transgender woman (also: trans woman, male-to female, transgender female): This refers to individuals assigned male at birth but who identify and live as women.
Transition: This refers to the process during which transgender persons change their physical, social, and/or legal characteristics consistent with the affirmed gender identity. Prepubertal children may choose to transition socially.
Transsexual: This is an older term that originated in the medical and psychological communities to refer to individuals who have permanently transitioned through medical interventions or desired to do so.

exstrophy and penile agenesis, the occurrence of transgender male changes was significantly more prevalent than in the general population (30, 31). However, the fact that a high percentage of individuals with the same conditions did not change gender suggests that cultural factors may play a role as well.

With respect to genetics and gender identity, several studies have suggested heritability of GD/gender incongruence (32, 33). In particular, a study by Heylens *et al.* (33) demonstrated a 39.1% concordance rate for gender identity disorder (based on the DSM-IV criteria) in 23 monozygotic twin pairs but no concordance in 21 same-sex dizygotic or seven opposite-sex twin pairs. Although numerous investigators have sought to identify specific genes associated with GD/gender incongruence, such studies have been inconsistent and without strong statistical significance (34–38).

Studies focusing on brain structure suggest that the brain phenotypes of people with GD/gender incongruence differ in various ways from control males and females, but that there is not a complete sex reversal in brain structures (39).

In summary, although there is much that is still unknown with respect to gender identity and its expression, compelling studies support the concept that biologic factors, in addition to environmental factors, contribute to this fundamental aspect of human development.

Natural History of Children With GD/Gender Incongruence

With current knowledge, we cannot predict the psychosexual outcome for any specific child. Prospective follow-up studies show that childhood GD/gender incongruence does not invariably persist into adolescence and adulthood (so-called "desisters"). Combining all outcome studies to date, the GD/gender incongruence of a minority of prepubertal children appears to persist in adolescence (20, 40). In adolescence, a significant number of these desisters identify as homosexual or bisexual. It may be that children who only showed some gender nonconforming characteristics have been included in the follow-up studies, because the DSM-IV text revision criteria for a diagnosis were rather broad. However, the persistence of GD/gender incongruence into adolescence is more likely if it had been extreme in childhood (41, 42). With the newer, stricter criteria of the DSM-5 (Table 2), persistence rates may well be different in future studies.

1.0 Evaluation of Youth and Adults

Gender-affirming treatment is a multidisciplinary effort. After evaluation, education, and diagnosis, treatment may include mental health care, hormone therapy, and/or surgical therapy. Together with an MHP, hormone-prescribing clinicians should examine the psychosocial impact of the potential changes on people's lives, including mental health, friends, family, jobs, and their role in society. Transgender individuals should be encouraged to experience living in the new gender role and assess whether this improves their quality of life. Although the focus of this guideline is gender-affirming hormone therapy, collaboration with appropriate professionals responsible for each aspect of treatment maximizes a successful outcome.

Diagnostic assessment and mental health care

GD/gender incongruence may be accompanied with psychological or psychiatric problems (43–51). It is therefore necessary that clinicians who prescribe hormones and are involved in diagnosis and psychosocial assessment meet the following criteria: (1) are competent in using the DSM and/or the ICD for diagnostic purposes, (2) are able to diagnose GD/gender incongruence and make a distinction between GD/gender incongruence and conditions that have similar features (*e.g.*, body dysmorphic disorder), (3) are trained in diagnosing psychiatric conditions, (4) undertake or refer for appropriate treatment, (5) are able to do a psychosocial assessment of the patient's understanding, mental health, and social conditions that can impact gender-affirming hormone therapy, and (6) regularly attend relevant professional meetings.

Because of the psychological vulnerability of many individuals with GD/gender incongruence, it is important that mental health care is available before, during, and sometimes also after transitioning. For children and adolescents, an MHP who has training/experience in child and adolescent gender development (as well as child and adolescent psychopathology) should make the diagnosis, because assessing GD/gender incongruence in children and adolescents is often extremely complex.

During assessment, the clinician obtains information from the individual seeking gender-affirming treatment. In the case

Table 2. DSM-5 Criteria for Gender Dysphoria in Adolescents and Adults

A. A marked incongruence between one's experienced/expressed gender and natal gender of at least 6 mo in 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 designated gender)
   5. A strong desire to be treated as the other gender (or some alternative gender different from one's designated 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 designated gender)

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

Specify if:
   1. The condition exists with a disorder of sex development.
   2. The condition is posttransitional, in that 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 sex-related medical procedure or treatment regimen—namely, regular sex hormone treatment or gender reassignment surgery confirming the desired gender (*e.g.*, penectomy, vaginoplasty in natal males; mastectomy or phalloplasty in natal females).

Reference: American Psychiatric Association (14).

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of adolescents, the clinician also obtains information from the parents or guardians regarding various aspects of the child's general and psychosexual development and current functioning. On the basis of this information, the clinician:

- decides whether the individual fulfills criteria for treatment (see Tables 2 and 3) for GD/gender incongruence (DSM-5) or transsexualism (DSM-5 and/or ICD-10);
- informs the individual about the possibilities and limitations of various kinds of treatment (hormonal/surgical and nonhormonal), and if medical treatment is desired, provides correct information to prevent unrealistically high expectations;
- assesses whether medical interventions may result in unfavorable psychological and social outcomes.

In cases in which severe psychopathology, circumstances, or both seriously interfere with the diagnostic work or make satisfactory treatment unlikely, clinicians should assist the adolescent in managing these other issues. Literature on postoperative regret suggests that besides poor quality of surgery, severe psychiatric comorbidity and lack of support may interfere with positive outcomes (52–56).

For adolescents, the diagnostic procedure usually includes a complete psychodiagnostic assessment (57) and an assessment of the decision-making capability of the youth. An evaluation to assess the family's ability to endure stress, give support, and deal with the complexities of the adolescent's situation should be part of the diagnostic phase (58).

Social transitioning

A change in gender expression and role (which may involve living part time or full time in another gender role that is consistent with one's gender identity) may test the person's resolve, the capacity to function in the affirmed gender, and the adequacy of social, economic, and psychological supports. It assists both the individual and the clinician in their judgments about how to proceed (16). During social transitioning, the person's feelings about the social transformation (including coping with the responses of others) is a major focus of the counseling. The optimal timing for social transitioning may differ between individuals. Sometimes people wait until they start gender-affirming hormone treatment to make social transitioning easier, but individuals increasingly start social transitioning long before they receive medically supervised, gender-affirming hormone treatment.

Criteria

Adolescents and adults seeking gender-affirming hormone treatment and surgery should satisfy certain criteria before proceeding (16). Criteria for gender-affirming hormone therapy for adults are in Table 4, and criteria for gender-affirming hormone therapy for adolescents are in Table 5. Follow-up studies in adults meeting these criteria indicate a high satisfaction rate with treatment (59). However, the quality of evidence is usually low. A few follow-up studies on adolescents who fulfilled these criteria also indicated good treatment results (60–63).

Recommendations for Those Involved in the Gender-Affirming Hormone Treatment of Individuals With GD/Gender Incongruence

1.1. We advise that only trained MHPs who meet the following criteria should diagnose GD/gender incongruence in adults: (1) competence in using the DSM and/or the ICD for diagnostic purposes, (2) the ability to diagnose GD/gender incongruence and make a distinction between GD/gender incongruence and conditions that have similar features (*e.g.*, body dysmorphic disorder), (3) training in diagnosing psychiatric conditions, (4) the ability to undertake or refer for appropriate treatment, (5) the ability to psychosocially assess the person's understanding, mental health, and social conditions that can impact gender-affirming hormone therapy, and (6) a practice of regularly attending relevant professional meetings. (Ungraded Good Practice Statement)

1.2. We advise that only MHPs who meet the following criteria should diagnose GD/gender incongruence in children and adolescents: (1) training in child and adolescent developmental psychology and psychopathology, (2) competence in using the DSM and/or ICD for diagnostic

Table 3. ICD-10 Criteria for Transsexualism

Transsexualism (F64.0) has three criteria:
1. The desire to live and be accepted as a member of the opposite sex, usually accompanied by the wish to make his or her body as congruent as possible with the preferred sex through surgery and hormone treatments.
2. The transsexual identity has been present persistently for at least 2 y.
3. The disorder is not a symptom of another mental disorder or a genetic, DSD, or chromosomal abnormality.

Table 4. Criteria for Gender-Affirming Hormone Therapy for Adults

1. Persistent, well-documented gender dysphoria/gender incongruence
2. The capacity to make a fully informed decision and to consent for treatment
3. The age of majority in a given country (if younger, follow the criteria for adolescents)
4. Mental health concerns, if present, must be reasonably well controlled

Reproduced from World Professional Association for Transgender Health (16).

purposes, (3) the ability to make a distinction between GD/gender incongruence and conditions that have similar features (*e.g.*, body dysmorphic disorder), (4) training in diagnosing psychiatric conditions, (5) the ability to undertake or refer for appropriate treatment, (6) the ability to psychosocially assess the person's understanding and social conditions that can impact gender-affirming hormone therapy, (7) a practice of regularly attending relevant professional meetings, and (8) knowledge of the criteria for puberty blocking and gender-affirming hormone treatment in adolescents. (Ungraded Good Practice Statement)

Evidence

Individuals with gender identity issues may have psychological or psychiatric problems (43–48, 50, 51, 64, 65). It is therefore necessary that clinicians making the diagnosis are able to make a distinction between GD/gender incongruence and conditions that have similar features. Examples of conditions with similar features are body dysmorphic disorder, body identity integrity disorder (a condition in which individuals have a sense that their anatomical configuration as an able-bodied person is somehow wrong or inappropriate) (66), or certain forms of eunuchism (in which a person is preoccupied with or engages in castration and/or penectomy for

Table 5. Criteria for Gender-Affirming Hormone Therapy for Adolescents

Adolescents are eligible for GnRH agonist treatment if:

1. A qualified MHP has confirmed that:
   - the adolescent has demonstrated a long-lasting and intense pattern of gender nonconformity or gender dysphoria (whether suppressed or expressed),
   - gender dysphoria worsened with the onset of puberty,
   - any coexisting psychological, medical, or social problems that could interfere with treatment (*e.g.*, that may compromise treatment adherence) have been addressed, such that the adolescent's situation and functioning are stable enough to start treatment,
   - the adolescent has sufficient mental capacity to give informed consent to this (reversible) treatment,
2. And the adolescent:
   - has been informed of the effects and side effects of treatment (including potential loss of fertility if the individual subsequently continues with sex hormone treatment) and options to preserve fertility,
   - has given informed consent and (particularly when the adolescent has not reached the age of legal medical consent, depending on applicable legislation) the parents or other caretakers or guardians have consented to the treatment and are involved in supporting the adolescent throughout the treatment process,
3. And a pediatric endocrinologist or other clinician experienced in pubertal assessment
   - agrees with the indication for GnRH agonist treatment,
   - has confirmed that puberty has started in the adolescent (Tanner stage ≥G2/B2),
   - has confirmed that there are no medical contraindications to GnRH agonist treatment.

Adolescents are eligible for subsequent sex hormone treatment if:

1. A qualified MHP has confirmed:
   - the persistence of gender dysphoria,
   - any coexisting psychological, medical, or social problems that could interfere with treatment (*e.g.*, that may compromise treatment adherence) have been addressed, such that the adolescent's situation and functioning are stable enough to start sex hormone treatment,
   - the adolescent has sufficient mental capacity (which most adolescents have by age 16 years) to estimate the consequences of this (partly) irreversible treatment, weigh the benefits and risks, and give informed consent to this (partly) irreversible treatment,
2. And the adolescent:
   - has been informed of the (irreversible) effects and side effects of treatment (including potential loss of fertility and options to preserve fertility),
   - has given informed consent and (particularly when the adolescent has not reached the age of legal medical consent, depending on applicable legislation) the parents or other caretakers or guardians have consented to the treatment and are involved in supporting the adolescent throughout the treatment process,
3. And a pediatric endocrinologist or other clinician experienced in pubertal induction:
   - agrees with the indication for sex hormone treatment,
   - has confirmed that there are no medical contraindications to sex hormone treatment.

Reproduced from World Professional Association for Transgender Health (16).

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reasons that are not gender identity related) (11). Clinicians should also be able to diagnose psychiatric conditions accurately and ensure that these conditions are treated appropriately, particularly when the conditions may complicate treatment, affect the outcome of gender-affirming treatment, or be affected by hormone use.

Values and preferences

The task force placed a very high value on avoiding harm from hormone treatment in individuals who have conditions other than GD/gender incongruence and who may not benefit from the physical changes associated with this treatment and placed a low value on any potential benefit these persons believe they may derive from hormone treatment. This justifies the good practice statement.

1.3. We advise that decisions regarding the social transition of prepubertal youths with GD/gender incongruence are made with the assistance of an MHP or another experienced professional. (Ungraded Good Practice Statement).

1.4. We recommend against puberty blocking and gender-affirming hormone treatment in prepubertal children with GD/gender incongruence. (1 ⊕◯◯◯)

Evidence

In most children diagnosed with GD/gender incongruence, it did not persist into adolescence. The percentages differed among studies, probably dependent on which version of the DSM clinicians used, the patient's age, the recruitment criteria, and perhaps cultural factors. However, the large majority (about 85%) of prepubertal children with a childhood diagnosis did not remain GD/gender incongruent in adolescence (20). If children have completely socially transitioned, they may have great difficulty in returning to the original gender role upon entering puberty (40). Social transition is associated with the persistence of GD/gender incongruence as a child progresses into adolescence. It may be that the presence of GD/gender incongruence in prepubertal children is the earliest sign that a child is destined to be transgender as an adolescent/adult (20). However, social transition (in addition to GD/gender incongruence) has been found to contribute to the likelihood of persistence.

This recommendation, however, does not imply that children should be discouraged from showing gender-variant behaviors or should be punished for exhibiting such behaviors. In individual cases, an early complete social transition may result in a more favorable outcome, but there are currently no criteria to identify the GD/gender-incongruent children to whom this applies. At the present time, clinical experience suggests that persistence of GD/gender incongruence can only be reliably assessed after the first signs of puberty.

Values and preferences

The task force placed a high value on avoiding harm with gender-affirming hormone therapy in prepubertal children with GD/gender incongruence. This justifies the strong recommendation in the face of low-quality evidence.

1.5. We recommend that clinicians inform and counsel all individuals seeking gender-affirming medical treatment regarding options for fertility preservation prior to initiating puberty suppression in adolescents and prior to treating with hormonal therapy of the affirmed gender in both adolescents and adults. (1 ⊕⊕⊕◯)

Remarks

Persons considering hormone use for gender affirmation need adequate information about this treatment in general and about fertility effects of hormone treatment in particular to make an informed and balanced decision (67, 68). Because young adolescents may not feel qualified to make decisions about fertility and may not fully understand the potential effects of hormonal interventions, consent and protocol education should include parents, the referring MHP(s), and other members of the adolescent's support group. To our knowledge, there are no formally evaluated decision aids available to assist in the discussion and decision regarding the future fertility of adolescents or adults beginning gender-affirming treatment.

Treating early pubertal youth with GnRH analogs will temporarily impair spermatogenesis and oocyte maturation. Given that an increasing number of transgender youth want to preserve fertility potential, delaying or temporarily discontinuing GnRH analogs to promote gamete maturation is an option. This option is often not preferred, because mature sperm production is associated with later stages of puberty and with the significant development of secondary sex characteristics.

For those designated male at birth with GD/gender incongruence and who are in early puberty, sperm production and the development of the reproductive tract are insufficient for the cryopreservation of sperm. However, prolonged pubertal suppression using GnRH analogs is reversible and clinicians should inform these individuals that sperm production can be initiated following prolonged gonadotropin suppression. This can be accomplished by spontaneous gonadotropin recovery after

cessation of GnRH analogs or by gonadotropin treatment and will probably be associated with physical manifestations of testosterone production, as stated above. Note that there are no data in this population concerning the time required for sufficient spermatogenesis to collect enough sperm for later fertility. In males treated for precocious puberty, spermarche was reported 0.7 to 3 years after cessation of GnRH analogs (69). In adult men with gonadotropin deficiency, sperm are noted in seminal fluid by 6 to 12 months of gonadotropin treatment. However, sperm numbers when partners of these patients conceive are far below the "normal range" (70, 71).

In girls, no studies have reported long-term, adverse effects of pubertal suppression on ovarian function after treatment cessation (72, 73). Clinicians should inform adolescents that no data are available regarding either time to spontaneous ovulation after cessation of GnRH analogs or the response to ovulation induction following prolonged gonadotropin suppression.

In males with GD/gender incongruence, when medical treatment is started in a later phase of puberty or in adulthood, spermatogenesis is sufficient for cryopreservation and storage of sperm. *In vitro* spermatogenesis is currently under investigation. Restoration of spermatogenesis after prolonged estrogen treatment has not been studied.

In females with GD/gender incongruence, the effect of prolonged treatment with exogenous testosterone on ovarian function is uncertain. There have been reports of an increased incidence of polycystic ovaries in transgender males, both prior to and as a result of androgen treatment (74–77), although these reports were not confirmed by others (78). Pregnancy has been reported in transgender males who have had prolonged androgen treatment and have discontinued testosterone but have not had genital surgery (79, 80). A reproductive endocrine gynecologist can counsel patients before gender-affirming hormone treatment or surgery regarding potential fertility options (81). Techniques for cryopreservation of oocytes, embryos, and ovarian tissue continue to improve, and oocyte maturation of immature tissue is being studied (82).

2.0 Treatment of Adolescents

During the past decade, clinicians have progressively acknowledged the suffering of young adolescents with GD/gender incongruence. In some forms of GD/gender incongruence, psychological interventions may be useful and sufficient. However, for many adolescents with GD/gender incongruence, the pubertal physical changes are unbearable. As early medical intervention may prevent psychological harm, various clinics have decided to start treating young adolescents with GD/gender incongruence with puberty-suppressing medication (a GnRH analog). As compared with starting gender-affirming treatment long after the first phases of puberty, a benefit of pubertal suppression at early puberty may be a better psychological and physical outcome.

In girls, the first physical sign of puberty is the budding of the breasts followed by an increase in breast and fat tissue. Breast development is also associated with the pubertal growth spurt, and menarche occurs ~2 years later. In boys, the first physical change is testicular growth. A testicular volume ≥4 mL is seen as consistent with the initiation of physical puberty. At the beginning of puberty, estradiol and testosterone levels are still low and are best measured in the early morning with an ultrasensitive assay. From a testicular volume of 10 mL, daytime testosterone levels increase, leading to virilization (83). Note that pubic hair and/or axillary hair/odor may not reflect the onset of gonadarche; instead, it may reflect adrenarche alone.

2.1. We suggest that adolescents who meet diagnostic criteria for GD/gender incongruence, fulfill criteria for treatment (Table 5), and are requesting treatment should initially undergo treatment to suppress pubertal development. (2 ⊕⊕◯◯)

2.2. We suggest that clinicians begin pubertal hormone suppression after girls and boys first exhibit physical changes of puberty (Tanner stages G2/B2). (2 ⊕⊕◯◯)

Evidence

Pubertal suppression can expand the diagnostic phase by a long period, giving the subject more time to explore options and to live in the experienced gender before making a decision to proceed with gender-affirming sex hormone treatments and/or surgery, some of which is irreversible (84, 85). Pubertal suppression is fully reversible, enabling full pubertal development in the natal gender, after cessation of treatment, if appropriate. The experience of full endogenous puberty is an undesirable condition for the GD/gender-incongruent individual and may seriously interfere with healthy psychological functioning and well-being. Treating GD/gender-incongruent adolescents entering puberty with GnRH analogs has been shown to improve psychological functioning in several domains (86).

Another reason to start blocking pubertal hormones early in puberty is that the physical outcome is improved compared with initiating physical transition after puberty has been completed (60, 62). Looking like a man or woman when living as the opposite sex creates difficult

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barriers with enormous life-long disadvantages. We therefore advise starting suppression in early puberty to prevent the irreversible development of undesirable secondary sex characteristics. However, adolescents with GD/gender incongruence should experience the first changes of their endogenous spontaneous puberty, because their emotional reaction to these first physical changes has diagnostic value in establishing the persistence of GD/gender incongruence (85). Thus, Tanner stage 2 is the optimal time to start pubertal suppression. However, pubertal suppression treatment in early puberty will limit the growth of the penis and scrotum, which will have a potential effect on future surgical treatments (87).

Clinicians can also use pubertal suppression in adolescents in later pubertal stages to stop menses in transgender males and prevent facial hair growth in transgender females. However, in contrast to the effects in early pubertal adolescents, physical sex characteristics (such as more advanced breast development in transgender boys and lowering of the voice and outgrowth of the jaw and brow in transgender girls) are not reversible.

Values and preferences

These recommendations place a high value on avoiding an unsatisfactory physical outcome when secondary sex characteristics have become manifest and irreversible, a higher value on psychological well-being, and a lower value on avoiding potential harm from early pubertal suppression.

Remarks

Table 6 lists the Tanner stages of breast and male genital development. Careful documentation of hallmarks of pubertal development will ensure precise timing when initiating pubertal suppression once puberty has started. Clinicians can use pubertal LH and sex steroid levels to confirm that puberty has progressed sufficiently before starting pubertal suppression (88). Reference ranges for sex steroids by Tanner stage may vary depending on the assay used. Ultrasensitive sex steroid and gonadotropin assays will help clinicians document early pubertal changes.

Irreversible and, for GD/gender-incongruent adolescents, undesirable sex characteristics in female puberty are breasts, female body habitus, and, in some cases, relative short stature. In male puberty, they are a prominent Adam's apple; low voice; male bone configuration, such as a large jaw, big feet and hands, and tall stature; and male hair pattern on the face and extremities.

2.3. We recommend that, where indicated, GnRH analogues are used to suppress pubertal hormones. (1 ⊕⊕◯◯)

Evidence

Clinicians can suppress pubertal development and gonadal function most effectively via gonadotropin suppression using GnRH analogs. GnRH analogs are long-acting agonists that suppress gonadotropins by GnRH receptor desensitization after an initial increase of gonadotropins during ~10 days after the first and (to a lesser degree) the second injection (89). Antagonists immediately suppress pituitary gonadotropin secretion (90, 91). Long-acting GnRH analogs are the currently preferred treatment option. Clinicians may consider long-acting GnRH antagonists when evidence on their safety and efficacy in adolescents becomes available.

During GnRH analog treatment, slight development of secondary sex characteristics may regress, and in a later phase of pubertal development, it will stop. In girls, breast tissue will become atrophic, and menses will stop. In boys, virilization will stop, and testicular volume may decrease (92).

An advantage of using GnRH analogs is the reversibility of the intervention. If, after extensive exploration of his/her transition wish, the individual no longer desires transition, they can discontinue pubertal suppression. In subjects with

Table 6. Tanner Stages of Breast Development and Male External Genitalia

The description of Tanner stages for breast development:
1. Prepubertal
2. Breast and papilla elevated as small mound; areolar diameter increased
3. Breast and areola enlarged, no contour separation
4. Areola and papilla form secondary mound
5. Mature, nipple projects, areola part of general breast contour

For penis and testes:
1. Prepubertal, testicular volume <4 mL
2. Slight enlargement of penis, enlarged scrotum, pink, texture altered, testes 4–6 mL
3. Penis longer, testes larger (8–12 mL)
4. Penis and glans larger, including increase in breadth, testes larger (12–15 mL), scrotum dark
5. Penis adult size; testicular volume > 15 ml

Adapted from Lawrence (56).

precocious puberty, spontaneous pubertal development has been shown to resume after patients discontinue taking GnRH analogs (93).

Recommendations 2.1 to 2.3 are supported by a prospective follow-up study from The Netherlands. This report assessed mental health outcomes in 55 transgender adolescents/young adults (22 transgender females and 33 transgender males) at three time points: (1) before the start of GnRH agonist (average age of 14.8 years at start of treatment), (2) at initiation of gender-affirming hormones (average age of 16.7 years at start of treatment), and (3) 1 year after "gender-reassignment surgery" (average age of 20.7 years) (63). Despite a decrease in depression and an improvement in general mental health functioning, GD/gender incongruence persisted through pubertal suppression, as previously reported (86). However, following sex hormone treatment and gender-reassignment surgery, GD/gender incongruence was resolved and psychological functioning steadily improved (63). Furthermore, well-being was similar to or better than that reported by age-matched young adults from the general population, and none of the study participants regretted treatment. This study represents the first long-term follow-up of individuals managed according to currently existing clinical practice guidelines for transgender youth, and it underscores the benefit of the multidisciplinary approach pioneered in The Netherlands; however, further studies are needed.

Side effects

The primary risks of pubertal suppression in GD/gender-incongruent adolescents may include adverse effects on bone mineralization (which can theoretically be reversed with sex hormone treatment), compromised fertility if the person subsequently is treated with sex hormones, and unknown effects on brain development. Few data are available on the effect of GnRH analogs on BMD in adolescents with GD/gender incongruence. Initial data in GD/gender-incongruent subjects demonstrated no change of absolute areal BMD during 2 years of GnRH analog therapy but a decrease in BMD z scores (85). A recent study also suggested suboptimal bone mineral accrual during GnRH analog treatment. The study reported a decrease in areal BMD z scores and of bone mineral apparent density z scores (which takes the size of the bone into account) in 19 transgender males treated with GnRH analogs from a mean age of 15.0 years (standard deviation = 2.0 years) for a median duration of 1.5 years (0.3 to 5.2 years) and in 15 transgender females treated from 14.9 (±1.9) years for 1.3 years (0.5 to 3.8 years), although not all changes were statistically significant (94). There was incomplete catch-up at age 22 years after sex hormone treatment from age 16.6 (±1.4) years for a median duration of 5.8 years (3.0 to 8.0 years) in transgender females and from age 16.4 (±2.3) years for 5.4 years (2.8 to 7.8 years) in transgender males. Little is known about more prolonged use of GnRH analogs. Researchers reported normal BMD z scores at age 35 years in one individual who used GnRH analogs from age 13.7 years until age 18.6 years before initiating sex hormone treatment (65).

Additional data are available from individuals with late puberty or GnRH analog treatment of other indications. Some studies reported that men with constitutionally delayed puberty have decreased BMD in adulthood (95). However, other studies reported that these men have normal BMD (96, 97). Treating adults with GnRH analogs results in a decrease of BMD (98). In children with central precocious puberty, treatment with GnRH analogs has been found to result in a decrease of BMD during treatment by some (99) but not others (100). Studies have reported normal BMD after discontinuing therapy (69, 72, 73, 101, 102). In adolescents treated with growth hormone who are small for gestational age and have normal pubertal timing, 2-year GnRH analog treatments did not adversely affect BMD (103). Calcium supplementation may be beneficial in optimizing bone health in GnRH analog–treated individuals (104). There are no studies of vitamin D supplementation in this context, but clinicians should offer supplements to vitamin D–deficient adolescents. Physical activity, especially during growth, is important for bone mass in healthy individuals (103) and is therefore likely to be beneficial for bone health in GnRH analog–treated subjects.

GnRH analogs did not induce a change in body mass index standard deviation score in GD/gender-incongruent adolescents (94) but caused an increase in fat mass and decrease in lean body mass percentage (92). Studies in girls treated for precocious puberty also reported a stable body mass index standard deviation score during treatment (72) and body mass index and body composition comparable to controls after treatment (73).

Arterial hypertension has been reported as an adverse effect in a few girls treated with GnRH analogs for precocious/early puberty (105, 106). Blood pressure monitoring before and during treatment is recommended.

Individuals may also experience hot flashes, fatigue, and mood alterations as a consequence of pubertal suppression. There is no consensus on treatment of these side effects in this context.

It is recommended that any use of pubertal blockers (and subsequent use of sex hormones, as detailed below) include a discussion about implications for fertility (see recommendation 1.3). Transgender adolescents may

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want to preserve fertility, which may be otherwise compromised if puberty is suppressed at an early stage and the individual completes phenotypic transition with the use of sex hormones.

Limited data are available regarding the effects of GnRH analogs on brain development. A single cross-sectional study demonstrated no compromise of executive function (107), but animal data suggest there may be an effect of GnRH analogs on cognitive function (108).

Values and preferences

Our recommendation of GnRH analogs places a higher value on the superior efficacy, safety, and reversibility of the pubertal hormone suppression achieved (as compared with the alternatives) and a relatively lower value on limiting the cost of therapy. Of the available alternatives, depot and oral progestin preparations are effective. Experience with this treatment dates back prior to the emergence of GnRH analogs for treating precocious puberty in papers from the 1960s and early 1970s (109–112). These compounds are usually safe, but some side effects have been reported (113–115). Only two recent studies involved transgender youth (116, 117). One of these studies described the use of oral lynestrenol monotherapy followed by the addition of testosterone treatment in transgender boys who were at Tanner stage B4 or further at the start of treatment (117). They found lynestrenol safe, but gonadotropins were not fully suppressed. The study reported metrorrhagia in approximately half of the individuals, mainly in the first 6 months. Acne, headache, hot flashes, and fatigue were other frequent side effects. Another progestin that has been studied in the United States is medroxyprogesterone. This agent is not as effective as GnRH analogs in lowering endogenous sex hormones either and may be associated with other side effects (116). Progestin preparations may be an acceptable treatment for persons without access to GnRH analogs or with a needle phobia. If GnRH analog treatment is not available (insurance denial, prohibitive cost, or other reasons), postpubertal, transgender female adolescents may be treated with an antiandrogen that directly suppresses androgen synthesis or action (see adult section).

Remarks

Measurements of gonadotropin and sex steroid levels give precise information about gonadal axis suppression, although there is insufficient evidence for any specific short-term monitoring scheme in children treated with GnRH analogs (88). If the gonadal axis is not completely suppressed—as evidenced by (for example) menses, erections, or progressive hair growth—the interval of GnRH analog treatment can be shortened or the dose increased. During treatment, adolescents should be monitored for negative effects of delaying puberty, including a halted growth spurt and impaired bone mineral accretion. Table 7 illustrates a suggested clinical protocol.

Anthropometric measurements and X-rays of the left hand to monitor bone age are informative for evaluating growth. To assess BMD, clinicians can perform dual-energy X-ray absorptiometry scans.

2.4. In adolescents who request sex hormone treatment (given this is a partly irreversible treatment), we recommend initiating treatment using a gradually increasing dose schedule (see Table 8) after a multidisciplinary team of medical and MHPs has confirmed the persistence of GD/gender incongruence and sufficient mental capacity to give informed consent, which most adolescents have by age 16 years (Table 5). (1 ⊕◯◯◯)

2.5. We recognize that there may be compelling reasons to initiate sex hormone treatment prior to the age of 16 years in some adolescents with GD/gender incongruence, even though there are minimal published studies of gender-affirming hormone treatments administered before age 13.5 to 14 years. As with the care of adolescents ≥16 years of age, we recommend that an expert multidisciplinary team of medical and MHPs manage this treatment. (1 ⊕◯◯◯)

2.6. We suggest monitoring clinical pubertal development every 3 to 6 months and laboratory parameters every 6 to 12 months during sex hormone treatment (Table 9). (2 ⊕⊕◯◯)

Table 7. Baseline and Follow-Up Protocol During Suppression of Puberty

Every 3–6 mo
Anthropometry: height, weight, sitting height, blood pressure, Tanner stages
Every 6–12 mo
Laboratory: LH, FSH, E2/T, 25OH vitamin D
Every 1–2 y
Bone density using DXA
Bone age on X-ray of the left hand (if clinically indicated)

Adapted from Hembree *et al.* (118).

Abbreviations: DXA, dual-energy X-ray absorptiometry; E2, estradiol; FSH, follicle stimulating hormone; LH, luteinizing hormone; T, testosterone.

Table 8. Protocol Induction of Puberty

Induction of female puberty with oral 17β-estradiol, increasing the dose every 6 mo:
- 5 μg/kg/d
- 10 μg/kg/d
- 15 μg/kg/d
- 20 μg/kg/d
- Adult dose = 2–6 mg/d
- *In postpubertal transgender female adolescents, the dose of 17β-estradiol can be increased more rapidly:*
  - 1 mg/d for 6 mo
  - 2 mg/d

Induction of female puberty with transdermal 17β-estradiol, increasing the dose every 6 mo (new patch is placed every 3.5 d):
- 6.25–12.5 μg/24 h (cut 25-μg patch into quarters, then halves)
- 25 μg/24 h
- 37.5 μg/24 h
- Adult dose = 50–200 μg/24 h
- *For alternatives once at adult dose, see Table 11.*
- *Adjust maintenance dose to mimic physiological estradiol levels (see Table 15).*

Induction of male puberty with testosterone esters increasing the dose every 6 mo (IM or SC):
- 25 mg/m^2/2 wk (or alternatively, half this dose weekly, or double the dose every 4 wk)
- 50 mg/m^2/2 wk
- 75 mg/m^2/2 wk
- 100 mg/m^2/2 wk
- Adult dose = 100–200 mg every 2 wk
- *In postpubertal transgender male adolescents the dose of testosterone esters can be increased more rapidly:*
  - 75 mg/2 wk for 6 mo
  - 125 mg/2 wk
- *For alternatives once at adult dose, see Table 11.*
- *Adjust maintenance dose to mimic physiological testosterone levels (see Table 14).*

Adapted from Hembree *et al.* (118).

Abbreviations: IM, intramuscularly; SC, subcutaneously.

Evidence

Adolescents develop competence in decision making at their own pace. Ideally, the supervising medical professionals should individually assess this competence, although no objective tools to make such an assessment are currently available.

Many adolescents have achieved a reasonable level of competence by age 15 to 16 years (119), and in many countries 16-year-olds are legally competent with regard to medical decision making (120). However, others believe that although some capacities are generally achieved before age 16 years, other abilities (such as good risk assessment) do not develop until well after 18 years (121). They suggest that health care procedures should be divided along a matrix of relative risk, so that younger adolescents can be allowed to decide about low-risk procedures, such as most diagnostic tests and common therapies, but not about high-risk procedures, such as most surgical procedures (121).

Currently available data from transgender adolescents support treatment with sex hormones starting at age 16 years (63, 122). However, some patients may incur potential risks by waiting until age 16 years. These include the potential risk to bone health if puberty is suppressed

Table 9. Baseline and Follow-up Protocol During Induction of Puberty

Every 3–6 mo
- •Anthropometry: height, weight, sitting height, blood pressure, Tanner stages

Every 6–12 mo
- •In transgender males: hemoglobin/hematocrit, lipids, testosterone, 25OH vitamin D
- •In transgender females: prolactin, estradiol, 25OH vitamin D

Every 1–2 y
- •BMD using DXA
- •Bone age on X-ray of the left hand (if clinically indicated)

BMD should be monitored into adulthood (until the age of 25–30 y or until peak bone mass has been reached).
For recommendations on monitoring once pubertal induction has been completed, see Tables 14 and 15.

Adapted from Hembree *et al.* (118).

Abbreviation: DXA, dual-energy X-ray absorptiometry.

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for 6 to 7 years before initiating sex hormones (*e.g.*, if someone reached Tanner stage 2 at age 9-10 years old). Additionally, there may be concerns about inappropriate height and potential harm to mental health (emotional and social isolation) if initiation of secondary sex characteristics must wait until the person has reached 16 years of age. However, only minimal data supporting earlier use of gender-affirming hormones in transgender adolescents currently exist (63). Clearly, long-term studies are needed to determine the optimal age of sex hormone treatment in GD/gender-incongruent adolescents.

The MHP who has followed the adolescent during GnRH analog treatment plays an essential role in assessing whether the adolescent is eligible to start sex hormone therapy and capable of consenting to this treatment (Table 5). Support of the family/environment is essential. Prior to the start of sex hormones, clinicians should discuss the implications for fertility (see recommendation 1.5). Throughout pubertal induction, an MHP and a pediatric endocrinologist (or other clinician competent in the evaluation and induction of pubertal development) should monitor the adolescent. In addition to monitoring therapy, it is also important to pay attention to general adolescent health issues, including healthy life style choices, such as not smoking, contraception, and appropriate vaccinations (*e.g.*, human papillomavirus).

For the induction of puberty, clinicians can use a similar dose scheme for hypogonadal adolescents with GD/gender incongruence as they use in other individuals with hypogonadism, carefully monitoring for desired and undesired effects (Table 8). In transgender female adolescents, transdermal 17β-estradiol may be an alternative for oral 17β-estradiol. It is increasingly used for pubertal induction in hypogonadal females. However, the absence of low-dose estrogen patches may be a problem. As a result, individuals may need to cut patches to size themselves to achieve appropriate dosing (123). In transgender male adolescents, clinicians can give testosterone injections intramuscularly or subcutaneously (124, 125).

When puberty is initiated with a gradually increasing schedule of sex steroid doses, the initial levels will not be high enough to suppress endogenous sex steroid secretion. Gonadotropin secretion and endogenous production of testosterone may resume and interfere with the effectiveness of estrogen treatment, in transgender female adolescents (126, 127). Therefore, continuation of GnRH analog treatment is advised until gonadectomy. Given that GD/gender-incongruent adolescents may opt not to have gonadectomy, long-term studies are necessary to examine the potential risks of prolonged GnRH analog treatment. Alternatively, in transgender male adolescents, GnRH analog treatment can be discontinued once an adult dose of testosterone has been reached and the individual is well virilized. If uterine bleeding occurs, a progestin can be added. However, the combined use of a GnRH analog (for ovarian suppression) and testosterone may enable phenotypic transition with a lower dose of testosterone in comparison with testosterone alone. If there is a wish or need to discontinue GnRH analog treatment in transgender female adolescents, they may be treated with an antiandrogen that directly suppresses androgen synthesis or action (see section 3.0 "Hormonal Therapy for Transgender Adults").

Values and preferences

The recommendation to initiate pubertal induction only when the individual has sufficient mental capacity (roughly age 16 years) to give informed consent for this partly irreversible treatment places a higher value on the ability of the adolescent to fully understand and oversee the partially irreversible consequences of sex hormone treatment and to give informed consent. It places a lower value on the possible negative effects of delayed puberty. We may not currently have the means to weigh adequately the potential benefits of waiting until around age 16 years to initiate sex hormones vs the potential risks/harm to BMD and the sense of social isolation from having the timing of puberty be so out of sync with peers (128).

Remarks

Before starting sex hormone treatment, effects on fertility and options for fertility preservation should be discussed. Adult height may be a concern in transgender adolescents. In a transgender female adolescent, clinicians may consider higher doses of estrogen or a more rapid tempo of dose escalation during pubertal induction. There are no established treatments yet to augment adult height in a transgender male adolescent with open epiphyses during pubertal induction. It is not uncommon for transgender adolescents to present for clinical services after having completed or nearly completed puberty. In such cases, induction of puberty with sex hormones can be done more rapidly (see Table 8). Additionally, an adult dose of testosterone in transgender male adolescents may suffice to suppress the gonadal axis without the need to use a separate agent. At the appropriate time, the multidisciplinary team should adequately prepare the adolescent for transition to adult care.

3.0 Hormonal Therapy for Transgender Adults

The two major goals of hormonal therapy are (1) to reduce endogenous sex hormone levels, and thus reduce

the secondary sex characteristics of the individual's designated gender, and (2) to replace endogenous sex hormone levels consistent with the individual's gender identity by using the principles of hormone replacement treatment of hypogonadal patients. The timing of these two goals and the age at which to begin treatment with the sex hormones of the chosen gender is codetermined in collaboration with both the person pursuing transition and the health care providers. The treatment team should include a medical provider knowledgeable in transgender hormone therapy, an MHP knowledgeable in GD/gender incongruence and the mental health concerns of transition, and a primary care provider able to provide care appropriate for transgender individuals. The physical changes induced by this sex hormone transition are usually accompanied by an improvement in mental well-being (129, 130).

3.1. We recommend that clinicians confirm the diagnostic criteria of GD/gender incongruence and the criteria for the endocrine phase of gender transition before beginning treatment. (1 ⊕⊕⊕◯)

3.2. We recommend that clinicians evaluate and address medical conditions that can be exacerbated by hormone depletion and treatment with sex hormones of the affirmed gender before beginning treatment (Table 10). (1 ⊕⊕⊕◯)

3.3. We suggest that clinicians measure hormone levels during treatment to ensure that endogenous sex steroids are suppressed and administered sex steroids are maintained in the normal physiologic range for the affirmed gender. (2 ⊕⊕◯◯)

Evidence

It is the responsibility of the treating clinician to confirm that the person fulfills criteria for treatment. The treating clinician should become familiar with the terms and criteria presented in Tables 1–5 and take a thorough history from the patient in collaboration with the other members of the treatment team. The treating clinician must ensure that the desire for transition is appropriate; the consequences, risks, and benefits of treatment are well understood; and the desire for transition persists. They also need to discuss fertility preservation options (see recommendation 1.3) (67, 68).

Transgender males

Clinical studies have demonstrated the efficacy of several different androgen preparations to induce masculinization in transgender males (Appendix A) (113, 114, 131–134). Regimens to change secondary sex characteristics follow the general principle of hormone replacement treatment of male hypogonadism (135). Clinicians can use either parenteral or transdermal preparations to achieve testosterone values in the normal male range (this is dependent on the specific assay, but is typically 320 to 1000 ng/dL) (Table 11) (136). Sustained supraphysiologic levels of testosterone increase the risk of adverse reactions (see section 4.0 "Adverse Outcome Prevention and Long-Term Care") and should be avoided.

Similar to androgen therapy in hypogonadal men, testosterone treatment in transgender males results in increased muscle mass and decreased fat mass, increased facial hair and acne, male pattern baldness in those genetically predisposed, and increased sexual desire (137).

Table 10. Medical Risks Associated With Sex Hormone Therapy

Transgender female: estrogen
Very high risk of adverse outcomes:
•Thromboembolic disease
Moderate risk of adverse outcomes:
•Macroprolactinoma
•Breast cancer
•Coronary artery disease
•Cerebrovascular disease
•Cholelithiasis
•Hypertriglyceridemia
Transgender male: testosterone
Very high risk of adverse outcomes:
•Erythrocytosis (hematocrit > 50%)
Moderate risk of adverse outcomes
•Severe liver dysfunction (transaminases > threefold upper limit of normal)
•Coronary artery disease
•Cerebrovascular disease
•Hypertension
•Breast or uterine cancer

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Table 11. Hormone Regimens in Transgender Persons

Transgender females[a]	
Estrogen	
Oral	
Estradiol	2.0–6.0 mg/d
Transdermal	
Estradiol transdermal patch (New patch placed every 3–5 d)	0.025–0.2 mg/d
Parenteral	
Estradiol valerate or cypionate	5–30 mg IM every 2 wk 2–10 mg IM every week
Anti-androgens	
Spironolactone	100–300 mg/d
Cyproterone acetate[b]	25–50 mg/d
GnRH agonist	3.75 mg SQ (SC) monthly 11.25 mg SQ (SC) 3-monthly
Transgender males	
Testosterone	
Parenteral testosterone	
Testosterone enanthate or cypionate	100–200 mg SQ (IM) every 2 wk or SQ (SC) 50% per week
Testosterone undecanoate[c]	1000 mg every 12 wk
Transdermal testosterone	
Testosterone gel 1.6%[d]	50–100 mg/d
Testosterone transdermal patch	2.5–7.5 mg/d

Abbreviations: IM, intramuscularly; SQ, sequentially; SC, subcutaneously.

[a] Estrogens used with or without antiandrogens or GnRH agonist.

[b] Not available in the United States.

[c] One thousand milligrams initially followed by an injection at 6 wk then at 12-wk intervals.

[d] Avoid cutaneous transfer to other individuals.

In transgender males, testosterone will result in clitoromegaly, temporary or permanent decreased fertility, deepening of the voice, cessation of menses (usually), and a significant increase in body hair, particularly on the face, chest, and abdomen. Cessation of menses may occur within a few months with testosterone treatment alone, although high doses of testosterone may be required. If uterine bleeding continues, clinicians may consider the addition of a progestational agent or endometrial ablation (138). Clinicians may also administer GnRH analogs or depot medroxyprogesterone to stop menses prior to testosterone treatment.

Transgender females

The hormone regimen for transgender females is more complex than the transgender male regimen (Appendix B). Treatment with physiologic doses of estrogen alone is insufficient to suppress testosterone levels into the normal range for females (139). Most published clinical studies report the need for adjunctive therapy to achieve testosterone levels in the female range (21, 113, 114, 132–134, 139, 140).

Multiple adjunctive medications are available, such as progestins with antiandrogen activity and GnRH agonists (141). Spironolactone works by directly blocking androgens during their interaction with the androgen receptor (114, 133, 142). It may also have estrogenic activity (143). Cyproterone acetate, a progestational compound with antiandrogenic properties (113, 132, 144), is widely used in Europe. 5α-Reductase inhibitors do not reduce testosterone levels and have adverse effects (145).

Dittrich *et al.* (141) reported that monthly doses of the GnRH agonist goserelin acetate in combination with estrogen were effective in reducing testosterone levels with a low incidence of adverse reactions in 60 transgender females. Leuprolide and transdermal estrogen were as effective as cyproterone and transdermal estrogen in a comparative retrospective study (146).

Patients can take estrogen as oral conjugated estrogens, oral 17β-estradiol, or transdermal 17β-estradiol. Among estrogen options, the increased risk of thromboembolic events associated with estrogens in general seems most concerning with ethinyl estradiol specifically (134, 140, 141), which is why we specifically suggest that it not be used in any transgender treatment plan. Data distinguishing among other estrogen options are less well established although there is some thought that oral routes of administration are more thrombogenic due to the "first pass effect" than are transdermal and parenteral routes, and that the risk of thromboembolic events is dose-dependent. Injectable estrogen and sublingual

estrogen may benefit from avoiding the first pass effect, but they can result in more rapid peaks with greater overall periodicity and thus are more difficult to monitor (147, 148). However, there are no data demonstrating that increased periodicity is harmful otherwise.

Clinicians can use serum estradiol levels to monitor oral, transdermal, and intramuscular estradiol. Blood tests cannot monitor conjugated estrogens or synthetic estrogen use. Clinicians should measure serum estradiol and serum testosterone and maintain them at the level for premenopausal females (100 to 200 pg/mL and <50 ng/dL, respectively). The transdermal preparations and injectable estradiol cypionate or valerate preparations may confer an advantage in older transgender females who may be at higher risk for thromboembolic disease (149).

Values

Our recommendation to maintain levels of gender-affirming hormones in the normal adult range places a high value on the avoidance of the long-term complications of pharmacologic doses. Those patients receiving endocrine treatment who have relative contraindications to hormones should have an in-depth discussion with their physician to balance the risks and benefits of therapy.

Remarks

Clinicians should inform all endocrine-treated individuals of all risks and benefits of gender-affirming hormones prior to initiating therapy. Clinicians should strongly encourage tobacco use cessation in transgender females to avoid increased risk of VTE and cardiovascular complications. We strongly discourage the unsupervised use of hormone therapy (150).

Not all individuals with GD/gender incongruence seek treatment as described (e.g., male-to-eunuchs and individuals seeking partial transition). Tailoring current protocols to the individual may be done within the context of accepted safety guidelines using a multidisciplinary approach including mental health. No evidence-based protocols are available for these groups (151). We need prospective studies to better understand treatment options for these persons.

3.4. We suggest that endocrinologists provide education to transgender individuals undergoing treatment about the onset and time course of physical changes induced by sex hormone treatment. (2 ⊕◯◯◯)

Evidence

Transgender males

Physical changes that are expected to occur during the first 1 to 6 months of testosterone therapy include cessation of menses, increased sexual desire, increased facial and body hair, increased oiliness of skin, increased muscle, and redistribution of fat mass. Changes that occur within the first year of testosterone therapy include deepening of the voice (152, 153), clitoromegaly, and male pattern hair loss (in some cases) (114, 144, 154, 155) (Table 12).

Transgender females

Physical changes that may occur in transgender females in the first 3 to 12 months of estrogen and antiandrogen therapy include decreased sexual desire, decreased spontaneous erections, decreased facial and body hair (usually mild), decreased oiliness of skin, increased breast tissue growth, and redistribution of fat mass (114, 139, 149, 154, 155, 161) (Table 13). Breast development is generally maximal at 2 years after initiating hormones (114, 139, 149, 155). Over a long period of time, the prostate gland and testicles will undergo atrophy.

Although the time course of breast development in transgender females has been studied (150), precise information about other changes induced by sex hormones is lacking (141). There is a great deal of variability among individuals, as evidenced during pubertal development. We all know that a major concern for transgender females is breast development. If we work with estrogens, the result will be often not what the transgender female expects.

Alternatively, there are transgender females who report an anecdotal improved breast development, mood, or sexual desire with the use of progestogens. However, there have been no well-designed studies of the role of progestogens in feminizing hormone regimens, so the question is still open.

Our knowledge concerning the natural history and effects of different cross-sex hormone therapies on breast

Table 12. Masculinizing Effects in Transgender Males

Effect	Onset	Maximum
Skin oiliness/acne	1–6 mo	1–2 y
Facial/body hair growth	6–12 mo	4–5 y
Scalp hair loss	6–12 mo	—[a]
Increased muscle mass/strength	6–12 mo	2–5 y
Fat redistribution	1–6 mo	2–5 y
Cessation of menses	1–6 mo	—[b]
Clitoral enlargement	1–6 mo	1–2 y
Vaginal atrophy	1–6 mo	1–2 y
Deepening of voice	6–12 mo	1–2 y

Estimates represent clinical observations: Toorians *et al.* (149), Asscheman *et al.* (156), Gooren *et al.* (157), Wierckx *et al.* (158).

[a] Prevention and treatment as recommended for biological men.

[b] Menorrhagia requires diagnosis and treatment by a gynecologist.

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Table 13. Feminizing Effects in Transgender Females

Effect	Onset	Maximum
Redistribution of body fat	3–6 mo	2–3 y
Decrease in muscle mass and strength	3–6 mo	1–2 y
Softening of skin/decreased oiliness	3–6 mo	Unknown
Decreased sexual desire	1–3 mo	3–6 mo
Decreased spontaneous erections	1–3 mo	3–6 mo
Male sexual dysfunction	Variable	Variable
Breast growth	3–6 mo	2–3 y
Decreased testicular volume	3–6 mo	2–3 y
Decreased sperm production	Unknown	>3 y
Decreased terminal hair growth	6–12 mo	>3 y[a]
Scalp hair	Variable	—[b]
Voice changes	None	—[c]

Estimates represent clinical observations: Toorians *et al.* (149), Asscheman *et al.* (156), Gooren *et al.* (157).

[a]Complete removal of male sexual hair requires electrolysis or laser treatment or both.

[b]Familial scalp hair loss may occur if estrogens are stopped.

[c]Treatment by speech pathologists for voice training is most effective.

development in transgender females is extremely sparse and based on the low quality of evidence. Current evidence does not indicate that progestogens enhance breast development in transgender females, nor does evidence prove the absence of such an effect. This prevents us from drawing any firm conclusion at this moment and demonstrates the need for further research to clarify these important clinical questions (162).

Values and preferences

Transgender persons have very high expectations regarding the physical changes of hormone treatment and are aware that body changes can be enhanced by surgical procedures (*e.g.*, breast, face, and body habitus). Clear expectations for the extent and timing of sex hormone–induced changes may prevent the potential harm and expense of unnecessary procedures.

4.0 Adverse Outcome Prevention and Long-Term Care

Hormone therapy for transgender males and females confers many of the same risks associated with sex hormone replacement therapy in nontransgender persons. The risks arise from and are worsened by inadvertent or intentional use of supraphysiologic doses of sex hormones, as well as use of inadequate doses of sex hormones to maintain normal physiology (131, 139).

4.1. We suggest regular clinical evaluation for physical changes and potential adverse changes in response to sex steroid hormones and laboratory monitoring of sex steroid hormone levels every 3 months during the first year of hormone therapy for transgender males and females and then once or twice yearly. (2 ⊕⊕○○)

Evidence

Pretreatment screening and appropriate regular medical monitoring are recommended for both transgender males and females during the endocrine transition and periodically thereafter (26, 155). Clinicians should monitor weight and blood pressure, conduct physical exams, and assess routine health questions, such as tobacco use, symptoms of depression, and risk of adverse events such as deep vein thrombosis/pulmonary embolism and other adverse effects of sex steroids.

Transgender males

Table 14 contains a standard monitoring plan for transgender males on testosterone therapy (154, 159). Key issues include maintaining testosterone levels in the physiologic normal male range and avoiding adverse events resulting from excess testosterone therapy, particularly erythrocytosis, sleep apnea, hypertension, excessive weight gain, salt retention, lipid changes, and excessive or cystic acne (135).

Because oral 17-alkylated testosterone is not recommended, serious hepatic toxicity is not anticipated with parenteral or transdermal testosterone use (163, 164). Past concerns regarding liver toxicity with testosterone have been alleviated with subsequent reports that indicate the risk of serious liver disease is minimal (144, 165, 166).

Transgender females

Table 15 contains a standard monitoring plan for transgender females on estrogens, gonadotropin suppression, or antiandrogens (160). Key issues include avoiding supraphysiologic doses or blood levels of estrogen that may lead to increased risk for thromboembolic disease, liver dysfunction, and hypertension. Clinicians should monitor serum estradiol levels using laboratories participating in external quality control, as measurements of estradiol in blood can be very challenging (167).

VTE may be a serious complication. A study reported a 20-fold increase in venous thromboembolic disease in a large cohort of Dutch transgender subjects (161). This increase may have been associated with the use of the synthetic estrogen, ethinyl estradiol (149). The incidence decreased when clinicians stopped administering ethinyl estradiol (161). Thus, the use of synthetic estrogens and conjugated estrogens is undesirable because of the inability to regulate doses by measuring serum levels and the risk of thromboembolic disease. In a German gender clinic, deep vein thrombosis occurred in 1 of 60 of transgender females treated with a GnRH analog and oral

Table 14. Monitoring of Transgender Persons on Gender-Affirming Hormone Therapy: Transgender Male

1. Evaluate patient every 3 mo in the first year and then one to two times per year to monitor for appropriate signs of virilization and for development of adverse reactions.
2. Measure serum testosterone every 3 mo until levels are in the normal physiologic male range.[a]
   a. For testosterone enanthate/cypionate injections, the testosterone level should be measured midway between injections. The target level is 400–700 ng/dL to 400 ng/dL. Alternatively, measure peak and trough levels to ensure levels remain in the normal male range.
   b. For parenteral testosterone undecanoate, testosterone should be measured just before the following injection. If the level is <400 ng/dL, adjust dosing interval.
   c. For transdermal testosterone, the testosterone level can be measured no sooner than after 1 wk of daily application (at least 2 h after application).
3. Measure hematocrit or hemoglobin at baseline and every 3 mo for the first year and then one to two times a year. Monitor weight, blood pressure, and lipids at regular intervals.
4. Screening for osteoporosis should be conducted in those who stop testosterone treatment, are not compliant with hormone therapy, or who develop risks for bone loss.
5. If cervical tissue is present, monitoring as recommended by the American College of Obstetricians and Gynecologists.
6. Ovariectomy can be considered after completion of hormone transition.
7. Conduct sub- and periareolar annual breast examinations if mastectomy performed. If mastectomy is not performed, then consider mammograms as recommended by the American Cancer Society.

[a]Adapted from Lapauw *et al.* (154) and Ott *et al.* (159).

estradiol (141). The patient who developed a deep vein thrombosis was found to have a homozygous C677 T mutation in the methylenetetrahydrofolate reductase gene. In an Austrian gender clinic, administering gender-affirming hormones to 162 transgender females and 89 transgender males was not associated with VTE, despite an 8.0% and 5.6% incidence of thrombophilia (159). A more recent multinational study reported only 10 cases of VTE from a cohort of 1073 subjects (168). Thrombophilia screening of transgender persons initiating hormone treatment should be restricted to those with a personal or family history of VTE (159). Monitoring D-dimer levels during treatment is not recommended (169).

4.2. We suggest periodically monitoring prolactin levels in transgender females treated with estrogens. (2 ⊕⊕◯◯)

Evidence

Estrogen therapy can increase the growth of pituitary lactrotroph cells. There have been several reports of prolactinomas occurring after long-term, high-dose estrogen therapy (170–173). Up to 20% of transgender females treated with estrogens may have elevations in prolactin levels associated with enlargement of the pituitary gland (156). In most cases, the serum prolactin levels will return to the normal range with a reduction or discontinuation of the estrogen therapy or discontinuation of cyproterone acetate (157, 174, 175).

The onset and time course of hyperprolactinemia during estrogen treatment are not known. Clinicians should measure prolactin levels at baseline and then at least annually during the transition period and every 2 years thereafter. Given that only a few case studies reported prolactinomas, and prolactinomas were not reported in large cohorts of estrogen-treated persons, the risk is likely to be very low. Because the major presenting findings of microprolactinomas (hypogonadism and sometimes gynecomastia) are not apparent in transgender females, clinicians may perform radiologic examinations of the pituitary in those patients whose prolactin levels persistently increase despite stable or reduced estrogen levels. Some transgender individuals receive psychotropic medications that can increase prolactin levels (174).

Table 15. Monitoring of Transgender Persons on Gender-Affirming Hormone Therapy: Transgender Female

1. Evaluate patient every 3 mo in the first year and then one to two times per year to monitor for appropriate signs of feminization and for development of adverse reactions.
2. Measure serum testosterone and estradiol every 3 mo.
   a. Serum testosterone levels should be <50 ng/dL.
   b. Serum estradiol should not exceed the peak physiologic range: 100–200 pg/mL.
3. For individuals on spironolactone, serum electrolytes, particularly potassium, should be monitored every 3 mo in the first year and annually thereafter.
4. Routine cancer screening is recommended, as in nontransgender individuals (all tissues present).
5. Consider BMD testing at baseline (160). In individuals at low risk, screening for osteoporosis should be conducted at age 60 years or in those who are not compliant with hormone therapy.

This table presents strong recommendations and does not include lower level recommendations.

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4.3. We suggest that clinicians evaluate transgender persons treated with hormones for cardiovascular risk factors using fasting lipid profiles, diabetes screening, and/or other diagnostic tools. (2 ⊕○○○)

Evidence

Transgender males

Administering testosterone to transgender males results in a more atherogenic lipid profile with lowered high-density lipoprotein cholesterol and higher triglyceride and low-density lipoprotein cholesterol values (176–179). Studies of the effect of testosterone on insulin sensitivity have mixed results (178, 180). A randomized, open-label uncontrolled safety study of transgender males treated with testosterone undecanoate demonstrated no insulin resistance after 1 year (181, 182). Numerous studies have demonstrated the effects of sex hormone treatment on the cardiovascular system (160, 179, 183, 184). Long-term studies from The Netherlands found no increased risk for cardiovascular mortality (161). Likewise, a meta-analysis of 19 randomized trials in nontransgender males on testosterone replacement showed no increased incidence of cardiovascular events (185). A systematic review of the literature found that data were insufficient (due to very low–quality evidence) to allow a meaningful assessment of patient-important outcomes, such as death, stroke, myocardial infarction, or VTE in transgender males (176). Future research is needed to ascertain the potential harm of hormonal therapies (176). Clinicians should manage cardiovascular risk factors as they emerge according to established guidelines (186).

Transgender females

A prospective study of transgender females found favorable changes in lipid parameters with increased high-density lipoprotein and decreased low-density lipoprotein concentrations (178). However, increased weight, blood pressure, and markers of insulin resistance attenuated these favorable lipid changes. In a meta-analysis, only serum triglycerides were higher at ≥24 months without changes in other parameters (187). The largest cohort of transgender females (mean age 41 years, followed for a mean of 10 years) showed no increase in cardiovascular mortality despite a 32% rate of tobacco use (161).

Thus, there is limited evidence to determine whether estrogen is protective or detrimental on lipid and glucose metabolism in transgender females (176). With aging, there is usually an increase of body weight. Therefore, as with nontransgender individuals, clinicians should monitor and manage glucose and lipid metabolism and blood pressure regularly according to established guidelines (186).

4.4. We recommend that clinicians obtain BMD measurements when risk factors for osteoporosis exist, specifically in those who stop sex hormone therapy after gonadectomy. (1 ⊕⊕○○)

Evidence

Transgender males

Baseline bone mineral measurements in transgender males are generally in the expected range for their pretreatment gender (188). However, adequate dosing of testosterone is important to maintain bone mass in transgender males (189, 190). In one study (190), serum LH levels were inversely related to BMD, suggesting that low levels of sex hormones were associated with bone loss. Thus, LH levels in the normal range may serve as an indicator of the adequacy of sex steroid administration to preserve bone mass. The protective effect of testosterone may be mediated by peripheral conversion to estradiol, both systemically and locally in the bone.

Transgender females

A baseline study of BMD reported T scores less than −2.5 in 16% of transgender females (191). In aging males, studies suggest that serum estradiol more positively correlates with BMD than does testosterone (192, 193) and is more important for peak bone mass (194). Estrogen preserves BMD in transgender females who continue on estrogen and antiandrogen therapies (188, 190, 191, 195, 196).

Fracture data in transgender males and females are not available. Transgender persons who have undergone gonadectomy may choose not to continue consistent sex steroid treatment after hormonal and surgical sex reassignment, thereby becoming at risk for bone loss. There have been no studies to determine whether clinicians should use the sex assigned at birth or affirmed gender for assessing osteoporosis (*e.g.*, when using the FRAX tool). Although some researchers use the sex assigned at birth (with the assumption that bone mass has usually peaked for transgender people who initiate hormones in early adulthood), this should be assessed on a case-by-case basis until there are more data available. This assumption will be further complicated by the increasing prevalence of transgender people who undergo hormonal transition at a pubertal age or soon after puberty. Sex for comparison within risk assessment tools may be based on the age at which hormones were initiated and the length of exposure to hormones. In some cases, it may be

reasonable to assess risk using both the male and female calculators and using an intermediate value. Because all subjects underwent normal pubertal development, with known effects on bone size, reference values for birth sex were used for all participants (154).

4.5. We suggest that transgender females with no known increased risk of breast cancer follow breast-screening guidelines recommended for those designated female at birth. (2 ⊕⊕○○)

4.6. We suggest that transgender females treated with estrogens follow individualized screening according to personal risk for prostatic disease and prostate cancer. (2 ⊕○○○)

Evidence

Studies have reported a few cases of breast cancer in transgender females (197–200). A Dutch study of 1800 transgender females followed for a mean of 15 years (range of 1 30 years) found one case of breast cancer. The Women's Health Initiative study reported that females taking conjugated equine estrogen without progesterone for 7 years did not have an increased risk of breast cancer as compared with females taking placebo (137).

In transgender males, a large retrospective study conducted at the U.S. Veterans Affairs medical health system identified seven breast cancers (194). The authors reported that this was not above the expected rate of breast cancers in cisgender females in this cohort. Furthermore, they did report one breast cancer that developed in a transgender male patient after mastectomy, supporting the fact that breast cancer can occur even after mastectomy. Indeed, there have been case reports of breast cancer developing in subareolar tissue in transgender males, which occurred after mastectomy (201, 202).

Women with primary hypogonadism (Turner syndrome) treated with estrogen replacement exhibited a significantly decreased incidence of breast cancer as compared with national standardized incidence ratios (203, 204). These studies suggest that estrogen therapy does not increase the risk of breast cancer in the short term (<20 to 30 years). We need long-term studies to determine the actual risk, as well as the role of screening mammograms. Regular examinations and gynecologic advice should determine monitoring for breast cancer.

Prostate cancer is very rare before the age of 40, especially with androgen deprivation therapy (205). Childhood or pubertal castration results in regression of the prostate and adult castration reverses benign prostate hypertrophy (206). Although van Kesteren *et al.* (207) reported that estrogen therapy does not induce hypertrophy or premalignant changes in the prostates of transgender females, studies have reported cases of benign prostatic hyperplasia in transgender females treated with estrogens for 20 to 25 years (208, 209). Studies have also reported a few cases of prostate carcinoma in transgender females (210–214).

Transgender females may feel uncomfortable scheduling regular prostate examinations. Gynecologists are not trained to screen for prostate cancer or to monitor prostate growth. Thus, it may be reasonable for transgender females who transitioned after age 20 years to have annual screening digital rectal examinations after age 50 years and prostate-specific antigen tests consistent with U.S. Preventive Services Task Force Guidelines (215).

4.7. We advise that clinicians determine the medical necessity of including a total hysterectomy and oophorectomy as part of gender-affirming surgery. (Ungraded Good Practice Statement)

Evidence

Although aromatization of testosterone to estradiol in transgender males has been suggested as a risk factor for endometrial cancer (216), no cases have been reported. When transgender males undergo hysterectomy, the uterus is small and there is endometrial atrophy (217, 218). Studies have reported cases of ovarian cancer (219, 220). Although there is limited evidence for increased risk of reproductive tract cancers in transgender males, health care providers should determine the medical necessity of a laparoscopic total hysterectomy as part of a gender-affirming surgery to prevent reproductive tract cancer (221).

Values

Given the discomfort that transgender males experience accessing gynecologic care, our recommendation for the medical necessity of total hysterectomy and oophorectomy places a high value on eliminating the risks of female reproductive tract disease and cancer and a lower value on avoiding the risks of these surgical procedures (related to the surgery and to the potential undesirable health consequences of oophorectomy) and their associated costs.

Remarks

The sexual orientation and type of sexual practices will determine the need and types of gynecologic care required following transition. Additionally, in certain countries, the approval required to change the sex in a birth certificate for transgender males may be dependent on having a complete hysterectomy. Clinicians should help patients research nonmedical administrative criteria and

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provide counseling. If individuals decide not to undergo hysterectomy, screening for cervical cancer is the same as all other females.

5.0 Surgery for Sex Reassignment and Gender Confirmation

For many transgender adults, genital gender-affirming surgery may be the necessary step toward achieving their ultimate goal of living successfully in their desired gender role. The type of surgery falls into two main categories: (1) those that directly affect fertility and (2) those that do not. Those that change fertility (previously called sex reassignment surgery) include genital surgery to remove the penis and gonads in the male and removal of the uterus and gonads in the female. The surgeries that effect fertility are often governed by the legal system of the state or country in which they are performed. Other gender-conforming surgeries that do not directly affect fertility are not so tightly governed.

Gender-affirming surgical techniques have improved markedly during the past 10 years. Reconstructive genital surgery that preserves neurologic sensation is now the standard. The satisfaction rate with surgical reassignment of sex is now very high (187). Additionally, the mental health of the individual seems to be improved by participating in a treatment program that defines a pathway of gender-affirming treatment that includes hormones and surgery (130, 144) (Table 16).

Surgery that affects fertility is irreversible. The World Professional Association for Transgender Health Standards of Care (222) emphasizes that the "threshold of 18 should not be seen as an indication in itself for active intervention." If the social transition has not been satisfactory, if the person is not satisfied with or is ambivalent about the effects of sex hormone treatment, or if the person is ambivalent about surgery then the individual should not be referred for surgery (223, 224).

Gender-affirming genital surgeries for transgender females that affect fertility include gonadectomy, penectomy, and creation of a neovagina (225, 226). Surgeons often invert the skin of the penis to form the wall of the vagina, and several literatures reviews have reported on outcomes (227). Sometimes there is inadequate tissue to form a full neovagina, so clinicians have revisited using intestine and found it to be successful (87, 228, 229). Some newer vaginoplasty techniques may involve autologuous oral epithelial cells (230, 231).

The scrotum becomes the labia majora. Surgeons use reconstructive surgery to fashion the clitoris and its hood, preserving the neurovascular bundle at the tip of the penis as the neurosensory supply to the clitoris. Some surgeons are also creating a sensate pedicled-spot adding a G spot to the neovagina to increase sensation (232). Most recently, plastic surgeons have developed techniques to fashion labia minora. To further complete the feminization, uterine transplants have been proposed and even attempted (233).

Neovaginal prolapse, rectovaginal fistula, delayed healing, vaginal stenosis, and other complications do sometimes occur (234, 235). Clinicians should strongly remind the transgender person to use their dilators to maintain the depth and width of the vagina throughout the postoperative period. Genital sexual responsivity and other aspects of sexual function are usually preserved following genital gender-affirming surgery (236, 237).

Ancillary surgeries for more feminine or masculine appearance are not within the scope of this guideline. Voice therapy by a speech language pathologist is available to transform speech patterns to the affirmed gender (148). Spontaneous voice deepening occurs during testosterone treatment of transgender males (152, 238). No studies have compared the effectiveness of speech therapy, laryngeal surgery, or combined treatment.

Breast surgery is a good example of gender-confirming surgery that does not affect fertility. In all females, breast size exhibits a very broad spectrum. For transgender females to make the best informed decision, clinicians should delay breast augmentation surgery until the patient has completed at least 2 years of estrogen therapy, because the breasts continue to grow during that time (141, 155).

Another major procedure is the removal of facial and masculine-appearing body hair using either electrolysis or

Table 16. Criteria for Gender-Affirming Surgery, Which Affects Fertility

1. Persistent, well-documented gender dysphoria
2. Legal age of majority in the given country
3. Having continuously and responsibly used gender-affirming hormones for 12 mo (if there is no medical contraindication to receiving such therapy)
4. Successful continuous full-time living in the new gender role for 12 mo
5. If significant medical or mental health concerns are present, they must be well controlled
6. Demonstrable knowledge of all practical aspects of surgery (*e.g.*, cost, required lengths of hospitalizations, likely complications, postsurgical rehabilitation)

laser treatments. Other feminizing surgeries, such as that to feminize the face, are now becoming more popular (239–241).

In transgender males, clinicians usually delay gender-affirming genital surgeries until after a few years of androgen therapy. Those surgeries that affect fertility in this group include oophorectomy, vaginectomy, and complete hysterectomy. Surgeons can safely perform them vaginally with laparoscopy. These are sometimes done in conjunction with the creation of a neopenis. The cosmetic appearance of a neopenis is now very good, but the surgery is multistage and very expensive (242, 243). Radial forearm flap seems to be the most satisfactory procedure (228, 244). Other flaps also exist (245). Surgeons can make neopenile erections possible by reinervation of the flap and subsequent contraction of the muscle, leading to stiffening of the neopenis (246, 247), but results are inconsistent (248). Surgeons can also stiffen the penis by imbedding some mechanical device (*e.g.*, a rod or some inflatable apparatus) (249, 250). Because of these limitations, the creation of a neopenis has often been less than satisfactory. Recently, penis transplants are being proposed (233).

In fact, most transgender males do not have any external genital surgery because of the lack of access, high cost, and significant potential complications. Some choose a metaoidioplasty that brings forward the clitoris, thereby allowing them to void in a standing position without wetting themselves (251, 252). Surgeons can create the scrotum from the labia majora with good cosmetic effect and can implant testicular prostheses (253).

The most important masculinizing surgery for the transgender male is mastectomy, and it does not affect fertility. Breast size only partially regresses with androgen therapy (155). In adults, discussions about mastectomy usually take place after androgen therapy has started. Because some transgender male adolescents present after significant breast development has occurred, they may also consider mastectomy 2 years after they begin androgen therapy and before age 18 years. Clinicians should individualize treatment based on the physical and mental health status of the individual. There are now newer approaches to mastectomy with better outcomes (254, 255). These often involve chest contouring (256). Mastectomy is often necessary for living comfortably in the new gender (256).

5.1. We recommend that a patient pursue genital gender-affirming surgery only after the MHP and the clinician responsible for endocrine transition therapy both agree that surgery is medically necessary and would benefit the patient's overall health and/or well-being. (1 ⊕⊕◯◯)

5.2. We advise that clinicians approve genital gender-affirming surgery only after completion of at least 1 year of consistent and compliant hormone treatment, unless hormone therapy is not desired or medically contraindicated. (Ungraded Good Practice Statement)

5.3. We advise that the clinician responsible for endocrine treatment and the primary care provider ensure appropriate medical clearance of transgender individuals for genital gender-affirming surgery and collaborate with the surgeon regarding hormone use during and after surgery. (Ungraded Good Practice Statement)

5.4. We recommend that clinicians refer hormone-treated transgender individuals for genital surgery when: (1) the individual has had a satisfactory social role change, (2) the individual is satisfied about the hormonal effects, and (3) the individual desires definitive surgical changes. (1 ⊕◯◯◯)

5.5. We suggest that clinicians delay gender-affirming genital surgery involving gonadectomy and/or hysterectomy until the patient is at least 18 years old or legal age of majority in his or her country. (2 ⊕⊕◯◯).

5.6. We suggest that clinicians determine the timing of breast surgery for transgender males based upon the physical and mental health status of the individual. There is insufficient evidence to recommend a specific age requirement. (2 ⊕◯◯◯)

Evidence

Owing to the lack of controlled studies, incomplete follow-up, and lack of valid assessment measures, evaluating various surgical approaches and techniques is difficult. However, one systematic review including a large numbers of studies reported satisfactory cosmetic and functional results for vaginoplasty/neovagina construction (257). For transgender males, the outcomes are less certain. However, the problems are now better understood (258). Several postoperative studies report significant long-term psychological and psychiatric pathology (259–261). One study showed satisfaction with breasts, genitals, and femininity increased significantly and showed the importance of surgical treatment as a key therapeutic option for transgender females (262). Another analysis demonstrated that, despite the young average age at death following surgery and the relatively larger number of individuals with somatic morbidity, the study does not allow for determination of

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causal relationships between, for example, specific types of hormonal or surgical treatment received and somatic morbidity and mortality (263). Reversal surgery in regretful male-to-female transsexuals after sexual reassignment surgery represents a complex, multistage procedure with satisfactory outcomes. Further insight into the characteristics of persons who regret their decision postoperatively would facilitate better future selection of applicants eligible for sexual reassignment surgery. We need more studies with appropriate controls that examine long-term quality of life, psychosocial outcomes, and psychiatric outcomes to determine the long-term benefits of surgical treatment.

When a transgender individual decides to have gender-affirming surgery, both the hormone prescribing clinician and the MHP must certify that the patient satisfies criteria for gender-affirming surgery (Table 16).

There is some concern that estrogen therapy may cause an increased risk for venous thrombosis during or following surgery (176). For this reason, the surgeon and the hormone-prescribing clinician should collaborate in making a decision about the use of hormones before and following surgery. One study suggests that preoperative factors (such as compliance) are less important for patient satisfaction than are the physical postoperative results (56). However, other studies and clinical experience dictate that individuals who do not follow medical instructions and do not work with their physicians toward a common goal do not achieve treatment goals (264) and experience higher rates of postoperative infections and other complications (265, 266). It is also important that the person requesting surgery feels comfortable with the anatomical changes that have occurred during hormone therapy. Dissatisfaction with social and physical outcomes during the hormone transition may be a contraindication to surgery (223).

An endocrinologist or experienced medical provider should monitor transgender individuals after surgery. Those who undergo gonadectomy will require hormone replacement therapy, surveillance, or both to prevent adverse effects of chronic hormone deficiency.

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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

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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* (dosage-sensitive sex reversal, adrenal 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

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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

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(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.

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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

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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).

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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

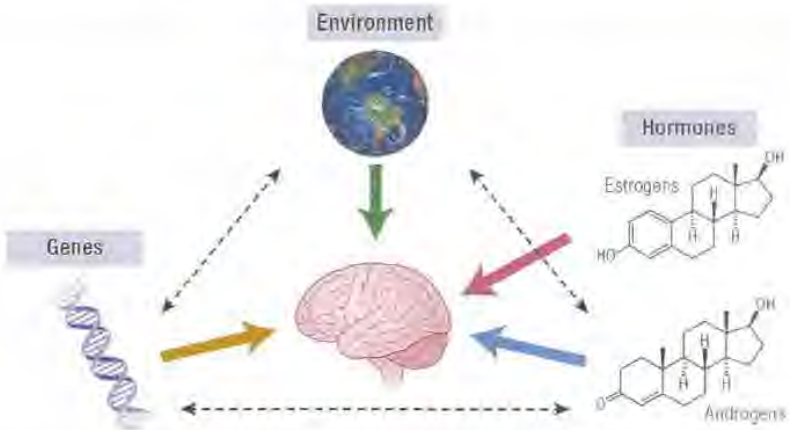

Figure 1. Simplified view of the factors influencing sex differences in the brain. Three broad groups of factors influence the sexually dimorphic brain, as indicated by the broad, colored arrows. 1) Genes and genetic factors that influence the brain include both those on sex chromosomes and autosomes,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

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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, nor 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

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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).

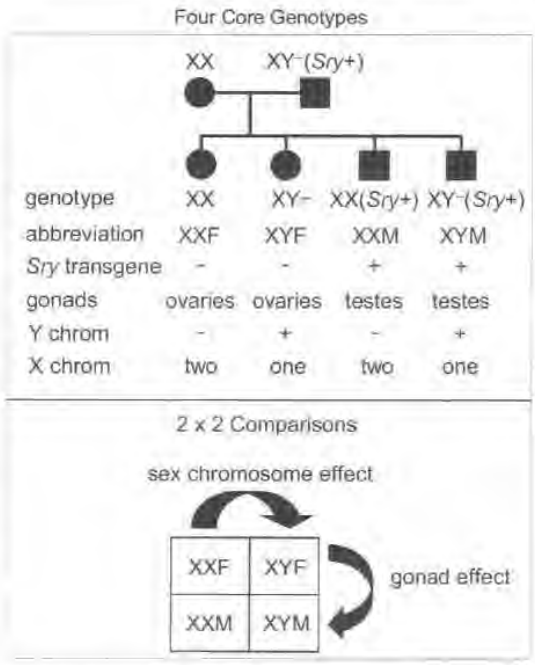

Figure 2. Schematic diagram of the Four Core Genotypes mouse model. The testis-determining gene *Sry* is deleted from the Y chromosome, producing the Y⁻ chromosome. An *Sry* transgene is inserted onto chromosome 3. Thus, the type of gonad is no longer linked to the sex chromosomes. The model produces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

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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

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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

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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

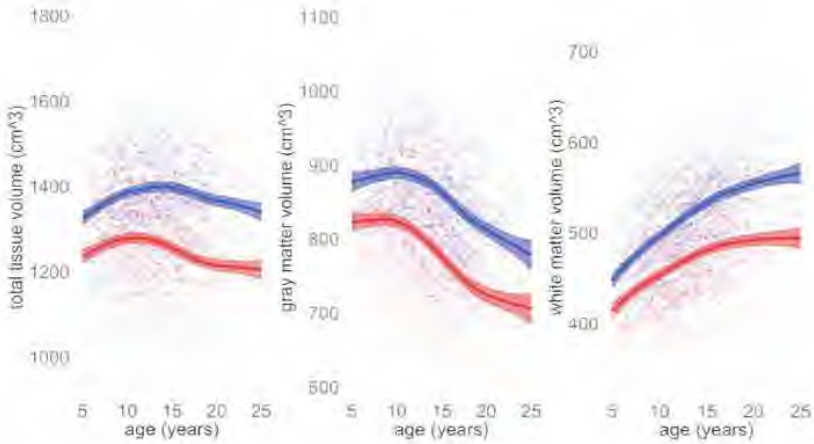

Figure 3. Developmental trajectories for total brain tissue volume, gray matter volume, and white matter volume in men and women over Development. Person-level data are shown for women (red) and men (blue) as points, with lines linking measures from the same person over time. Note the large interindividual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

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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

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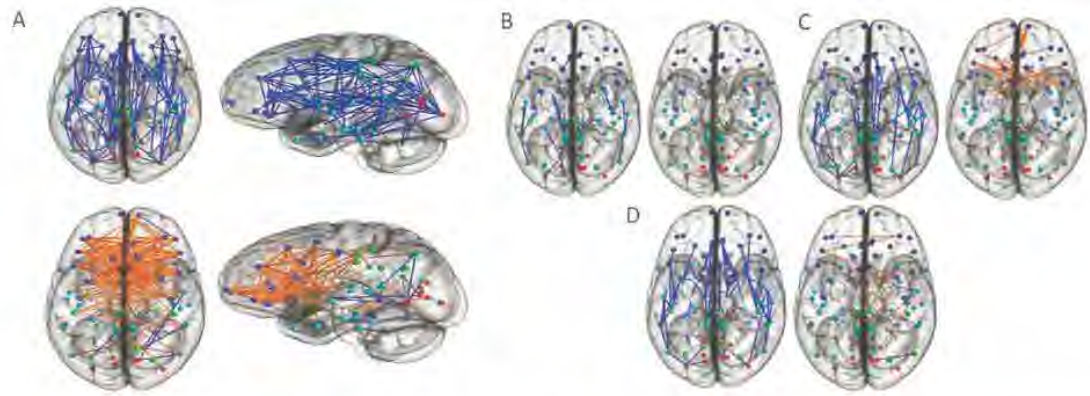

Figure 4. Sex differences in structural connectomes across development. Connectomes representing the white matter structural connectivity in the brain, with nodes indicating the brain regions and edges between the nodes representing the structural connectivity between the nodes. Node colors representing 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

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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

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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-linked 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

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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

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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,

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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

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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

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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

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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

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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

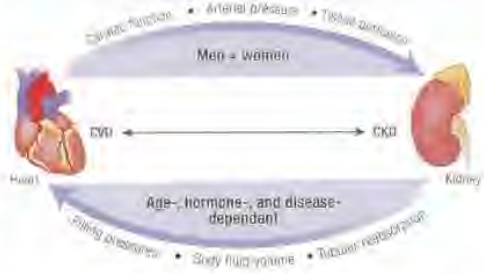

Figure 5. Heart and kidney functions are linked. Sex differences exist in many aspects of heart and kidney function at baseline and in CVD and CKD, as shown. Both organs feed-forward and influence each other's function. Genes, hormones, and age are some known factors that modulate this relationship in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

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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

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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

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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

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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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Protecting Transgender Health and Challenging Science Denialism in Policy

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can occur in TGD people taking hormones. The informed-consent process includes a discussion of these potential effects, and gamete preservation should be offered to TGD people seeking puberty blockers or sex hormones (although access to such services remains inequitable).

Finally, policymakers promoting such bans often misuse scientific theory and current evidence. To justify elimination of Medicaid coverage of gender-affirming care, Florida's Agency for Health Care Administration prepared a review limited to cherry-picked studies published between 2020 and early 2022, many of which had negative findings and some of which were heavily criticized. People with [illegible] expertise in [illegible] rather than transgender health, and no clinical experience in gender-affirming care testified in support of bans in Texas, Florida, and Alabama.[12]

A particularly flawed claim is that the absence of randomized, controlled trials (RCTs) of gender-affirming care negates any of its known benefits. There's no way to conduct such RCTs with clinical equipoise, since evidence demonstrates mental health benefits, reduced suicidality, improved body satisfaction, and healthier social functioning associated with this care. Furthermore, foundational evidence supporting the use of insulin, penicillin, and aspirin is derived from observational studies, yet those who use such logic to restrict gender-affirming care haven't similarly restricted these drugs.

Innovation and research in the area of gender-affirming care is robust. The newly released eighth version of the World Professional Association for Transgender Health Standards of Care cites the largest and most generalizable studies on this topic.[9] Federally funded, prospective observational studies involving adolescents with gender dysphoria are under way. Defenders of medical bans claim that gender-affirming care is "experimental" because the field is actively growing, but scientific consensus is ever evolving. The first hormone treatments for gender dysphoria were administered in the 1930s when estrogen and testosterone became commercially available, and the first use of puberty blockers for gender dysphoria was reported more than 20 years ago. Transgender health isn't a nascent field, but it is an evolving one that shouldn't be [illegible] by biased laws.

In litigation, lawyers and scientists have begun consolidating their expertise to expose and refute science denialism at the policy level. This type of interdisciplinary collaboration consists of written reports and expert testimony that summarize clinical research for judges, regulators, and the public. As of October 2022, courts had temporarily blocked legal measures that would have prohibited gender-affirming care for minors in Arkansas, Alabama, and Texas. These initial successes reflect the coordinated efforts of legal organizations defending the rights of TGD people, including the American Civil Liberties Union, GLBTQ Legal Advocates and Defenders, and others. These preliminary victories face further adjudication.

National medical societies, which traditionally haven't intervened in state politics, are now mobilizing. Twenty organizations filed an amicus brief in a 2022 lawsuit challenging Alabama's law. The court's opinion in this case acknowledged the importance of scientific expertise and consensus, noting that "medical providers have used transitioning medications for decades to treat medical conditions other than gender dysphoria" and that the defendants provided "no credible evidence to show that transitioning medications are experimental."[13]

We have written rapid-response, in-depth rebuttals of science-denialist claims that have been used in litigation and provided material for direct intervention in legal processes in the form of [illegible] and regulatory comments.[14] Reports such as these are composed by subject-matter experts without conflicts of interest and made publicly available to interested members of the media, the medical community, and legal organizations working on [illegible] these quickly evolving issues. Scientists, clinicians, and legal experts who face science denialism in other fields may consider similar undertakings.

Bans on gender-affirming care are grounded in science denialism, harm the health of marginalized people, and degrade medical authority. Collaboration between lawyers and scientists is critical to defeating such bans in the courts. The U.S. Supreme Court's recent ruling in Dobbs v. Jackson Women's Health Organization signals a new legal era in which states may attempt to restrict reproductive autonomy, marriage equality, and the right to intimacy [illegible]. The combined powers of medicine and law could safeguard the credibility of sci-

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ence and civil liberties in health policy.

Disclosure forms provided by the authors are available at NEJM.org.

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THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Psychosocial Functioning in Transgender Youth after 2 Years of Hormones

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ABSTRACT

BACKGROUND

Limited prospective outcome data exist regarding transgender and nonbinary youth receiving gender-affirming hormones (GAH; testosterone or estradiol).

METHODS

We characterized the longitudinal course of psychosocial functioning during the 2 years after GAH initiation in a prospective cohort of transgender and nonbinary youth in the United States. Participants were enrolled in a four-site prospective, observational study of physical and psychosocial outcomes. Participants completed the Transgender Congruence Scale, the Beck Depression Inventory–II, the Revised Children's Manifest Anxiety Scale (Second Edition), and the Positive Affect and Life Satisfaction measures from the NIH (National Institutes of Health) Toolbox Emotion Battery at baseline and at 6, 12, 18, and 24 months after GAH initiation. We used latent growth curve modeling to examine individual trajectories of appearance congruence, depression, anxiety, positive affect, and life satisfaction over a period of 2 years. We also examined how initial levels of and rates of change in appearance congruence correlated with those of each psychosocial outcome.

RESULTS

A total of 315 transgender and nonbinary participants 12 to 20 years of age (mean [±SD], 16±1.9) were enrolled in the study. A total of 190 participants (60.3%) were transmasculine (i.e., persons designated female at birth who identify along the masculine spectrum), 185 (58.7%) were non-Latinx or non-Latine White, and 25 (7.9%) had received previous pubertal suppression treatment. During the study period, appearance congruence, positive affect, and life satisfaction increased, and depression and anxiety symptoms decreased. Increases in appearance congruence were associated with concurrent increases in positive affect and life satisfaction and decreases in depression and anxiety symptoms. The most common adverse event was suicidal ideation (in 11 participants [3.5%]); death by suicide occurred in 2 participants.

CONCLUSIONS

In this 2-year study involving transgender and nonbinary youth, GAH improved appearance congruence and psychosocial functioning. (Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development.)

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TRANSGENDER AND NONBINARY YOUTH comprise 2 to 9% of high-school–aged persons in the United States.[1-3] Many transgender and nonbinary youth have gender dysphoria, the persistent distress arising from incongruence between gender identity and external phenotype. Increasingly, transgender and nonbinary youth receive medical care to alleviate gender dysphoria, including gonadotropin-releasing hormone (GnRH) agonists to suppress gender-incongruent puberty and gender-affirming hormones (GAH; testosterone or estradiol) to foster gender-congruent secondary sex characteristics. An important goal of such treatment is to attenuate gender dysphoria by increasing appearance congruence — that is, the degree to which youth experience alignment between their gender and their physical appearance.

The available prospective research indicates that gender-affirming medical care is associated with improvements in psychosocial functioning.[4-9] Previously published studies with modest sample sizes[5,6,9] have examined outcomes for relatively short follow-up periods (approximately 1 year on average),[5,6,9] focused exclusively on outcomes of GnRH agonists,[7,8] or examined outcomes for mixed samples of youth initiating GnRH agonists or GAH,[4,6,9] despite evidence that such cohorts have distinct psychosocial profiles.[10] Evidence has been lacking from longitudinal studies that explore potential mechanisms by which gender-affirming medical care affects gender dysphoria and subsequent well-being.

We characterized the longitudinal course of psychosocial functioning over a period of 2 years after GAH initiation in a prospective cohort of more than 300 transgender and nonbinary young people in the United States. We hypothesized that appearance congruence, positive affect, and life satisfaction would increase and that depression and anxiety symptoms would decrease. We also hypothesized that improvements would be secondary to treatment for gender dysphoria, such that increasing appearance congruence would be associated with concurrent improvements in psychosocial outcomes. We also explored the potential moderating effects of demographic and clinical characteristics, including age, designated sex at birth, racial and ethnic identity, and the initiation of GAH in early as compared with later stages of puberty.

METHODS

STUDY DESIGN AND PARTICIPANT RECRUITMENT

Participants were recruited from gender clinics at the Ann and Robert H. Lurie Children's Hospital of Chicago, UCSF Benioff Children's Hospitals, Boston Children's Hospital, and Children's Hospital Los Angeles from July 2016 through June 2019 for the Trans Youth Care–United States (TYCUS) Study,[11] a prospective, observational study evaluating the physical and psychosocial outcomes of medical treatment for gender dysphoria in two distinct cohorts of transgender and nonbinary youth — those initiating GnRH agonists and those initiating GAH as part of their clinical care. All participating clinics employ a multidisciplinary team that includes medical and mental health providers and that collaboratively determines whether gender dysphoria is present and whether gender-affirming medical care is appropriate. For minors, parental consent is required to initiate medical treatment. Publications by individual study teams provide details on site-specific approaches to care.[12-15]

Study visits occurred at baseline and at 6, 12, 18, and 24 months after treatment initiation. Details on study procedures have been published previously,[11] and the protocol is available with the full text of this article at NEJM.org. The present analyses focus on the GAH cohort; outcomes for the cohort initiating GnRH agonists are being analyzed separately, given differences in baseline functioning between the two cohorts[10] and distinct outcomes of GnRH agonists[8] as compared with GAH treatment.[4] Participants provided written informed consent or assent; parents provided permission for minors to participate. Procedures were approved by the institutional review board at each study site.

The first and second authors analyzed the data and wrote the initial draft of the manuscript. All the authors critically reviewed the manuscript. The authors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol. There were no agreements regarding confidentiality of the data among the sponsor (Eunice Kennedy Shriver National Institute of Child Health and Human Development), the authors, and the participating institutions. The sponsor had no role in the design of the study; the collection, analysis, or in-

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terpretation of data; the writing of the manuscipt; or the decision to submit the manuscript for publication.

MEASURES

Participants reported age, racial and ethnic identity, gender identity, and designated sex at birth (details are provided in the Supplementary Appendix, available at NEJM.org). A small subgroup had been treated with GnRH agonists in early puberty (Tanner stage 2 or 3) (20 participants) or had a relatively late age at onset of endogenous puberty, such that they began receiving GAH in Tanner stage 3 (at 13 to 15 years of age) even without previous treatment with GnRH agonists (4 participants). These 24 participants comprise a subcohort in that they did not undergo extensive gender-incongruent puberty. Participants with a history of GnRH agonist treatment that was initiated in Tanner stage 4 (5 participants) were not included in this subcohort, because their experience of substantial gender-incongruent puberty is more similar to that of youth initiating GAH in Tanner stage 4 or 5.

With respect to longitudinal outcomes, participants completed the Transgender Congruence Scale,[16] the Beck Depression Inventory–II,[17] the Revised Children's Manifest Anxiety Scale (Second Edition),[18] and the Positive Affect and Life Satisfaction measures from the NIH (National Institutes of Health) Toolbox Emotion Battery[19] at each study visit. Scoring information and sample items from each scale are provided in the Supplementary Appendix. Higher scores on these measures reflect greater appearance congruence, depression, anxiety, positive affect, and life satisfaction, respectively.

STATISTICAL ANALYSIS

Trajectories of psychosocial functioning were examined with the use of repeated-measures multivariate analysis of variance and mixed-effects models. Multivariate analysis of variance provided a preliminary omnibus test for significant within-person change over time. Owing to listwise deletion, 150 participants were excluded from the multivariate analysis of variance (the analysis involved 141 participants). Mixed-effects modeling was therefore selected owing to greater flexibility in accommodating missing data and nonnormal distributions and examining parallel processes. Specifically, we used latent growth curve modeling, which uses a structural equation modeling framework to examine changes in mean scores over time.[20] Repeated measures are treated as indicators of latent factors: an intercept factor (estimates of initial levels) and a slope factor (rate of change). Intercept and slope factors can be regressed on covariates in adjusted models to explore moderation effects. In addition, growth curves for two different outcomes can be combined to examine how intercepts and slopes of those constructs correlate with each other. Data were Winsorized at the 95th percentile to reduce the influence of outliers.

Analyses involving latent growth curve modeling proceeded in three steps. First, we modeled trajectories of appearance congruence and psychosocial outcomes (i.e., effects of time only). Second, we adjusted models to estimate the effects of covariates on baseline scores and rates of change over time. Third, because changes in appearance congruence and psychosocial outcomes occur as parallel, simultaneous processes during GAH treatment, we examined how initial levels and rates of change in appearance congruence correlated with those of each psychosocial outcome. Standardized β levels were used as indicators of effect sizes for longitudinal models using conventional ranges (small, 0.20; medium, 0.50; and large, 0.80). Our conceptual model is shown in Figure S1 in the Supplementary Appendix. All statistical analyses were conducted with the use of SPSS software, version 27, and Mplus software, version 8.8.

RESULTS

ANALYTIC SAMPLE

There were a total of 6114 observations from 315 participants, who were assessed up to five times over a period of 2 years (data were available for 81% of all possible observations). Most participants (238 [75.6%]) completed either four study visits (76 participants) or five visits (162 participants). Tables S1 and S2 show the number of completed visits by time point and data coverage for key variables. The analytic sample for longitudinal models included 291 participants with follow-up data on primary outcome variables (Fig. S2). The analytic sample did not differ substantially from the overall sample with respect to age, designated sex at birth, racial and ethnic

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identity, initiation of GAH in early puberty, or baseline scores on psychosocial measures (Table S3).

SAMPLE CHARACTERISTICS

We enrolled 315 eligible participants 12 to 20 years of age (mean [±SD], 16±1.9 years) (Table 1). Most were transmasculine (i.e., persons designated female at birth who identify along the masculine spectrum; 60.3%), designated female at birth (64.8%), and non-Latinx or non-Latine White (58.7%). Transmasculine, non-Latinx or non-Latine White, and multiracial participants were overrepresented and nonbinary and Black participants were underrepresented as compared with the study sample in the Williams Institute Executive Report[21] (Table S4); however, the study sample was representative of transgender and nonbinary youth presenting to pediatric subspecialty gender programs[22] and generalizable to this population. Two participants died by suicide during the study (one after 6 months of follow-up and the other after 12 months of follow-up), and 6 participants withdrew from the study. For these eight participants, data that had been collected before death or study withdrawal were included in the analyses. Data on adverse events are provided in Table 2.

APPEARANCE CONGRUENCE AND PSYCHOSOCIAL OUTCOMES OVER TIME

Table S5 depicts mean scores for appearance congruence, depression, anxiety, positive affect, and life satisfaction at baseline and 24 months. Results for multivariate analysis of variance indicated that there were significant within-participant changes over time for all psychosocial outcomes in hypothesized directions (Wilk's lambda, 0.32; F statistic with 20 and 122 degrees of freedom; 12.86; P<0.001). Specifically, scores for appearance congruence, positive affect, and life satisfaction increased significantly, and scores for depression and anxiety decreased significantly.

Means and variances of the variables for latent growth curve modeling, with estimated baseline levels and change over time for both time-only and adjusted models, are provided in Table 3. Scores for appearance congruence increased (annual increase on a 5-point scale, 0.48 points; 95% confidence interval [CI], 0.42 to 0.54; standardized β=1.47), as did T scores for positive affect (annual increase on a 100-point scale, 0.80 points; 95% CI, 0.08 to 1.54; β=0.19) and life satisfaction (annual increase on a 100-point scale, 2.32 points; 95% CI, 1.64 to 3.00; β=0.52). We observed decreased scores for depression (annual change on a 63-point scale, −1.27 points; 95% CI, −1.98 to −0.57; standardized β=−0.29) and decreased T scores for anxiety (annual change on a 100-point scale, −1.46 points; 95% CI, −2.13 to −0.79; β=−0.35) over a period of 2 years of GAH treatment.

Unadjusted models can be interpreted on their original scale. For instance, depression scores range from 0 to 63 (ranges of severity, minimal, 0 to 13; mild, 14 to 19; moderate, 20 to 28; and severe, 29 to 63). The model had an intercept (baseline mean) of 15.46 and estimated slope (change per year) of −1.27. Thus, on average, depression started in the mild range and decreased to the subclinical level by 24 months. Table S6 shows the percentages of youth scoring in the clinical range for depression and anxiety at each time point. Of 27 participants with depression scores in the severe range at baseline, 18 (67%) reported a depression score in the minimal or moderate ranges at 24 months. Similarly, 21 of 33 participants (64%) with depression scores in the moderate range at baseline reported a depression score in the minimal or moderate ranges at 24 months (chi-square statistic with 9 degrees of freedom, 49.85; P<0.001). With respect to anxiety, 47 of 122 participants (38.5%) with baseline scores in the clinical range (T scores, >60) were in the nonclinical range at 24 months (chi-square statistic with 1 degree of freedom, 22.05; P<0.001).

ASSOCIATIONS BETWEEN APPEARANCE CONGRUENCE AND PSYCHOSOCIAL OUTCOMES

Figure 1 depicts parallel processes between appearance congruence and each psychosocial outcome as analyzed by means of latent growth curve modeling. As described above, we used linear latent growth curve modeling to estimate baseline scores (intercepts) and linear rates of change (slopes) of each outcome (see Table 3 for details of each model). In parallel-process models, we examined how the components for latent growth curve modeling for appearance congruence related to those for scores for depression (Fig. 1A) and T scores for anxiety (Fig. 1B), positive affect (Fig. 1C), and life satisfaction

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Table 1. Demographic and Clinical Characteristics of the Participants.*

Characteristic	Participants (N=315)
	no. (%)
Gender identity†	
Transmasculine	190 (60.3)
Transfeminine	106 (33.7)
Nonbinary	19 (6.0)
Designated sex at birth	
Female	204 (64.8)
Male	111 (35.2)
Racial and ethnic identity	
Non-Latinx or non-Latine White	185 (58.7)
Latinx or Latine non-White	50 (15.9)
Latinx or Latine White	25 (7.9)
Black	11 (3.5)
Asian or Pacific Islander	10 (3.2)
Multiracial	32 (10.2)
Other	1 (0.3)
Unknown	1 (0.3)
Age at baseline	
12 yr	6 (1.9)
13 yr	23 (7.3)
14 yr	38 (12.1)
15 yr	67 (21.3)
16 yr	55 (17.5)
17 yr	51 (16.2)
18 yr	48 (15.2)
19 yr	15 (4.8)
20 yr	12 (3.8)
Tanner stage at GAH initiation‡	
1	2 (0.6)
2	13 (4.1)
3	9 (2.9)
4	29 (9.2)
5	262 (83.2)
Past use of GnRH agonist	
No	290 (92.1)
Yes	25 (7.9)
Tanner stage at initiation of GnRH agonist	
2	12 (3.8)
3	8 (2.5)
4	5 (1.6)
Not applicable	290 (92.1)
Initiation of GAH in early puberty subcohort§	
No	291 (92.4)
Yes	24 (7.6)

* The table does not include demographic and clinical characteristics for one participant who was accidentally enrolled and did not meet criteria for study eligibility. Percentages may not total 100 because of rounding. GAH denotes gender-affirming hormones, and GnRH gonadotropin-releasing hormone.

† Transmasculine refers to persons designated female at birth who identify along the masculine spectrum. Transfeminine refers to persons designated male at birth who identify along the feminine spectrum.

‡ Three participants began receiving GnRH agonists in either Tanner stage 2 or 3 and subsequently had pubertal regression to Tanner stage 1 or 2 by the time of GAH initiation.

§ This subcohort includes 20 participants who began receiving GnRH agonists at Tanner stage 2 or 3 and 4 participants who had not previously received GnRH agonists but had begun receiving GAH in Tanner stage 3 owing to a relatively late onset of puberty (13 to 15 years of age) and thus did not have physical changes associated with later stages of endogenous puberty. This subcohort does not include 5 participants with a history of initiation of GnRH agonists in Tanner stage 4 and who thus did undergo substantial gender-incongruent puberty.

(Fig. 1D). Higher appearance congruence at baseline was associated with lower baseline scores for depression ($r=-0.60$) and T scores for anxiety ($r=-0.40$), and increases in appearance congruence were associated with decreases in scores for depression ($r=-0.68$) and T scores for anxiety ($r=-0.52$) over time. In addition, higher appearance congruence at baseline was associated with higher baseline T scores for positive affect ($r=0.46$) and life satisfaction ($r=0.72$), and increases in appearance congruence were associated with increases in T scores for positive affect ($r=0.74$) and life satisfaction ($r=0.84$) over time.

MODERATING EFFECTS OF DEMOGRAPHIC AND CLINICAL COVARIATES

Table 3 shows the effects of covariates on scores for appearance congruence and depression and T scores for anxiety, positive affect, and life satisfaction. Age was not associated with any outcomes at baseline or over time.

Designated Sex at Birth

Depression and anxiety scores decreased among youth designated female at birth but not among those designated male at birth. Similarly, T scores for life satisfaction increased among youth designated female at birth but not among those designated male at birth (Fig. S3). Designated sex at birth was not associated with any other outcomes at baseline or over time.

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Table 2. Adverse Events.

Event	No. of Events in Sample
Any event	15
Death by suicide	2
Suicidal ideation reported during study visit	11
Severe anxiety triggered by study visit	2

Effects of Racial and Ethnic Identity

At baseline, youth of color had higher scores for appearance congruence, lower scores for depression, and higher scores for positive affect than non-Latinx or non-Latine White youth. With respect to change over time, non-Latinx or non-Latine White youth had greater decreases in depression scores than youth of color (Fig. S4). Racial and ethnic identity were not associated with any other outcomes at baseline or over time.

Initiation of GAH in Early Puberty

Youth who had initiated GAH in early puberty had higher scores for appearance congruence, positive affect, and life satisfaction at baseline and lower scores for depression and anxiety at baseline than those who had initiated GAH in later puberty. Tables S7, S8, and S9 provide more information regarding differences between youth initiating GAH in early puberty and those initiating GAH in late puberty. With respect to change over time, youth initiating GAH in later puberty had greater improvements in appearance congruence than those initiating GAH in early puberty (Fig. 2).

DISCUSSION

Understanding the effect of GAH on the psychosocial outcomes of transgender and nonbinary youth would appear crucial, given the documented mental health disparities observed in this population,[10,15,23,24] particularly in the context of increasing politicization of gender-affirming medical care.[25] In our U.S.-based cohort of transgender and nonbinary youth treated with GAH, we found decreases in depression and anxiety symptoms and increases in positive affect and life satisfaction as assessed through validated instruments. Our findings are consistent with those of other longitudinal studies involving transgender and nonbinary youth receiving GAH, which showed reductions in depression[6,7] and anxiety[6] and increases in overall well-being[7] with small-to-moderate effects over a follow-up period of up to 1 year. We replicated these findings in a larger sample of racially and ethnically diverse transgender and nonbinary youth recruited from four geographically distinct regions in the United States and found sustained improvements over a period of 2 years.

Increasing appearance congruence is a primary goal of GAH, and we observed appearance congruence improve over 2 years of treatment. This was a moderate effect, and the strongest effect observed across our outcomes, consistent with the effect seen in research involving other samples, which has noted large effects of GAH on body image and small-to-moderate effects on mental health.[6] Appearance congruence was also associated with each psychosocial outcome assessed at baseline and during the follow-up period, such that increases in appearance congruence were associated with decreases in depression and anxiety symptoms and increases in positive affect and life satisfaction. These findings suggest that appearance congruence is a candidate mechanism by which GAH influences psychosocial functioning.

The importance of appearance congruence for psychosocial well-being is further highlighted by the effect of avoiding gender-incongruent pubertal changes. Youth who had not undergone substantial gender-incongruent puberty had higher scores for appearance congruence, positive affect, and life satisfaction and lower scores for depression and anxiety at baseline than youth who had undergone substantial endogenous puberty. These observations align with other published reports that earlier access to gender-affirming medical care is associated with more positive psychosocial functioning.[10,26] Alternatively, youth who first recognize their gender incongruence in adolescence may represent a distinct subgroup of transgender and nonbinary youth who have more psychosocial complexities than youth recognizing gender incongruence in childhood.[27]

The effects of GAH on some psychosocial outcomes varied on the basis of designated sex

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Table 3. Variable Estimates for Individual Latent Growth Curve Models of 2-Year Outcomes.*

Model	Appearance Congruence†	Depression‡	Anxiety§	Positive Affect¶	Life Satisfaction‖
			value (95% confidence interval)		
Unconditional model: time					
Intercept mean	2.99 (2.90 to 3.08)	15.46 (14.27 to 16.70)	59.58 (58.22 to 60.68)	42.93 (41.82 to 44.03)	40.12 (38.99 to 41.26)
Intercept variance	0.35 (0.27 to 0.50)	86.23 (68.13 to 106.85)	17.84 (11.38 to 24.54)	63.50 (46.23 to 81.79)	75.21 (59.76 to 93.98)
Slope mean	0.48 (0.42 to 0.54)	−1.27 (−1.98 to −0.57)	−1.46 (−2.13 to −0.79)	0.80 (0.08 to 1.54)	2.32 (1.64 to 3.00)
Slope variance	0.11 (0.07 to 0.15)	19.44 (12.23 to 27.14)	17.84 (11.38 to 24.54)	17.98 (9.25 to 27.57)	20.33 (14.12 to 27.70)
Conditional model					
Time					
Intercept mean	2.59 (1.91 to 3.27)	20.01 (10.79 to 29.48)	60.82 (53.56 to 67.95)	47.27 (38.93 to 55.81)	38.86 (29.90 to 47.75)
Intercept variance	0.32 (0.25 to 0.42)	80.92 (63.35 to 100.47)	114.74 (91.96 to 138.23)	56.96 (41.19 to 74.75)	71.93 (57.15 to 90.22)
Slope mean	0.51 (0.07 to 0.96)	−0.92 (−3.82 to −0.06)	−1.95 (−3.81 to −0.09)	1.79 (0.14 to 3.43)	4.54 (2.66 to 6.43)
Slope variance	0.10 (0.06 to 0.14)	18.81 (11.71 to 26.34)	18.37 (11.78 to 25.63)	17.97 (9.29 to 27.66)	19.74 (13.61 to 27.06)
Time-invariant effects on intercept					
Baseline age	0.02 (−002 to 0.06)	−0.23 (−0.08 to 0.36)	−0.20 (−0.78 to 0.38)	−0.32 (−0.84 to 0.21)	0.06 (−0.49 to 0.62)
Designated sex at birth**	−0.12 (−0.31 to 0.06)	1.74 (−0.69 to 4.09)	0.05 (−2.37 to 2.49)	−1.26 (−3.53 to 0.91)	−2.36 (−4.89 to 0.18)
Racial and ethnic identity††	0.19 (0.03 to 0.36)	−2.60 (−4.82 to −0.32)	−2.22 (−4.48 to 0.06)	2.30 (0.22 to 4.38)	1.70 (−0.58 to 3.98)
Early gender-affirming care‡‡	0.70 (0.35 to 1.04)	−5.88 (−9.67 to −1.96)	−7.41 (−11.30 to −3.52)	5.34 (1.70 to 8.98)	7.55 (2.87 to 12.28)
Time-invariant effects on slope					
Baseline age	0.00 (−0.03 to 0.03)	−0.04 (−0.18 to 0.10)	−0.02 (−0.15 to 0.12)	−0.03 (−0.15 to 0.10)	−0.09 (−0.22 to 0.05)
Designated sex at birth**	0.03 (−0.09 to 0.15)	1.91 (0.33 to 3.50)	1.56 (0.01 to 3.10)	−0.43 (−2.10 to 1.31)	−1.86 (−3.49 to −0.24)
Racial and ethnic identity††	−0.10 (−0.20 to 0.01)	1.70 (0.23 to 3.15)	0.62 (−0.77 to 1.98)	−1.42 (−2.98 to 0.13)	−1.08 (−2.52 to 0.36)
Early gender-affirming care‡‡	−0.42 (−0.66 to −0.19)	−0.73 (−3.41 to 1.93)	0.04 (−2.53 to 2.59)	−0.78 (−3.56 to 2.06)	−1.08 (−4.01 to 1.86)

* Shown are unstandardized variable estimates with 95% confidence intervals. Slope means indicate change over time, and slope variances indicate heterogeneity within the sample.
† Scores on the Appearance Congruence subscale of the Transgender Congruence Scale range from 1 to 5, with higher scores indicating greater appearance congruence.
‡ Scores on the Beck Depression Inventory–II range from 0 to 63, with scores of 20 to 28 indicating moderate depression and scores of 29 to 63 indicating severe depression.
§ T scores on the Revised Children's Manifest Anxiety Scale (Second Edition) have a mean of 50 and a standard deviation of 10, with scores of 60 or more indicating clinical levels of anxiety.
¶ T scores for the Positive Affect measure from the NIH (National Institutes of Health) Toolbox Emotion Battery have a mean of 50 and a standard deviation of 10, with higher scores indicating greater positive affect.
‖ T scores for the Life Satisfaction measure from the NIH Toolbox Emotion Battery have a mean of 50 and a standard deviation of 10, with higher scores indicating greater life satisfaction.
** Coding for designated sex at birth was as follows: 0=assigned female at birth (reference) and 1=assigned male at birth.
†† Coding for racial and ethnic identity was as follows: 0=non-Latinx or non-Latine White (reference) and 1=other racial and ethnic identities.
‡‡ Coding for early gender-affirming care was as follows: 0=initiated GAH in later puberty (Tanner stage 4 or 5) (reference) and 1=initiated GAH in early puberty (Tanner stage 2 or 3).

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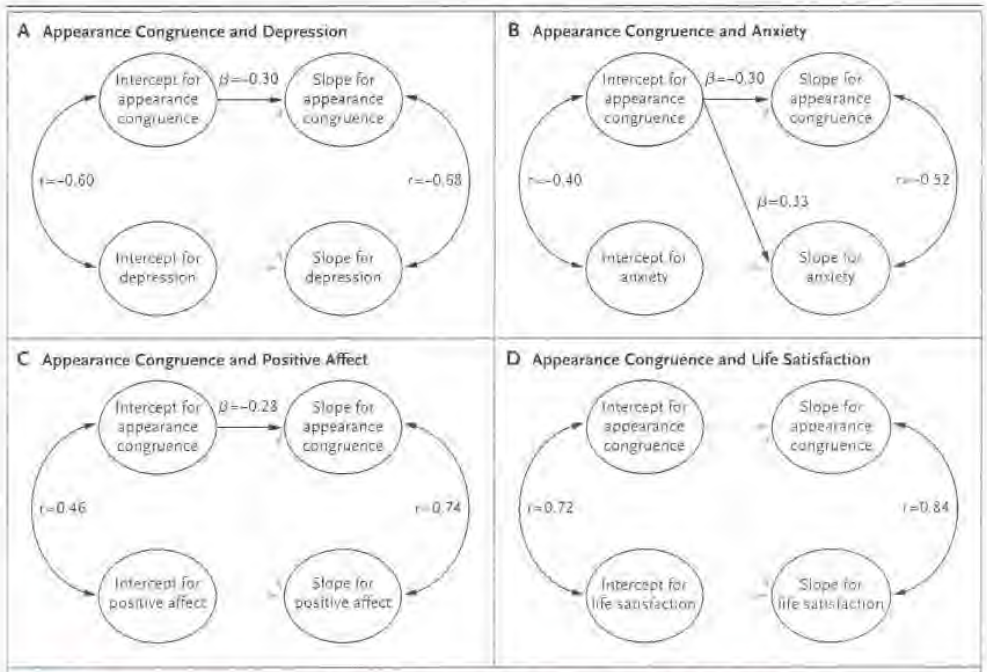

Figure 1. Appearance Congruence and Depression, Anxiety, Positive Affect, and Life Satisfaction.

Parallel-process latent growth curve models are depicted. A linear latent growth curve model was fitted for each outcome, with model-based estimates of baseline scores (intercept) and rates of linear change over time (slope). Parallel-process models can provide tests of how aspects of trajectories relate to each other. Each panel provides estimates for correlations between baseline scores of appearance congruence and each outcome (intercept correlations, arcs displayed on the left side of each panel), correlations between rate of change of appearance congruence and rate of change of each outcome (slope correlations, arcs displayed on the right side of each panel), and effects of baseline scores on slopes (straight lines in the middle of each panel). Solid black lines and arcs indicate significant effects (confidence intervals for variable estimates do not contain 0); nonsignificant effects are shown with dashed gray lines. All models were controlled for age, designated sex at birth, racial and ethnic identity, and early gender-affirming care (not shown for ease of interpretation).

at birth. Depression and anxiety symptoms decreased significantly, and life satisfaction increased significantly, among youth designated female at birth but not among those designated male at birth. Given that some key estrogen-mediated phenotypic changes can take between 2 and 5 years to reach their maximum effect (e.g., breast growth),[28] we speculate that a longer follow-up period may be necessary to see an effect on depression, anxiety, and life satisfaction. Furthermore, changes that are associated with an endogenous testosterone-mediated puberty (e.g., deeper voice) may be more pronounced and observable than those associated with an endogenous estrogen-mediated puberty. Thus, we hypothesize that observed differences in depression, anxiety, and life satisfaction among youth designated female at birth as compared with those designated male at birth may be related to differential experiences of gender minority stress, which could arise from differences in societal acceptance of transfeminine (i.e., persons designated male at birth who identity along the feminine spectrum) as compared with transmasculine persons. Indeed, gender minority stress is consistently associated with more negative mental health outcomes,[29] and research suggests that transfeminine youth may experience more minority stress than transmasculine youth.[30]

Our study has certain limitations. Because participants were recruited from four urban pediatric gender centers, the findings may not be generalizable to youth without access to comprehensive interdisciplinary services or to transgen-

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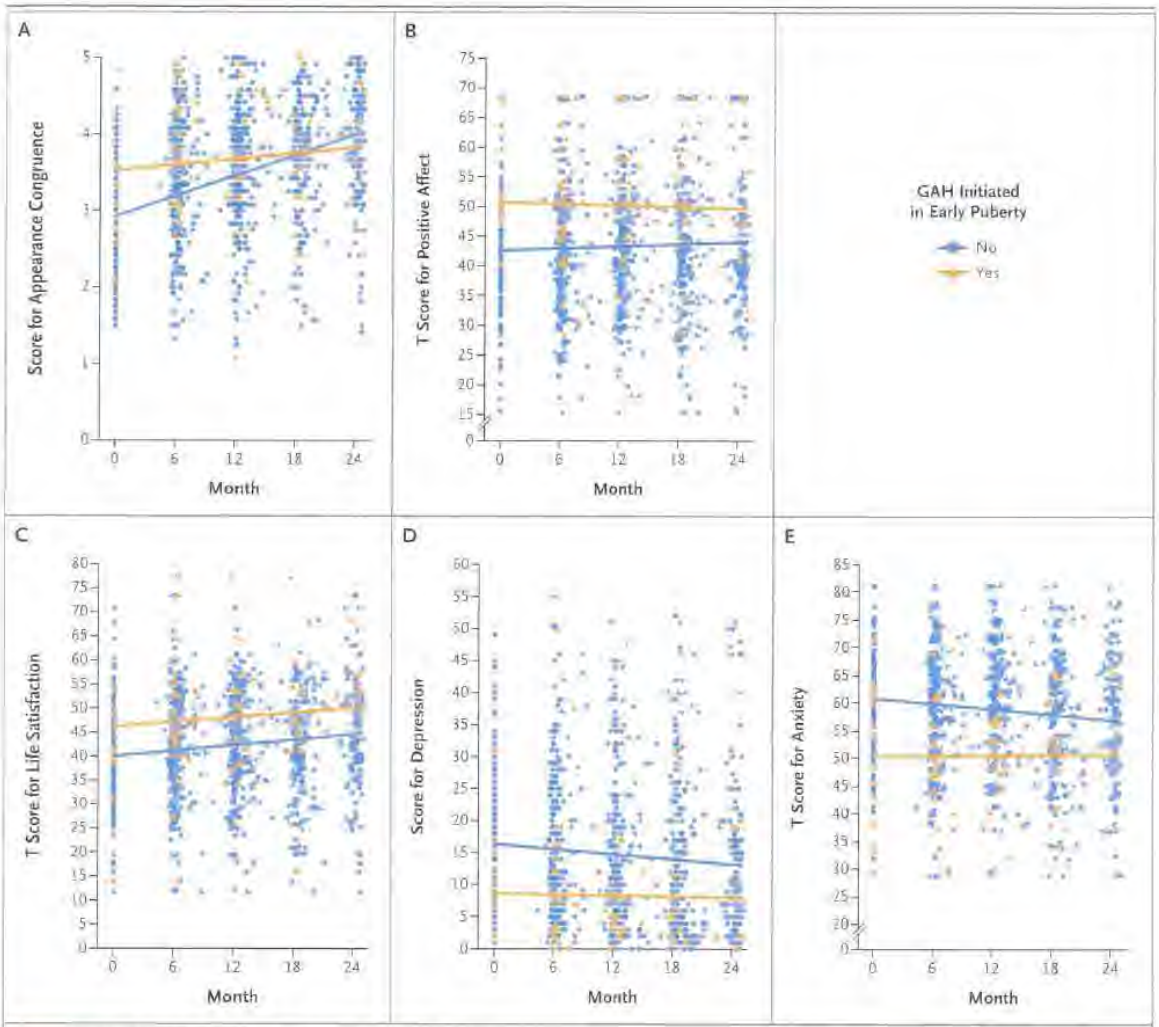

Figure 2. Psychosocial Outcomes during 2 Years of GAH.

Shown are changes in participant-reported measures over a period of 2 years of treatment with gender-affirming hormones (GAH). Scores on the Appearance Congruence subscale of the Transgender Congruence Scale (Panel A) range from 1 to 5, with higher scores indicating greater appearance congruence. T scores for the Positive Affect measure from the NIH (National Institutes of Health) Toolbox Emotion Battery (Panel B) range from 0 to 100, with higher scores indicating greater positive affect. T scores for the Life Satisfaction measure from the NIH Toolbox Emotion Battery (Panel C) range from 0 to 100, with higher scores indicating greater life satisfaction. Scores on the Beck Depression Inventory–II (Panel D) range from 0 to 63, with higher scores indicating greater depression. T scores on the Revised Children's Manifest Anxiety Scale (Second Edition) (Panel D), range from 0 to 100, with higher scores indicating greater anxiety. Individual scores are depicted with orange triangles for youth initiating GAH in early puberty ("Yes") and with blue circles for youth who did not initiate GAH in early puberty ("No"). Lines indicate mean scores for each group, with gray shaded bands for 95% confidence intervals.

der and nonbinary youth who are self-medicating with GAH. In addition, despite improvement across psychosocial outcomes on average, there was substantial variability around the mean trajectory of change. Some participants continued to report high levels of depression and anxiety and low positive affect and life satisfaction, despite the use of GAH. We plan to examine other factors that are known to contribute to psychosocial functioning among transgender and non-

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binary youth and may not be affected by GAH, such as parental support,[31,32] in this cohort. Finally, our study lacked a comparison group, which limits our ability to establish causality. However, the large effects in parallel-process models examining associations between improvements in appearance congruence and improvements in psychosocial outcomes provide support for the concept that GAH may affect psychosocial outcomes through increasing gender congruence.

Despite these limitations, our findings showed improvements in psychosocial functioning across 2 years of GAH treatment, which supports the use of GAH as effective treatment for transgender and nonbinary youth. We are now following this cohort to see whether gains in functioning are sustained over a longer follow-up period, and — given substantial variability in outcomes even after controlling for a number of factors — we hope to discover additional predictors of change to identify youth for whom GAH alone is not adequate to address mental health challenges. We intend to initiate further work with this cohort to focus on understanding reasons for discontinuing GAH among the small subgroup of youth who stopped medical treatment. Overall, our results provide evidence that GAH improved appearance congruence and psychosocial functioning in transgender and nonbinary youth.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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Supplementary Appendix

Supplement to: Chen D, Berona J, Chan Y-M, et al. Psychosocial functioning in transgender youth after 2 years of hormones. N Engl J Med 2023;388:240-50. DOI: 10.1056/NEJMoa2206297

This appendix has been provided by the authors to give readers additional information about the work.

Table of Contents

METHODS

Measures

Demographic and Clinical Characteristics

Participants self-reported age, race/ethnicity, gender identity, and designated sex at birth. For age, participants were asked "How old are you?" For race/ethnicity, between the start of the study and May 2018, participants were asked "With which racial or ethnic group do you most closely identify? (Choose one) and provided with the following options: (a) American Indian or Alaska Native; (b) Asian; (c) Black or African American; (d) Hispanic or Latino; (e) Native Hawaiian or Other Pacific Islander; (f) White; (g) Other. After May 2018, participants were asked "What race or ethnicity are you? Check all that apply" and provided with the following options: (a) American Indian or Alaska Native; (b) Asian; (c) Black or African American; (d) Hispanic or Latino; (e) Native Hawaiian or other Pacific Islander; (f) White; (g) other. Those selecting "other" were asked to specify race or ethnicity in free text form. Participant responses were recoded into the following: (a) non-Latinx/Latine White; (b) Latinx/Latine, non-White; (c) Latinx/Latine, White; (d) Black/African American; (e) Asian/Pacific Islander; (f) Multiracial; (g) other; and (h) Unknown.

For gender identity, youth either selected from eight response options [male, female, transgender female (male-to-female), transgender male (female-to-male), gender fluid, gender queer, bigender, or nonbinary] or indicated "other" and specified. Responses were recoded into three categories: transmasculine, transfeminine, and nonbinary. For designated sex at birth, participants were asked "What was your assigned sex at birth?" with male and female as response options.

Longitudinal Outcomes

Appearance Congruence. Appearance congruence was captured through the 9-item appearance congruence subscale of the Transgender Congruence Scale.[1] Each item was rated on a 5-point scale from "strongly disagree" to "strongly agree" and averaged. Example items include: "My outward appearance represents my gender identity" and "I am happy with the way my appearance expresses my gender identity". Higher scores reflect greater appearance congruence.

Depression Symptoms. Depression symptoms were assessed using the 21-item Beck Depression Inventory-II (BDI-II).[2] Each item was rated on a 4-point scale, summed and compared to standardized cutoffs reflecting minimal (0-13), mild (14-19), moderate (20-28), or severe depression symptoms (29-63).

Anxiety Symptoms. Anxiety symptoms were assessed by the Revised Children's Manifest Anxiety Scale, Second Edition (RCMAS2).[3] Forty-nine items were rated "yes"/ "no". "Yes" responses were tallied and transformed into a *T* score; for this scale *T* scores >60 are considered clinically significant.

Positive Affect. Positive affect was assessed using the 10-item Positive Affect measure from the National Institutes of Health (NIH) Toolbox—Emotion Battery.[4] Participants were asked to rate how frequently they experienced a variety of positive feelings over the past seven days. Example items include "I felt joyful" and "I felt content". Each item was rated on a 5-point scale from 1 = "not at all" to 5 = "very much". Raw scores were summed and converted to *T* scores; higher scores indicate greater positive affect.

Life Satisfaction. Life satisfaction was assessed using the 10-item General Life Satisfaction measure from the NIH Toolbox—Emotion Battery.[4] Participants were asked to rate how much they agree or disagree with statements about their personal well-being. Example items

include "If I could live my life over, I would change almost nothing," "I have what I want in life," and "My life is going well." Each item was rated on a 5-point scale from "strongly disagree" to "strongly agree". Raw scores were summed and converted to *T* scores; higher scores indicate greater life satisfaction.

Rationale for Selecting Primary Mental Health Outcome Measures

The Trans Youth Care—United States (TYCUS) study used various measures to assess different domains of mental health and psychosocial functioning,[1] including the Youth Self-Report (YSR),[2] a widely used child-report measure that assesses problem behaviors along two "broadband scales" (Internalizing, Externalizing) and eight empirically-based syndrome and DSM-oriented scales and provides a Total Problems score, and the age-appropriate version of the MINI International Neuropsychiatric Interview (MINI)[3] or the MINI International Neuropsychiatric Interview for Children and Adolescents (MINI-KID).[4] We chose to use the BDI-II and RCMAS2 as our primary mental health outcome measures in this paper as they are more granular than the YSR and have clinical thresholds that aid in interpretation of findings. Furthermore, the YSR and MINI/MINI-KID were administered annually (baseline, 12-month, and 24-month) versus the BDI-II and RCMAS2 which were administered every 6 months. Having more datapoints to model change across time allowed us to explore whether change in these outcomes were non-linear in nature. Future work using the YSR and MINI/MINI-KID data will allow for comparison across samples, as these measures are widely used among other study teams.[5,6]

Statistical Analysis Plan

Missing Data

At least four out of five total time points were available for 75% of participants (Table S1). As a result, there was high covariance coverage with data available for the majority of the sample for each variable of interest at all time points (range of data present: 0.66-0.99; Table S2). Within our sample, data exhibited skew and were determined to be missing at random (Little's MCAR test: χ^2 [751] = 803.25, p = 0.09).[5,6] This type of missing data can be appropriately handled using maximum likelihood estimation methods (described below).

Longitudinal Modeling Approach

Analyses were conducted in a latent growth curve modeling (LGCM) framework using Mplus 8.8.[7] This approach provides a unified modeling framework with several pertinent computational techniques including specification of hierarchical data structure, accommodation of missing data, and integration of both maximum likelihood and Bayesian estimation techniques. Consistent with NEJM recommendations, we handled missing data using model-based methods.[8] More specifically, LGCM was conducted with a two-stage estimation process in which starting values were generated for parameter estimates using full-information maximum likelihood estimation (FIML) followed by optimization using the Bayes estimator. The Bayes estimator was used in the second stage optimization as it is recommended for use when variables of interest exhibit non-normal distributions.[9,10] Bayesian estimation uses Markov chain Monte Carlo (MCMC) resampling algorithms and do not require large sample sizes.[11,12] These methods accommodate multilevel models that would otherwise be computationally intractable due to small sample sizes, modest effect sizes, and skewed response distributions.[13]

Model Specifications

Latent growth curves were generated for each variable of interest. Linear and quadratic effects of time were explored for inclusion. In all cases, quadratic effects were either non-significant (i.e., confidence intervals included 0) or had small parameter estimates that did not alter interpretation of results. For parsimony, all growth curves included intercepts and linear slopes. Intercept priors were estimated based on median values from observed data. Models employed MCMC algorithms to generate a series of 50,000 random draws from 4 stationary Markov chains to approximate the multivariate posterior distribution of our sample, with a burn-in period of 2,500 iterations. Model convergence was determined by the Gelman-Rubin potential scale reduction factor (PSR) values, with values close to 1 indicating convergence.[14] Trace plots were also inspected to evaluate model fit. All PSR values (range: 1.01-1.03) and trace plots indicated that the models converged and fit the data well.

Table S1. Count of Visits Completed

Visits	n	Proportion present
1	12	0.04
2	27	0.09
3	38	0.11
4	76	0.24
5	162	0.51

Proportion present is out of N=315 eligible participants.

Table S2. Data Coverage for Key Variables

	Baseline		Month 6		Month 12		Month 18		Month 24	
Variable	n	present*	n	present	n	present	n	present	n	present
AC	310	0.98	283	0.90	249	0.79	212	0.67	221	0.70
BDI	307	0.97	281	0.89	248	0.79	210	0.67	219	0.70
RCMAS	308	0.98	282	0.90	248	0.79	209	0.66	216	0.69
NPA	311	0.99	284	0.90	250	0.79	211	0.67	223	0.71
NLS	312	0.99	282	0.90	250	0.79	210	0.67	224	0.71

Note. Proportion present is out of N=315 eligible participants. AC = appearance congruence. BDI = Beck Depressive Inventory. RCMAS = Revised Children's Manifest Anxiety Scale. NPA = NIH Toolbox Positive Affect. NLS = NIH Toolbox Life Satisfaction
*present= proportion present.

Table S3. Comparison of Analytic Sample (n=291) and Participants Excluded from Longitudinal Analysis (n=24)

	t	df	*p*	Cohen's *d*
Baseline Age	0.28	26.27	0.78	0.06
Appearance Congruence	-0.63	25.58	0.54	-0.13
Depression	1.99	22.17	0.06	0.48
Anxiety	1.02	21.42	0.32	0.24
Positive Affect	-0.09	23.07	0.93	-0.02
Life Satisfaction	-1.56	24.03	0.13	-0.35
	c^2	df	*p*	f
Designated sex	0.47	1	0.49	0.04
Early gender-affirming care	0.44	1	0.51	0.04
Racial/ethnic identity	0.002	1	0.97	0.002

Note. For continuous variables, negative *t*-scores and Cohen's *d* indicate higher scores among participants excluded from longitudinal analysis.

Table S4. Representativeness of Study Participants

Category	Example
Disease, problem, or condition under investigation	People who identify as transgender in the U.S.
Special considerations related to:	
Sex and gender	Of the estimated 1.3 million transgender adults, 38.5% are transgender women, 35.9% are transgender men, and 25.6% are nonbinary.
Age	Youth ages 13 to 17 comprise 7.6% of the U.S. population and represent 18% of the transgender population in the U.S. Youth ages 18 to 24 comprise 11% of the U.S. population and represent 24.4% of the transgender population in the U.S. Approximately 1.4% of youth ages 13 to 17 and 1.3% of youth ages 18 to 24 identify as transgender.
Race or ethnic group	The racial/ethnic distribution of youth and adults who identify as transgender appears generally similar to the U.S. population, though transgender youth and adults are more likely to report being Latinx and less likely to report being White compared to the U.S. population. Among youth ages 13 to 17, white youth represent 51.3% of the U.S. population and 46.3% of transgender youth are white. Black youth represent 13.4% of the U.S. population and 13.2% of transgender youth are Black. Asian youth represent 5% of the U.S. population and 3.6% of transgender youth are Asian. American Indian or Alaska Native (AIAN) youth represent 0.8% of the U.S. population and 1% of transgender youth are AIAN. Latinx youth represent 24.8% of the U.S. population and 31% of transgender youth are Latinx. Multiracial youth represent 4.7% of the U.S. population and 5% of transgender youth are multiracial.
Geography	Percentage of residents in U.S. regions who identify as transgender range from 1.8% in the Northeast to 1.2% in the Midwest for youth ages 13 to 17. At the state level, estimates range from 3% of youth ages 13-17 identifying as transgender in New York to 0.6% in Wyoming.
Other considerations	In the last decade, the number of youth presenting for gender-affirming medical care has increased exponentially. In addition, the number of youth reporting a nonbinary identity also has increased significantly in recent years.
Overall representativeness of this trial	Transmasculine participants are over-represented in our study and non-binary participants are under-represented. Non-Latinx white and multiracial participants are over-represented in our sample, whereas Black participants are vastly under-represented in our sample. The proportion of Latinx and Asian participants are

	comparable to population estimates. Because study recruitment occurred at 4 study sites in the Northeast, Midwest, and California, youth in the Southeastern and Southwestern United States are not represented in the sample.

Note. Numbers are predominately pulled from the most recent Williams Institute Executive Summary "How many adults and youth identify as transgender in the United States" published in June 2022 by Jody L. Herman, Andrew R. Flores, and Kathryn K. O'Neill.

Table S5. Paired Samples *t*-tests Comparing Scores at Baseline and 24 Months

	n	baseline	24 Months	*p*-value	effect size
Appearance congruence	213	2.86 (0.74)	3.86 (0.76)	<0.001	-1.12
Depression	211	16.39 (11.88)	13.95 (12.76)	<0.001	0.20
Anxiety	208	60.25 (11.18)	57.38 12.00)	<0.001	0.25
Positive affect	215	42.90 (10.05)	43.72 (12.03)	0.37	-0.05
Life satisfaction	217	39.92 (10.55)	44.61 (12.29)	<0.001	-0.39

Note. Variables are presented as mean (SD). Results are based on *t*-tests (baseline minus 24-months). Negative *t*-test values indicate increases in appearance congruence, positive affect, and life satisfaction. Effect sizes are Cohen's *d* (ranges: 0.20, small; 0.50, medium; 0.80, large).

Table S6. Proportions of Youth Scoring in the Clinical Range for Depression and Anxiety at Each Timepoint

	Baseline	**6-month**	**12-month**	**18-month**	**24-month**
Beck Depression Inventory-II n (%)	*n*=307	*n*=281	*n*=248	*n*=210	*n*=219
Minimal Depression	149 (48.5)	152 (54.1)	143 (57.7)	125 (59.5)	126 (57.5)
Mild Depression	53 (17.3)	46 (16.4)	41 (16.5)	25 (11.9)	41 (18.7)
Moderate Depression	57 (18.6)	43 (15.3)	24 (9.7)	30 (14.3)	22 (10)
Severe Depression	48 (15.6)	40 (14.2)	40 (16.1)	30 (14.3)	30 (13.7)
Revised Children's Manifest Anxiety Scale 2	*n*=308	*n*=282	*n*=248	*n*=209	*n*=216
M (*SD*)	60.0 (11.5)	58.6 (11.6)	58.6 (11.3)	56.8 (11.4)	57.4 (12.1)
n (%) in Clinical range (*T*>60)	181 (58.8)	145 (51.4)	115 (46.4)	90 (43.1)	103 (47.7)

Note. % calculated as valid percent using the n for each timepoint as the denominator.

Table S7. Independent Samples *t*-tests Comparing Baseline Scores between Youth Initiating GAH in Early versus Late Puberty

	Total sample	Early gender-affirming care			
		Yes	No		
	N=315	n = 24	n = 291	*p*-value	effect size
Appearance congruence	2.36 (0.88)	3.08 (0.95)	2.31 (0.85)	<0.001	0.86
Depression	16.44 (12.11)	9.57 (8.26)	17.00 (12.21)	<0.001	0.71
Anxiety	60.03 (11.48)	51.54 (12.20)	60.75 (11.15)	<0.001	0.79
Positive affect	43.05 (10.78)	50.27 (12.08)	42.47 (10.49)	<0.001	0.69
Life satisfaction	39.76 (10.85)	44.90 (14.13)	39.35 (10.46)	0.08	0.45

Note. Variables are presented as mean (SD). Results are based on *t*-tests. Effect sizes are Cohen's *d* (ranges: 0.20, small; 0.50, medium; 0.80, large).

Table S8. Independent Samples *t*-tests Comparing Baseline Scores between Youth Initiating GAH in Early versus Late Puberty Among Youth Designated Male at Birth

	DMAB	Early gender-affirming care			
		Yes	No		
	n=111	n = 20	n = 91	*p*-value	Effect Size
Appearance congruence	2.27 (1.03)	3.09 (1.02)	2.10 (0.95)	<0.001	1.00
Depression	17.52 (13.35)	9.41 (8.70)	19.23 (13.56)	<0.001	0.86
Anxiety	59.12 (11.47)	52.30 (11.94)	60.67 (10.85)	0.008	0.73
Positive affect	42.06 (12.68)	51.24 (12.70)	40.14 (11.87)	0.002	0.90
Life satisfaction	38.82 (13.47)	45.71 (15.20)	37.38 (12.71)	0.04	0.59

Note. DMAB = designated male at birth. Variables are presented as mean (SD). Results are based on *t*-tests. Effect sizes are Cohen's *d* (ranges: 0.20, small; 0.50, medium; 0.80, large).

Table S9. Independent Samples *t*-tests Comparing Baseline Scores between Youth Initiating GAH in Early versus Late Puberty among Youth Designated Female at Birth

	DFAB	Early gender-affirming care			
		Yes	No		
	n=204	n = 4	n = 200	*p*-value	Effect Size
Appearance congruence	2.42 (0.78)	3.04 (0.56)	2.40 (0.77)	0.11	0.94
Depression	15.85 (11.36)	10.32 (6.69)	15.96 (11.42)	0.19	0.60
Anxiety	60.52 (11.48)	47.75 (14.66)	60.78 (11.30)	0.17	1.00
Positive affect	43.59 (9.59)	45.65 (8.19)	43.55 (9.62)	0.65	0.24
Life satisfaction	40.27 (9.10)	41.08 (7.43)	40.25 (9.14)	0.84	0.10

Note. DFAB = designated female at birth. Variables are presented as mean (SD). Results are based on *t*-tests. Effect sizes are Cohen's *d* (ranges: 0.20, small; 0.50, medium; 0.80, large).

Figure S1 Conceptual Model of Parallel Process Latent Growth Curve Models

Conceptual model of parallel process latent growth curve models. Rectangles indicate measured variables. Ovals represent model-based estimates of baseline scores (intercepts) and linear rates of change (slopes). Straight arrows indicate regression paths to model (1) moderating effects of baseline covariates on growth curve intercepts and slopes and (2) effects of intercepts on slopes. Curved arrows represent correlations between intercepts and slopes.

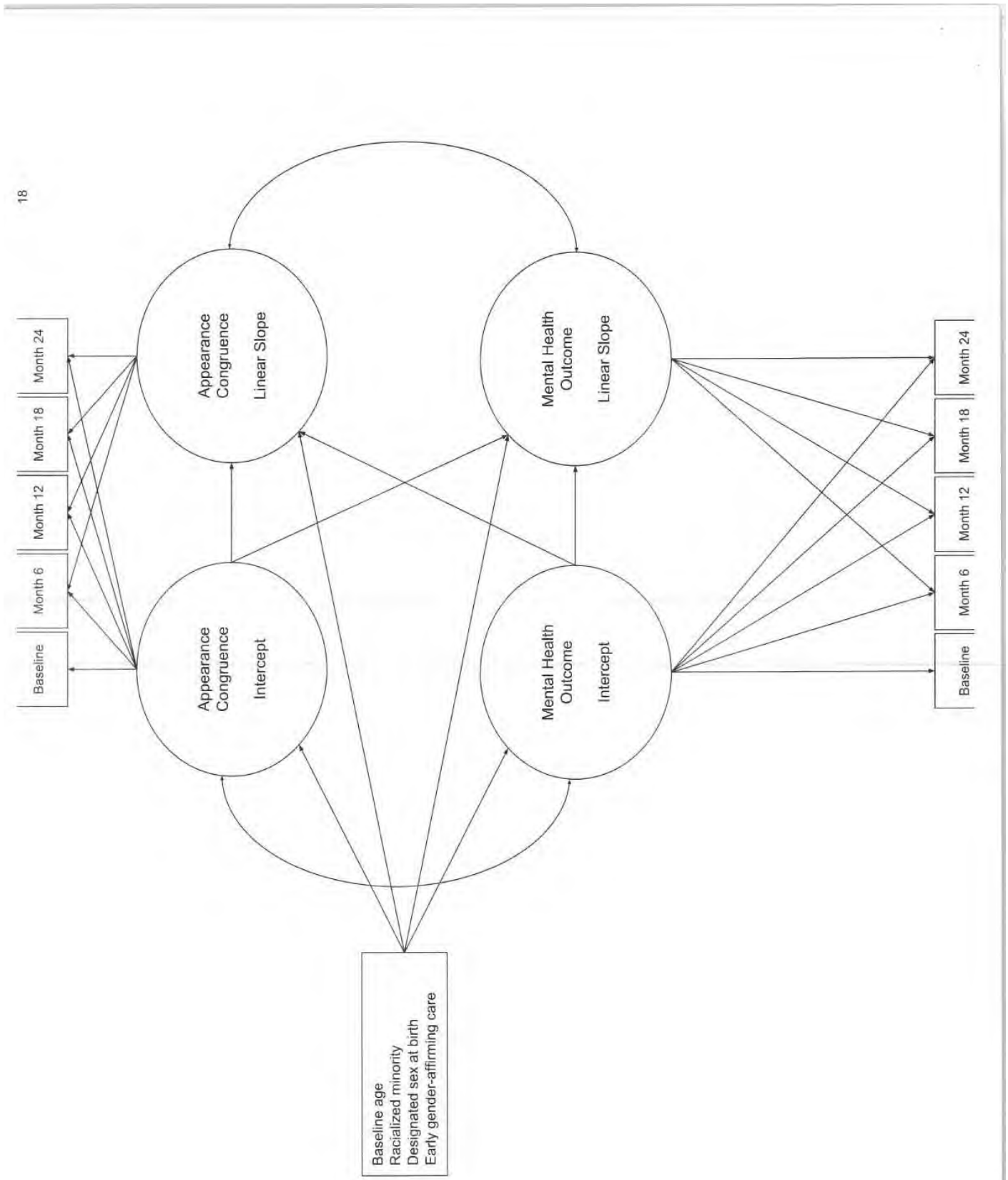
18
Appearance Congruence Linear Slope
Mental Health Outcome Linear Slope
Appearance Congruence Intercept
Mental Health Outcome Intercept
Baseline
Month 6
Month 12
Month 18
Month 24
Baseline age
Racialized minority
Designated sex at birth
Early gender-affirming care

Figure S2 Consort Diagram

Flow diagram of the progress through the phases of a prospective, observational study, including enrollment, follow-up, and data analysis for latent growth curve models.

Figure S2. Consort diagram

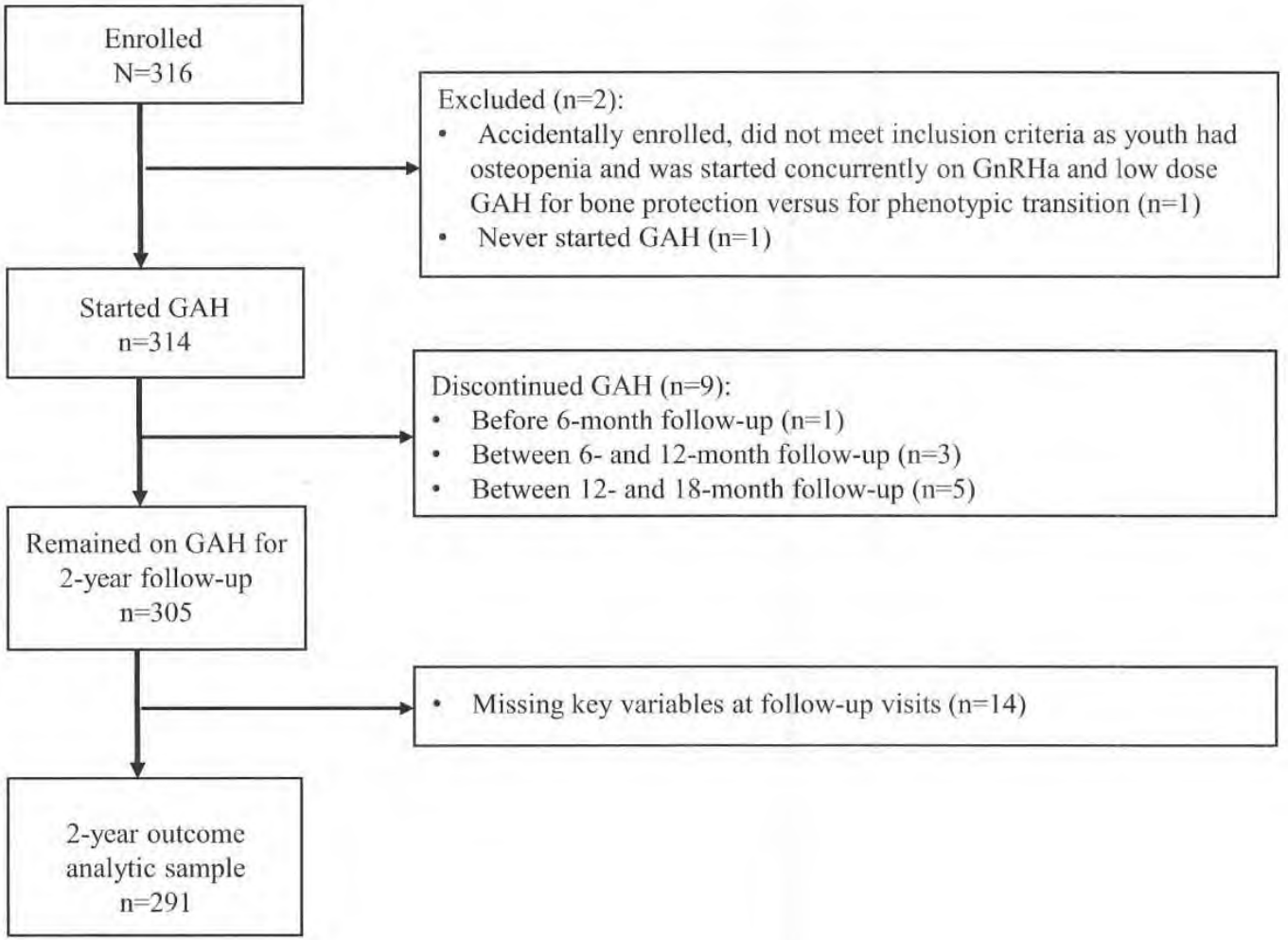

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Figure S3 Change in Psychosocial Outcomes by Designated Sex at Birth

Figure panels display changes in psychosocial outcomes over two years of GAH by designated sex at birth (designated female at birth: blue circles; designated male at birth: orange triangles). Lines indicate mean scores for each group with gray shaded bands for 95% confidence intervals. Outcomes shown are as follows: (S3-A) Transgender Congruence Scale, range: 1-5; (S3-B) Positive Affect Scale T-Score (NIH Toolbox), range: 0-100; (S3-C) Life Satisfaction T-Score (NIH Toolbox), range 0-100); (S3-D) Beck Depression Inventory-II, range: 0-63; (S3-E) Revised Children's Manifest Anxiety Scale, Second Edition T-Score, range: 0-100.

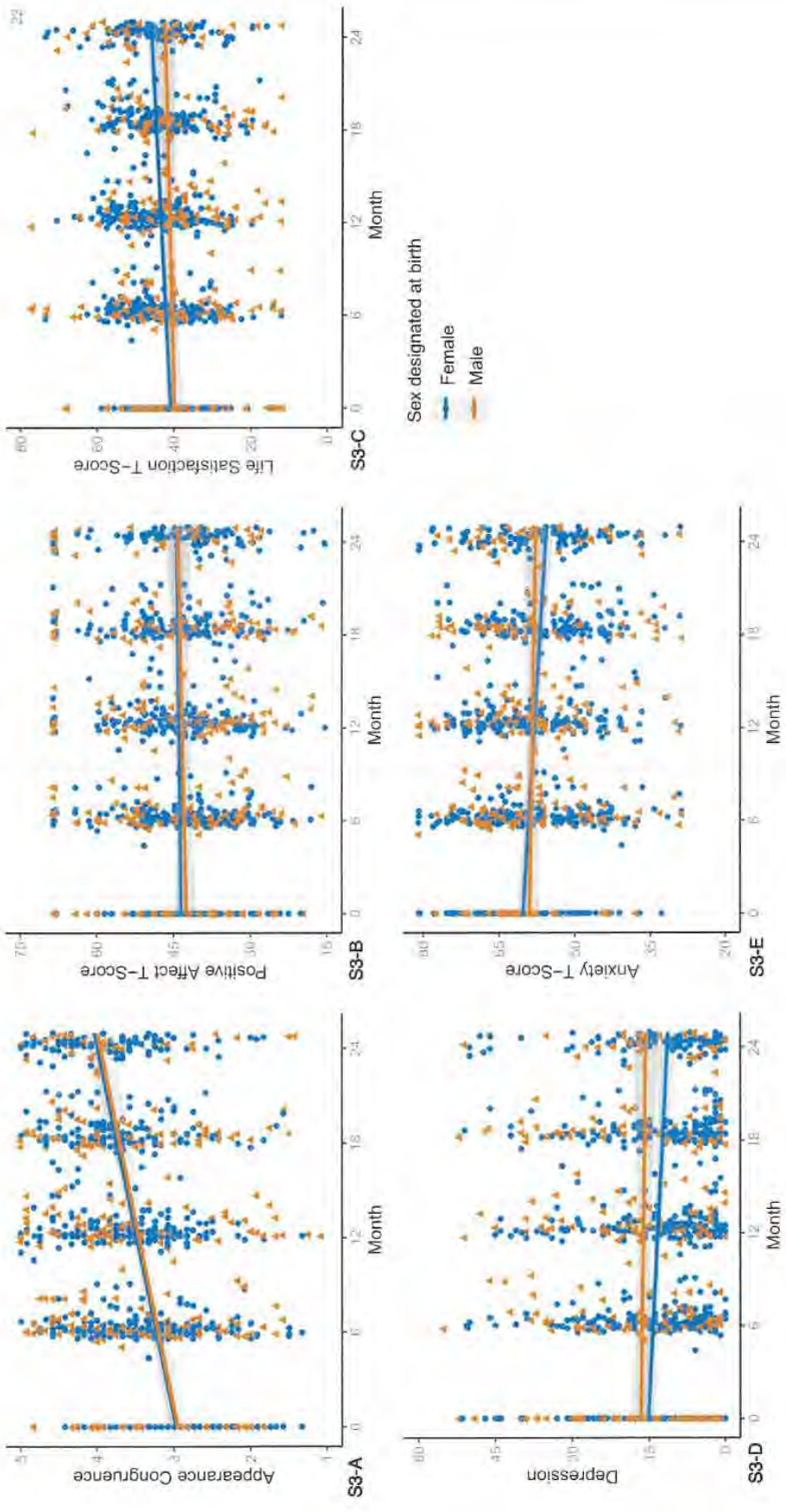
S3-A
Appearance Congruence
S3-B
Positive Affect T-Score
S3-C
Life Satisfaction T-Score
S3-D
Depression
S3-E
Anxiety T-Score
Month
Sex designated at birth
Female
Male

Figure S4 Change in Psychosocial Outcomes by Racial/Ethnic Identity

Figure panels display changes in psychosocial outcomes over two years of GAH by racial/ethnic identity (Non-Latinx White: blue circles; youth of color: orange triangles). Lines indicate mean scores for each group with gray shaded bands for 95% confidence intervals. Outcomes shown are as follows: (S4-A) Transgender Congruence Scale, range: 1-5; (S4-B) Positive Affect Scale T-Score (NIH Toolbox), range: 0-100; (S4-C) Life Satisfaction T-Score (NIH Toolbox), range 0-100); (S4-D) Beck Depression Inventory-II, range: 0-63; (S4-E) Revised Children's Manifest Anxiety Scale, Second Edition T-Score, range: 0-100.

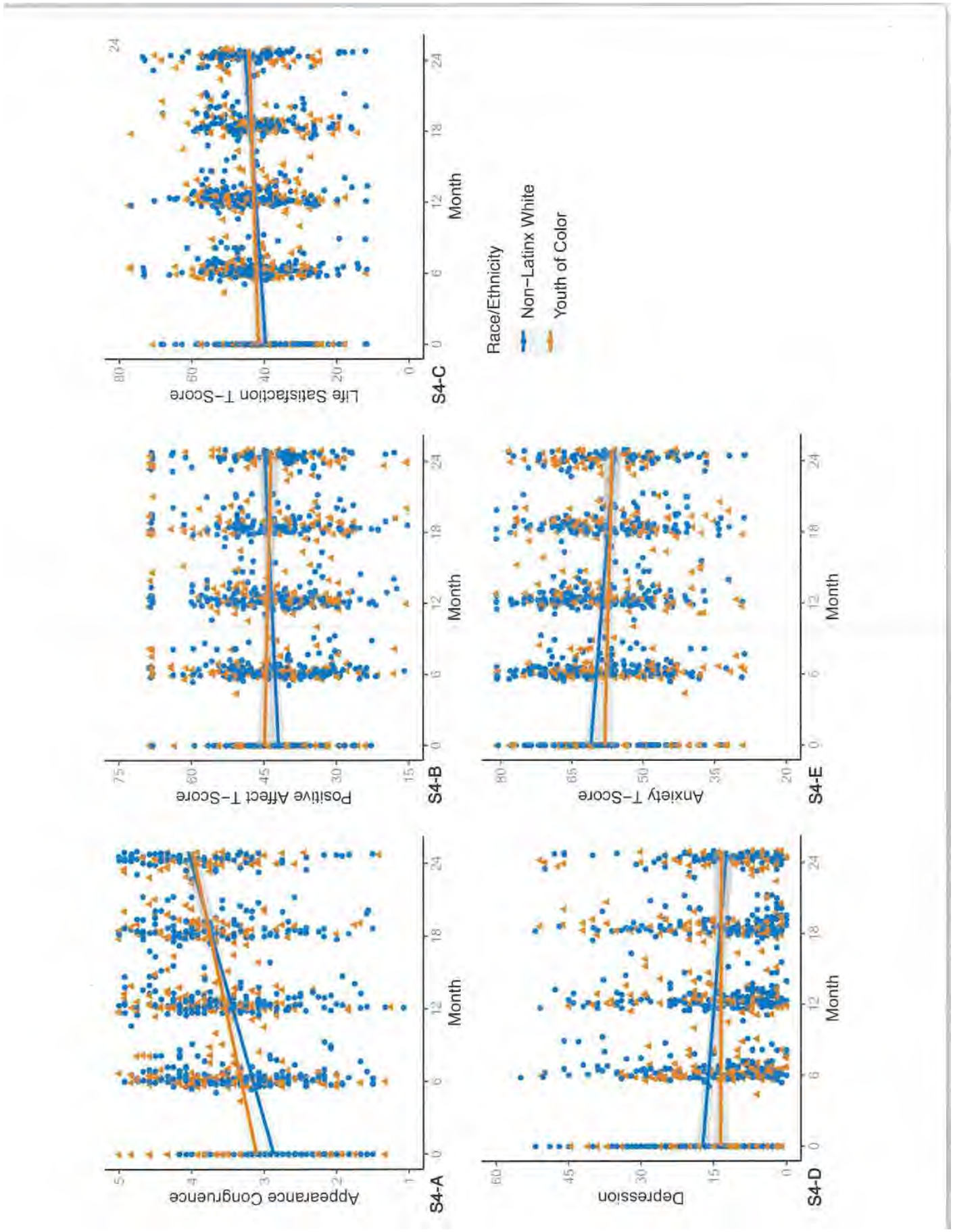
Race/Ethnicity
Non−Latinx White
Youth of Color
Life Satisfaction T−Score
S4-C
Positive Affect T−Score
S4-B
Anxiety T−Score
S4-E
Appearance Congruence
S4-A
Depression
S4-D
Month

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EXHIBIT 62

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ORIGINAL ARTICLE

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

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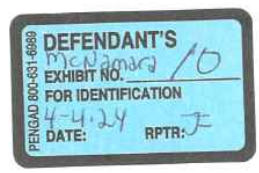

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Abstract

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

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REVIEW ARTICLE

Gender dysphoria in adolescence

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ABSTRACT

Adolescents presenting with gender-related concerns are increasingly seeking support from providers from a variety of disciplines within health care settings across the world. For those treating young people who meet the criteria for the DSM 5 diagnosis of gender dysphoria (GD), complex decisions in clinical care are common. Defining best practice with this population with respect to interventions that span mental health, physical, and surgical domains can be challenging, given a relative dearth of empirical data available; yet practice guidelines have emerged from different professional organizations which can aid with this. For this review paper, a broad literature search was performed to identify relevant studies pertaining to the care of adolescents with GD. In addition, an overview of trends in clinical practice, including shifts in conceptualization of how clinicians and patients define care that is considered affirming when working with this population, is described. This paper explores the characteristics of referral patterns to specialized clinics, provides a brief overview of gender identity development in adolescence, and then describes the phenomenology of known aetiological factors and co-occurring psychiatric issues in adolescents with GD. Additionally, clinical management considerations that detail assessment aims and common treatment interventions across disciplines will be explored.

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Introduction

Adolescents with gender identities and/or expressions that differ from societal expectations based on the gender that they were assigned at birth are a heterogeneous population. Some present with longstanding behaviours since childhood, while others are presenting with feelings and behaviours emerging during or after the onset of the physical changes brought on by puberty. Care for these transgender adolescents takes place in a variety of discipline settings across the world. The field is fast evolving in terms of defining best assessment and treatment practices that include interventions spanning both psychological and physical domains.

The point in time that marks the beginning of adolescence might vary depending on whether it is physical pubertal advancement or a youth's age that one uses to define the precise developmental border (Berenbaum, Beltz, & Corley, 2015). Understanding this distinction is important when considering physical interventions for young people presenting with gender identity concerns, as both physical maturation and psychological maturity associated with age are important determinants of eligibility and readiness for certain treatments as described in the World Professional Association of Transgender Health Standards of Care seventh edition (WPATH SOC7) (Coleman et al., 2011). The potential gap between physical and emotional maturity characterizes the potential dilemmas in assessing and treating transgender adolescents (for example, some individuals enter puberty as young as age nine or even earlier, and some pubertally advanced individuals are not emotionally mature enough to be able to give informed consent at ages as old as 16) (Steinberg, Cauffman, Woolard, Graham, & Banich, 2009).

In this review we conducted a PubMed search using the terms 'transgender adolescent', 'gender nonconforming youth', 'gender identity disorder adolescents', and 'gender dysphoria adolescent'. Search results were limited to articles within the last 10 years and yielded 70, 3, 42, and 30 articles respectively, with some overlap. Articles that were considered reviews or were out of scope for this review were not included (e.g. not focusing on adolescents, not in English, main scope not related to gender dysphoria (GD) or gender nonconformity). The authors also used additional references that were

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considered to be of great importance or relevance to the current state of affairs in the clinical management of transgender adolescents.

Terminology

With terminology constantly evolving, a cursory review of the most widely used definitions is important. 'Gender identity' refers to one's subjective sense of self as male, female, another gender, or a combination of aspects of maleness and/or femaleness. This is distinct from 'sex', which refers to anatomical features, including genitalia, that are typically used to assign an individual's gender at birth. When gender identity and sex are not aligned, the discrepancy has been referred to as 'gender discordance' (or incongruence) (Adelson, 2012) that may sometimes result in a negative affective disturbance known as 'gender dysphoria'. The same term describing this phenomenon has also more recently been used as the DSM5 diagnosis gender dysphoria (American Psychiatric Association, 2013), with two distinct sets of criteria, one set for children and another for adolescents/adults. In distinguishing the phenomenon from the diagnosis, we will use the acronym GD henceforth only when describing the latter. The International Classification of Diseases (ICD) currently classifies adolescents who desire to be another gender, accompanied by a wish to align their bodies through physical interventions with that gender, as 'transsexualism', and details of the history of these diagnoses are better illustrated elsewhere (Drescher, Cohen-Kettenis, & Winter, 2012). Currently, revision proposals of the ICD have suggested replacing the term transsexualism with 'gender incongruence'. 'Gender role or expression' refers to an individual's outward behavioural demonstration of gender, through mannerisms, clothing, posture, and other factors that a specific culture might associate with a specific gender. 'Gender nonconformity' (GNC) refers to gender expression that is non-stereotypical for a particular culture based on an individual's assigned gender.

The above terms refer to concepts and experiences often used clinically and in the literature; however, it is also important to define the terms that are used by individuals when describing associated identities. 'Transgender' has more recently become an umbrella term to describe a broad set of individuals whose gender identity differs from the gender that they were assigned at birth based on their sex. Some adolescents identify as 'gender non-binary' (or 'genderqueer'), which refers to identities that are neither exclusively male nor exclusively female (Richards, Bouman, Seal, Barker, Nieder, & T'Sjoen, 2016; Simmons & White, 2014). 'Cisgender' is the opposite of transgender – referring to the majority of the population whose gender identity and expression matches their gender assigned at birth. Cisgender has become an increasingly popular term as it raises awareness that all humans have a connection between their gender identity and physical anatomy.

Trends in treating Transgender and/or Gender Nonconforming (TGNC) adolescents

Model of care delivery

Clinics specializing in gender identity services for adolescents have markedly increased in number in the last decade, both within the USA and internationally (Antonio et al., 2013; Barrett, 2014; Hewitt et al., 2012; Hsieh & Leininger, 2014). In recent years, the affirmative model has been described regarding the approach to transgender youth (Hidalgo et al., 2013). The premise of such a model has been defined by Hidalgo et al. as one that does not view a transgender identity as inherently pathological and that a person should be supported by their clinician to live in the gender that feels most comfortable to them. The definition of an affirming care model with transgender adolescents has been expanded to include the importance of comprehensive psychological assessment before moving forward with physical interventions for this vulnerable and complex population (Edwards-Leeper, Leibowitz, & Sangganjanavanich, unpublished). There is no scientific data regarding how the term 'affirming' has been used among patients and providers, and whether a specific model of care delivery – specifically as it pertains to the degree of mental health evaluation required for physical interventions – would be considered affirming for youth. Given that physical interventions are being considered at younger ages (Olson & Garofalo, 2014), and that adolescents are presenting with increasing complexity and psychiatric co-morbidities (Leibowitz & Spack, 2011; Tishelman et al., 2015), defining best practices requires additional prospective medical and psychological data.

Referral rates and sex ratios

Two studies from the Gender Identity Service in the Child, Youth, and Family Program at Toronto's Centre for Addiction and Mental Health, and the Amsterdam Centre for Expertise on Gender Dysphoria illustrate the marked increase of adolescent referrals (compared to children) to their specialized gender identity treatment clinics in the last decade (de Vries & Cohen-Kettenis, 2012; Wood et al., 2013). In the Toronto study, the number of adolescent referrals surpassed the number of child referrals for the first time in the 2008–2011 cohort

since the clinic began collecting data in 1976. The authors surmise that with increased access to the Internet and social media, more adolescents seek treatment because they are better able to put a name to their experience and have increased awareness that treatment interventions exist.

The sex ratio of presenting adolescents has been previously described to be much closer to 1:1 than in children, with natal boys presenting at substantially higher rates than natal girls in the younger age group (Wood et al., 2013). Despite the more equal ratio of sexes presenting for treatment in adolescence, previous reports indicate that natal boys presented at slightly higher rates than natal girls in the past (Cohen-Kettenis & Pfafflin, 2003). However, a more recent study of the combined referral rates from the Toronto and Amsterdam clinics, looking at 748 adolescents combined over a period from 2006 to 2013, demonstrates that there was a significant inversion of the sex ratio of referred adolescents in both clinics favouring natal girls (Aitken et al., 2015). The inversion takes place within the context of increased numbers of adolescent referrals (compared to children) in both clinics in recent years, the relatively recent availability of pubertal suppression in the last decade, and the increased visibility of transgender issues in the media and Internet. The authors postulate that such an inversion may be the result of both increased visibility of transgender identities in the media (leading more young people to declare such an identity), and the disproportionately higher stigma that natal boys continue to experience compared to natal girls (making it easier for natal girls to come out and seek transition).

Development of gender identity in adolescents

Adolescence is considered a crucial period of gender identity development in gender non-conforming young people (Steensma, Kreukels, de Vries, & Cohen-Kettenis, 2013). Adolescents in a qualitative study reported that ages 10–13 years were essential for them when it came to determining whether GD persisted or desisted. This was influenced by the changes in social interactions with peers, the emerging physical characteristics of puberty, and the first romantic experiences during this time in their lives (Steensma, Biemond, Boer, & Cohen-Kettenis, 2011).

After the onset of puberty, it is considered that the likelihood that GD will persist into adulthood is high. Indeed, in three Dutch clinical follow-up studies of adolescents who, after comprehensive psycho-diagnostic assessment, received puberty suppression and/or cross-gender hormones, none of the participating adolescents refrained from gender affirming surgery after they had started pubertal suppression (GnRH agonists) or cross-gender hormones (Cohen-Kettenis & van Goozen, 1997; de Vries et al., 2014; Smith, Van Goozen, Kuiper, & Cohen-Kettenis, 2005a).

Some adolescents may also present with GD after the start of puberty. This adolescent late onset type of GD is described and studied in adult transgender populations (Lawrence, 2010), but few reports exist in the adolescent research. One study demonstrated that natal female adolescents presenting with GD recalled, on average, more cross-gender behaviour in childhood compared to their natal male counterparts (Zucker, et al., 2012). The authors suggest that this may reflect the fact that it is mostly natal boys who present with a late onset type of GD in adolescence, similar to adults (Lawrence, 2010). However, 65% of Finnish gender clinic-referred adolescents of mainly natal female assignation (41 of 47 adolescents studied) belonged to this late onset group (Kaltiala-Heino, Sumia, Työläjärvi, & Lindberg, 2015). Clearly, there is a need for further empirical studies on the time of onset of GD in adolescents.

Studies that report on sexual orientation of adolescents with GD show variable results. In one Dutch study almost all of the adolescents who were evaluated to be eligible for pubertal suppression reported being sexually attracted to individuals of their natal sex (de Vries, Steensma, Doreleijers, & Cohen-Kettenis, 2011). Another study showed that, compared to natal male adolescents with early- onset GD, natal male adolescents with late onset GD were more often sexually attracted to a gender different from their natal sex (Zucker et al., 2012). Finally, in a third study on clinic-referred transgender boys and girls, 40–45% of them were sexually attracted to individuals of a gender different from their natal sex with no significant difference between the binary genders of male or female (Spack et al., 2012).

The relevance of sex ratio, age of onset, and sexual orientation lies in the fact that adult studies have revealed that natal maleness, late onset GD, and a sexual attraction to individuals of a different gender from the adolescent's natal sex may predict a more challenging treatment course and outcome (Lawrence, 2010; Smith et al., 2005a; Smith, van Goozen, Kuiper, & Cohen-Kettenis, 2005b). Additionally, trends in the onset of GD in relation to sexual attraction in these populations may be of interest in understanding aetiological and phenomenological underpinnings of GD.

Aetiological factors related to gender dysphoria in adolescents

Adolescents have only recently become the subject of aetiological studies on GD. It is most likely that the same

factors that are considered to be involved in GD of childhood and adulthood play a role in adolescence. Currently, it is thought that psychological, social, and biological factors are all involved. Earlier studies have reported correlations of GD with certain psychological factors in children (e.g. separation anxiety) (Coates & Person, 1985; Zucker, Bradley, & Lowry Sullivan, 1996); yet other studies interpreted these factors as a consequence, not cause, of gender variance (Pleak, Meyer-Bahlburg, O'Brien, Bowen, & Morganstein, 1989; Wallien, Zucker, Steensma, & Cohen-Kettenis, 2008).

Biological factors that have been studied in adolescents with GD are fraternal birth order and sibling sex ratio (Schagen, Delemarre-van de Waal, Blanchard, & Cohen-Kettenis, 2012; Vanderlaan, Blanchard, Wood, & Zucker, 2014). The maternal immunization hypothesis suggests that mothers develop more anti-male antibodies (against male-specific antigens linked to the Y chromosome) during each pregnancy of a male fetus. The resulting anti male immune-reaction then affects male brain development of the fetus and results in higher chances of a sexual orientation attracted to individuals of the natal sex of the child (Blanchard, Zucker, Cohen-Kettenis, Gooren, & Bailey, 1996). Two studies in adolescents with GD – who were assumed to be sexually attracted to individuals of their natal sex – confirmed this hypothesis, one in natal boys, the other one in both natal boys and natal girls (the latter according to their sisternal birth order) (Schagen et al., 2012; Vanderlaan, Blanchard et al., 2014).

Recently, the first series of structural and functional brain imaging studies in adolescents with GD have been published. These studies examine whether sexual differentiation of the brain is more in line with the experienced gender identity of the transgender adolescent than it is with natal sex. In one such study on whole-brain grey matter in 55 adolescent natal girls and 38 adolescent natal boys with GD, compared to 44 boys and 52 girls without GD, differences in brain volumes were only found between the sexes, however not between the groups with and without GD for each sex. However, in certain sex dimorphic structures, subtle differences were found between adolescents with and without GD (Hoekzema et al., 2015), and this was true in both medically treated and untreated subgroups. Another study on functioning of the brain showed that on a verbal fluency task, adolescent natal boys with GD performed better (as expected) than the control boys (but also better than the adolescent natal girls with GD and controls girls), but this was not represented in any significant differences in brain activity between the groups (Soleman et al., 2013). In a third study of prepubertal children as well as adolescents with and without GD, androstadienone – an odorous stimulus that evokes a sex dimorphic response in the hypothalamus in adults without GD (Burke, Veltman, Gerber, Hummel, & Bakker, 2012) – was used to test the role of puberty hormones on typical and atypical sexual differentiation of the brain. Results in prepubertal children were that those without GD already showed this sex dimorphic response, while prepubertal girls with GD showed activation levels that were in between control boys and girls without GD. No differences were found between prepubertal boys with GD compared to their natal sex peers (Burke, Cohen-Kettenis, Veltman, Klink, & Bakker, 2014). Adolescents with GD participating in this study responded convincingly like their cisgender peers. In summary, some of the neuroimaging studies revealed that adolescents with GD had structural and functional characteristics that were in the direction of, or similar to, controls of their experienced gender, while other studies failed to find these differences.

Co-occurring psychological functioning

Perhaps due to the fact that adolescents with GD have long been an underserved population, studies investigating their psychological functioning are limited. It is only quite recently that studies of specialized gender identity clinics have published findings in this area. Understanding the psychological functioning and trends of co-occurring psychiatric illness in adolescents with GD is important both for determining an appropriate treatment plan that potentially includes irreversible interventions as well as to define future areas of research.

With regard to gender identity clinic-referred adolescents, methods and results of these studies differ greatly. Most studies have used chart review to report on psychiatric co-morbidity. For example, Spack et al. described that in their sample of 97 referred adolescents, 44.3% had a prior history of psychiatric diagnoses, 37.1% were taking psychotropic medications, and 21.6% had a history of self-injurious behaviour (Spack et al., 2012). Another such study of 84 Canadian adolescents reported mood and anxiety disorders in 44% and 33% within their birth-assigned female and male referrals respectively (Khatchadourian et al., 2014); and sadly 10 adolescents in this clinical sample had attempted suicide. All 21 adolescents assessed for eligibilty of medical intervention at an Australian clinic were reported to have symptoms of anxiety or depression (Hewitt et al., 2012). Of 218 referred cases (both children and adolescents) to a London gender identity service, available information before assessment revealed that low mood/depression occurred in 42% and self-harming in 39% (Holt et al., 2014). In a clinical sample of Finnish referrals for

medical intervention, exceptionally high rates of co-ocurring psychiatric disorders were reported, with 64% being treated for depression and 53% for suidical and self-harming behaviours. Additionally, psychotic symptoms, substance use, autism, and attention deficit hyperactivity disorder (ADHD) were also reported (Kaltiala-Heino et al., 2015).

These studies utilized an archival chart review methodology, so therefore it is difficult to interpret these prevalence findings. Using the standardized Diagnostic Interview Schedule for Children (DISC) (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), it was found that the majority (67.6%) of a cohort of 105 Dutch adolescent gender dysphoric referrals did not suffer from any psychiatric disorder (de Vries, Doreleijers, Steensma, & Cohen-Kettenis, 2011). In this sample, anxiety, mood, and disruptive behaviour disorders occurred in 21%, 12.4%, and 11.4% of the adolescents, respectively.

There is only one study directly comparing adolescents to adults with gender dysphoria. This study compared 86 adolescents with 293 adults by use of the Minnesota Multiphasic Personality Inventory (MMPI) and revealed that adults showed significantly more problems in the clinical range than adolescents (de Vries, Kreukels, Steensma, Doreleijers, & Cohen-Kettenis, 2011). This might give some evidence that early coming out and seeking care in adolescence (versus adulthood) might prevent some of the psychological burden that transgender individuals experience during their lives.

Some of the studies make use of similar measures that allow comparisons to be made between different clinics. For example, a study describing the baseline characteristics of 101 transgender adolescents seeking medical intervention (although it was not made clear whether they all started treatment) found clinically relevant depression scores as measured by the Beck Depression Inventory (BDI) (Beck, Steer, & Brown, 1996) in 35% of the sample (24% in the mild–moderate range and 11% in the severe range) (Olson, Schrager, Belzer, Simons, & Clark, 2015). The same measure was used in a follow-up study of 70 adolescents receiving puberty suppression. The mean baseline depression BDI score in that sample was well below the clinical range (de Vries, Steensma et al., 2011). The factors that explain this apparent cross-clinic difference are yet to be determined; however, cultural differences in tolerance may be an underlying factor.

Various gender identity service sample studies have used the Child Behavior Checklist (CBCL) (Achenbach & Edelbrock, 1983); the Youth Self-Report (YSR) (Achenbach, 1991); and the Teacher's Report Form (TRF) (Achenbach & Edelbrock, 1986). One such study of adolescents with GD showed that 67.1% of the 71 natal girls and 81.9% of the 83 natal boys had clinical range emotional and behavioural problems using the CBCL (Zucker, Bradley et al., 2012). When using the YSR, these percentages were much lower at 47% of natal girls and 49% of natal boys in a London-based gender identity clinic sample ($n = 141$) (Skagerberg, Davidson, & Carmichael, 2013). The reason for this difference may lie in the method used, with self-report being more likely to reveal lower percentages of emotional difficulties than adult-report (Achenbach, McConaughy, & Howell, 1987).

In a direct cross-national, cross-clinic, comparison between adolescents referred to a Canadian ($n = 177$) and a Dutch ($n = 139$) gender identity clinic using both the CBCL and the YSR, clinically significant behavioural and emotional problems occurred in 55.4%/40.6% respectively of the Amsterdam cohort and 77.5%/39.9% respectively of the Toronto cohort (de Vries, Steensma, VanderLaan, Cohen-Kettenis, & Zucker, 2015). In another such study, the TRF was used in a sample of 174 adolescents attending an Amsterdam or Toronto based clinic (Steensma et al., 2014). Teachers reported significantly more problem behaviour in the Toronto adolescents compared to those in Amsterdam, with clinical range behavioural and emotional problems occurring in 57.1% of Toronto adolescents and 33.3% in Amsterdam. Both the CBCL/YSR and the TRF studies thus showed that the Toronto-referred adolescents had more co-occurring psychological problems than the Amsterdam samples. Poor peer relations turned out to be the overall strongest predictor for emotional and behaviour problems (de Vries et al., 2015; Steensma et al., 2014). This finding is in line with general population studies that show that peer victimization (e.g. rejection by peers, teasing by peers) is an important factor leading to psychological distress in LGBT youth (Birkett, Newcomb, & Mustanski, 2015; Liu & Mustanski, 2012; Robinson, Espelage, & Rivers, 2013; Toomey, Ryan, Diaz, Card, & Russell, 2010). Cross-clinic differences may thus reflect that in some societies there is more peer acceptance and tolerance to gender non-conforming youth than in others. This knowledge is important when developing programmes that help to improve the sometimes worrisome psychological functioning of transgender youth.

Autism spectrum disorders (ASD) are reported to occur in higher than expected percentages in gender dysphoric adolescents when compared to the general population prevalence (which is around 1%). Of the adolescents assessed at a specialized gender identity clinic, 9.4% had a co-occurring confirmed ASD diagnosis (de Vries et al., 2010). Other studies using instruments

measuring autistic traits in adolescents with GD also report this overrepresentation (Miesen, de Vries, Steensma, & Hartman, unpublished). In a sample of children and adolescents with ASD, gender variance (defined as the parent-reported expressed wish to be the other gender on the CBCL) was expressed 7.59 times more frequently compared to non-referred controls (Strang et al., 2014). In a sample of 559 adolescents and 803 adults, gender variance (defined as the self-reported wish to be the other gender on the YSR and ASR) was expressed 1.84 and 2.62 times more frequently respectively as compared to the normative samples (Miesen, Hurley, Bal, & de Vries, unpublished). Apart from the theoretical question of why ASD and GD would co-occur at increased rates than ASD would in the general population, adolescents with autistic features pose clinicians challenging diagnostic and management issues when they seek gender reassignment treatment (see Miesen, Hurley, & de Vries, this issue).

As mentioned above in the different clinical chart reports, suicidality can be of specific concern in transgender adolescents. Using a convenience sample recruited at a community centre of 55 transgender adolescents, nearly half reported serious suicidal thoughts and a quarter reported suicide attempts (Grossman & D'Augelli, 2007). In a clinic chart study of adolescents referred to a gender identity clinic in London, 24% reported self-injurious behaviour, 14% had ideations to self-injure, and 10% had made a suicide attempt (Skagerberg, Parkinson, & Carmichael, 2013). Indeed, Olson et al. (2015) report suicidal thoughts (ever) at 51%, and suicide attempts (ever) at 30% of their sample of adolescents seeking medical care. These data speak to the need for providing clinically competent transgender health-care services when treating severe depression that manifests in such a way.

Clinical management

Interdisciplinary collaboration

Providing optimal care to adolescents with GD preferably involves collaboration between different disciplines. The importance of an interdisciplinary approach in working with transgender individuals is emphasized within clinical practice guidelines from major professional organizations including the WPATH SOC7 (Coleman et al., 2011), the American Academy of Child and Adolescent Psychiatry (AACAP) (Adelson, 2012), the American Psychological Association (APA) (2015), and the Endocrine Society (Hembree et al., 2009). Interventions span the social (advocacy efforts in schools and elsewhere), psychological (individual psychotherapy, family psychotherapy, parent guidance, completing psychological readiness evaluations), hormonal (menses cessation, pubertal suppression, cross-gender hormone therapy), psychiatric (psychopharmacology management, potential higher level of care), adjunctive (fertility consultation, voice training), and/or surgical (chest surgery most commonly in this age group) domains.

Gender identity treatment clinics have different psychological assessment and treatment protocols outlined in their approach to caring for young people with GD (de Vries & Cohen-Kettenis, 2012; Edwards-Leeper & Spack, 2012; Menvielle, 2012, Sherer, Rosenthal, Ehrensaft, & Baum, 2012; Zucker, Wood, Singh, & Bradley, 2012); however, they all rely on interdisciplinary perspectives and services. Interdisciplinary teams that function to provide high-quality care have been described in general (Ndoro, 2014). Ndoro explains that high-quality care relies on the six Cs, which include care, compassion, competence, communication, courage, and commitment. Presumably, gender health providers share the common goals of being compassionate and competent, and possessing courage and commitment. According to Ndoro, poor communication among team members has been implicated in poor outcomes. Improved communication among gender health providers likely helps to establish role clarity, trust, and the understanding of any ideological position discrepancies. Recognizing the variability in logistics and resources available to different institutions, it is important for providers to overcome the barriers in care that may present when working in different institutions without having the benefit for routine clinical rounds and team discussion.

Assessment

Assessment of transgender adolescents involves several key tasks, broadly organized into gender-related concerns and general psychological functioning. The main aims of an assessment are highlighted in Table 1 and providers can look to the WPATH SOC7, which has an extensive section on child and adolescent mental health (Coleman et al., 2011).

A clinical interview that incorporates time spent with the family unit together, the individual adolescent alone, and the parents alone, may be necessary to elicit the relevant information that will determine next steps. According to these SOC, interactions between clinician, staff, patients, and families, should be affirming of the adolescent's asserted gender identity to help establish therapeutic rapport and provide respectful care. This may include an individual assessment of the adolescent's preferred name and pronoun within the therapy setting,

Table 1. Assessment aims in adolescents presenting with gender issues.

Gender-specific	DSM 5 criteria for gender dysphoria
	Presence of desire to be or be perceived as another gender
	Desire for secondary sexual characteristics of another gender
	Desire to be rid of secondary sexual characteristics of birth sex
	Psychometrics
	Utrecht Gender Dysphoria Scale (UGDS)
	Recalled Childhood Gender Identity Scale (RCGI)
	Body Image Scale (BIS)
	Gender Minority Support and Resilience (GMSR)
	Internal conceptualizations of gender (some constructs in GMSR)
	Understanding of the difference between gender identity and role
	Gender one imagines oneself to be in the future
	Sense of pride in gender identity and expression
	Presence of internalized transphobia
	Relationship between gender identity and sexual orientation
	Perceived community connectedness
	External factors related to gender
	Parent and family support, ambivalence, or rejection
	School climate
	Community climate
	Gender development
	Childhood gender development
	Timeline and onset of gender dysphoria
	Physical exam by a paediatrician (when appropriate) to determine pubertal advancement
General functioning	Psychodiagnostic
	No co-occurring mental health diagnoses
	Co-occurring mental health diagnoses that complicate understanding of gender dysphoria
	Co-occurring mental health diagnoses that may be the result of the presence of gender dysphoria
	Co-occurring mental health diagnoses that do not impact understanding the presence of gender dysphoria but may complicate readiness for physical interventions
	Psychological, psychosocial, and psychiatric functioning
	Emotional functioning
	Cognitive functioning
	Resiliency and adaptive ego strengths
	Social support system and ability to develop supports

although there has been no research to establish how and when pronoun use and preferred name are associated with the development of therapeutic rapport.

An assessment can determine the main concerns of the referred adolescent and parents. Although many adolescents nowadays come with a wish for physical interventions and gender reassignment, some may seek help with the social aspects of gender identity and expression (Tishelman et al., 2015).

Assessing eligibility for physical interventions includes determining the presence of the DSM5 diagnostic classification of GD, which involves a 6-month duration and persistence of criteria (American Psychiatric Association, 2013). Criteria that are specific to certain physical interventions will be discussed below. The use of validated measures can inform the assessment and include the following: (1) Utrecht Gender Dysphoria Scale, a dimensional assessment of current GD (Steensma, Kreukels, Jurgensen et al., unpublished) (2) the Recalled Childhood Gender Identity Scale, a series of retrospective questions about childhood memories of gender role (Zucker et al., 2006) and (3) the Body Image Scale- a dimensional assessment of body dysphoria that asks the adolescent to assess 30 different anatomical features of their body in addition to whether the body part should be changed or not (Lindgren & Pauly, 1975). Other measures have been published, such as the Gender Minority Stress and Resilience Scale (GMSR), which assesses nine constructs related to minority stress and life satisfaction for individuals who identify as transgender (Testa, Habarth, Peta, Balsam, & Bockting, 2014).

The APA guidelines encourage completing a comprehensive evaluation of these young people (American Psychological Association, 2015) and the information gathered during the clinical interviews may take place over several visits with a patient and family. Factors such as the history of childhood gender development and the time of onset of GD are likely to inform treatment steps as the clinician can determine whether an intensification of anatomical distress has occurred with pubertal advancement. As described above, it can be helpful to explore in more detail several constructs related to gender identity that are used in the GMSR: (1) degree of feeling affirmed by others, (2) future expectations, (3) the

degree and source of pride they feel related to their gender identity, (4) the presence of internalized transphobia (self-loathing due to a transgender identity), and (5) how much community connectedness they may feel with other transgender adolescents (either through social media or in person). Understanding these constructs in detail may help the clinician understand any underlying motivations for the sought interventions and to more accurately pinpoint the presence of the DSM criteria for GD when it is unclear; however, use of this measure has not been studied in clinical populations to date.

According to the WPATH SOC7, the assessment should also include a broad psycho-diagnostic evaluation to determine the presence of any other co-occurring psychiatric diagnoses (Coleman et al., 2011). The presence of a co-occurring mental health diagnosis should not automatically preclude an appropriate adolescent who meets criteria for GD from being eligible for physical interventions, but psychological functioning should be stable enough and should not interfere with determining the presence of GD. Psychiatric symptoms that interfere with readiness criteria for physical interventions may include active suicidality, mania, psychosis, and/or other potential reinforcers of unsafe behaviour within a family system (e.g. a patient engaging in self-injury and family members agreeing to consent to irreversible hormonal interventions on the condition that the self-injury stops). Determining the relationship between any co-occurring mental health issue(s) and GD is important in determining appropriate treatment interventions. For example, if an adolescent meets criteria for a depressive disorder and GD, it is entirely possible that the depression could be a manifestation of underlying GD. Alternatively, if an adolescent meets criteria for an ASD and reports aspects of GD, it might be possible that the ASD complicates the assessment and impairs the adolescent's ability to understand the differences between gender identity and gender role, or gender identity and sexual orientation. Determining an adolescent's emotional and cognitive functioning are also important aspects of the assessment as specified in the WPATH SOC7.

In order to make a fully informed decision regarding puberty suppression and/or cross-gender hormones, the adolescent should be able to understand the information on treatment. It is also important to assess the degree of social supports that an adolescent may have and how resilient the individual is when faced with adversity or change. WPATH SOC7 specifies that support from family or others is a necessary criterion of eligibility for physical interventions with adolescents (Coleman et al., 2011).

For adolescents with non-binary gender identities (yet meeting criteria for GD), there is no systematic data or empirical evidence regarding the use of cross-gender hormones. In these adolescents it may be particularly important to understand the degree to which the adolescent is experiencing a core gender identity discrepancy versus having a motivation to simply challenge the societal gender binary through the use of irreversible physical changes to their body. There is no specific long-term data on the effects of physical interventions when used in gender non-binary adolescents (who meet the recently published DSM 5 GD criteria), so the authors conclude that it is extremely important to understand these adolescents' expectations of physical changes to make sure that they are realistic and accurate, and that they address the actual mind–body discrepancy that is causing distress to the person.

For individuals meeting criteria for GD, collaborating with a medical provider who can obtain a physical exam and determine pubertal advancement is helpful as physical treatment interventions are considered based on pubertal stage.

Management and treatment

As previously noted, the management of adolescents with GD may require interventions that span different domains. An overview of each of these interventions, and the existing evidence to use them, is provided below. Physical and mental health interventions can be used in conjunction with each other, and the timeframe for when such interventions might be recommended is highly individual and depends upon several factors. In general, physical interventions pertaining to gender transition are best organized according to the degree of reversibility that they effect on an individual's body. Therefore, the guidelines referenced above suggest that significant exploration of the risks and benefits of such interventions should be prioritized in order to obtain informed consent, particularly for those treatments that have more irreversible effects.

Mental health interventions

Supportive therapy may be indicated to further explore aspects of the adolescent's gender identity and gender transition. Given the higher rates of co-occurring mental health diagnoses often present in transgender adolescents, prioritizing treatment of those conditions is often appropriate. In some situations – especially should any significant safety concerns be elicited in treatment – an adolescent may need to be referred for a higher level of care. Special consideration of the gender aspects of care

should be taken into account in those situations as certain institutions may pair the individual according to natal sex or may not be familiar with the importance of using an adolescent's preferred name and pronoun. In some situations, additional advocacy efforts may be required on the part of the treating clinician, such as calling schools or referring to any local community support – as specified in the AACAP Practice Parameter (Adelson, 2012).

Supportive therapy can serve as a mechanism to help build adaptive ego strengths for transgender adolescents, including those who declare non-binary gender identities. As recommended in the APA guidelines (American Psychiatric Association, 2015) and the AACAP Practice Parameter (Adelson, 2012), this includes (1) heightening an adolescent's ability to accurately detect any unsafe situations in the social environment, (2) understanding the benefits and risks of self-disclosure across varying contexts, (3) exploration of hypothetical reactions of loved ones to any physical changes that might take place with sought physical interventions, (4) understanding how one's gender identity intersects with one's ethnic/cultural identity, and (5) the development of healthy coping strategies in the presence of potential adversity and stigma. Adolescents affirming a non-binary gender identity may have particular struggles in explaining their preferred pronoun, desire to not be perceived as exclusively male or female, and concept of an alternative gender to others (Simmons & White, 2014).

The WPATH SOC7 lists the following non-physical interventions to help alleviate GD in an adolescent: (1) in-person and online support groups or organizations that provide social support and advocacy, (2) in-person and online resources for friends and family, (3) breast binding (for natal girls) or padding (natal boys), genital tucking or penile prostheses, or padding of the hips and buttocks, and (4) changes in name and gender markers on identity documents. Additionally, the WPATH SOC7 lists interventions that might be considered in conjunction with meeting eligibility criteria for physical interventions. These include voice and communication therapy to help the individual develop both verbal and nonverbal communication skills that facilitate comfort with their gender identity, and hair removal through electrolysis, laser treatment, or waxing (for natal boys affirming a female gender identity).

Additionally, mental health support can be important for adolescents during the periods of physical change that take place with hormonal and/or surgical intervention. Helping an adolescent adjust to a changing body can potentially mitigate the negative effects that false expectations of anticipated changes may incur (Cohen-Kettenis & Pfäfflin, 2003; Cohen-Kettenis, Steensma, & de Vries, 2011).

Reversible physical interventions

Pubertal suppression with gonadotropin releasing hormone agonists (GnRHa) is a reversible hormonal intervention that prevents the development of unwanted secondary sexual characteristics of an adolescent's natal sex (Delemarre-van de Waal & Cohen-Kettenis, 2006). The evolution of the use of pubertal suppression, first used in this population in Amsterdam, is best summarized in Kreukels & Cohen-Kettenis, 2011, and is described in a long-term follow-up case report (Cohen-Kettenis, Schagen, Steensma, de Vries, & Delemarre-van de Waal, 2011). Table 2 describes the benefits, limitations, and currently known areas of scientific knowledge related to pubertal suppression.

The WPATH SOC7 (Coleman et al., 2011) and the most recent Endocrine Society Guidelines (Hembree et al., 2009) support its use in appropriately screened adolescents with GD. Suppressing pubertal development allows for an extended diagnostic period in younger adolescents who are at pubertal Tanner stages 2 or 3, prior to using hormonal or surgical interventions that carry irreversible changes. Should an adolescent receive pubertal suppression, and then ultimately receive other more irreversible physical interventions later on, they would more closely resemble the phenotypic gender with which they identify. Passing in society as the gender one most closely identifies with has been associated with better psychological adjustment and outcomes in adulthood (Lawrence, 2003). A natal boy would not develop broad shoulders, a deepening voice, or male patterns of body or facial hair. A natal girl would not experience breast development or the fat distribution associated with female bodies. Some surgical procedures such as a mastectomy (more commonly referred to as top surgery in this population), which are often invasive and costly, would be prevented as a result. One prospective study that evaluated 55 transgender adults after sexual reassignment surgery (22 trans women and 33 trans men), all of whom had received pubertal suppression as adolescents, demonstrated an alleviation of GD and healthy psychological adjustment that was comparable to their cisgender adult peers (de Vries et al., 2014).

Pubertal suppression has both hypothetical and actual limitations. One clinical challenge of pubertal suppression is related to the duration that one can medically suppress puberty safely. Depending on when a specific youth physiologically enters puberty (with some natal girls beginning as early as age 8 or 9 and some natal boys starting as early as age 10 or 11; Sizonenko, 1987), it is

Table 2. Pubertal Suppression – an overview.

What is known	What is not known
Pubertal suppression in adolescents with gender dysphoria	
• Prospective data on psychological outcomes into early adulthood indicates that it is a successful intervention when provided at ages typically above 12 years old (de Vries et al., 2014) • Preliminary research with prospective data on bone development indicates some potential reduction in bone mass density (Klink et al., 2015) • Pubertal suppression when later followed by cross-gender hormone administration promotes the development of an appearance more consistent with the other gender (Coleman et al., 2011, Hembree et al., 2009) • Data exist that correlate the degree to which gender dysphoric young adults physically appear as the opposite gender to healthier psychological outcomes (Lawrence, 2003) • Sex hormones are thought to be trophic on brain development in areas that affect cognitive growth and affect regulation (Berenbaum, Beltz, & Corley, 2015) • Preliminary fMRI research on the effects of pubertal suppression on brain development in gender dysphoric adolescents (when started at around age 12), indicates no detrimental effects on executive functioning in those without co-occurring neurodevelopmental disorders (Staphorsius et al., 2015) • Waiting for Tanner 2 pubertal stage before starting pubertal suppression is important in understanding an adolescent's response to their changing body (Hembree et al., 2009) • WPATH SOC 7 specifies criteria for using pubertal suppression (Coleman et al., 2011)	• Unclear long-term effects on brain development in this population • Lack of consensus among gender specialists in the field regarding the ideal time to start pubertal suppression (whether to use age, degree of pubertal advancement, or both) • The effect of pubertal suppression on brain development in young adolescents with co-morbid neurodevelopmental disorders has not been studied (yet there is a lack of understanding of sex hormone influences on brain development and behaviour in general) • Lack of data suggesting the benefits of using pubertal suppression for older (more pubertally advanced) gender dysphoric adolescents when used in conjunction with cross-gender hormone therapy. Anecdotally being used in this way to lower the necessary dose of cross-gender hormones in order to achieve a feminizing or masculinizing effect • The relevance on the impact on bone mass density to fracture risk has not been studied
Potential benefits	**Potential limitations**
• May alleviate immediate psychological distress of the young adolescent with emerging secondary sexual characteristics • Minimizes the need for costly surgical interventions later in life, when applicable • Helps an individual have the long-term physical appearance of their affirmed gender identity, which often impacts the way society views them	• May lead parents and families to automatically assume that a transgender outcome is inevitable which may prevent exploration of other possibilities • Unclear effect on brain development and processes affecting cognitive development and affect regulation, especially in young people with co-occurring neurodevelopmental disorders • May reinforce, on a societal level, the notion that they must physically appear as the gender they feel they are when that deviates from their natal sex

possible to miss the optimal window of lifelong beneficial effects should a strict age requirement be used to start pubertal suppression (age 12, for example). However, should an individual enter puberty at a younger age, it is unclear whether they can demonstrate sufficient psychological maturity to understand the effects that pubertal suppression might have on their reproductive system, if they eventually begin cross-gender hormone therapy. Additionally, providing pubertal suppression at younger ages introduces the potential need to introduce an exogenous sex hormone (oestrogen for natal boys and testosterone for natal girls) at a younger age than might be ideal considering there is no empirical data available to suggest the medical safety of prolonged pubertal suppression (greater than four years) or psychological benefits of starting testosterone or oestrogen at younger ages (less than 14 years of age). To date there are no studies that help to determine at which age adolescents are able to make these choices – and if psychological maturity is thought to be a better indicator of such a decision, how that should be determined. It is important to note that the only prospective data demonstrating positive psychological adjustment of pubertal suppression use, cited above, had a mean age of 13.6 for the initiation of pubertal suppression (de Vries et al., 2014); thus, no scientific evidence exists on the long-term psychological adjustment of adolescents who were provided pubertal suppression at much earlier ages. Anecdotal evidence in addition to one long-term case study on a natal girl initially suggests positive outcomes (Cohen-Kettenis et al., 2011); however, the individual in the case report was 13.7 years old at the time of GnRHa initiation (Tanner 3 at the time), and this needs further study.

The Endocrine Society guidelines and WPATH SOC 7 recommend that pubertal suppression be considered when an adolescent with GD is profoundly distressed by the changes that puberty brings to their body (Hembree

et al., 2009; Coleman et al., 2011). A discussion of the benefits and risks of using puberty suppression includes informing families of the lack of long-term medical safety data for the intervention in this population at this time (American Psychiatric Association, 2015). A referral to a medical provider, such as an endocrinologist who is aware of the medical management of GD is the mainstay treatment and such medical providers monitor the effects of pubertal suppression on growth and bone development according to WPATH SOC7. Prospective data on bone mass density in transgender young adults treated in the Amsterdam clinic with pubertal suppression several years prior demonstrated a loss of bone density by 22 years of age, with the effect more pronounced in natal boys (Klink, Caris, Heijboer, van Trotsenburg, & Rotteveel, 2015). These findings should be interpreted cautiously given numerous other potential factors that could be implicated which include the low sample size ($n = 34$), a lack of systematic data collection of Vitamin D levels, and the possibility that a different cross-gender hormone regimen might have led to different results. Of note, the median age of initiation of pubertal suppression of the individuals in this study was 14.9 years of age and 15 years of age for transwomen and transmen, respectively. The results cannot be extrapolated to individuals who are on pubertal suppression for longer periods of time (greater than 1.5 years as was the median duration of GnRHa treatment in the study), nor has any effect on lifetime fracture risk been studied. The study highlights the importance of continuous monitoring of bone mass development in this population by clinicians trained to manage their care.

There is no empirical evidence or prospective data evaluating the use of pubertal suppression in adolescents with GD who may have co-occurring neurodevelopmental disorders, such as ADHD or a tic disorder. As sex hormones are likely trophic on brain development in puberty (Giedd, 2008), there is a theoretical risk associated with suppressing the action of these hormones in youth who have other brain-related disorders. The first fMRI study on executive functioning in adolescents with GD (without co-occurring neurodevelopmental disorders) suggests that there was no detrimental effect of GnRH analogues when initiated after age 12, compared to typically developing adolescents (Staphorsius et al., 2015). Also, the subject in the case study described earlier (Cohen-Kettenis et al., 2011) demonstrated no clinical signs of impairment in brain development after GnRHa treatment, and no significant co-occurring neurodevelopmental disorders were reported present in this individual initially.

Another concern with the use of pubertal suppression may be related to surgical outcomes on genital surgery due to much reduced development of genitals in this population. There is currently no prospective data related to sexual functioning in young trans women who were previously treated with pubertal suppression and later received vaginoplasty (creation of a vagina).

Other reversible physical interventions described in the WPATH SOC7 include androgen blockers (spironolactone) for natal boys and menses suppression (progestins) for natal girls. These interventions are not as efficacious as GnRHa in minimizing the secondary sexual characteristics brought on by endogenous production of sex hormones of puberty, and can be used with some degree of relief for adolescents presenting in later stages of puberty while exploring whether the use of more irreversible interventions in therapy are warranted. The indications for the use of these medications are described in more detail in both the WPATH SOC7 and current Endocrine Society guidelines.

Partially reversible interventions

In older adolescents with GD, cross-gender hormone therapy (oestrogen for natal boys and testosterone for natal girls – also referred to as cross-sex hormones) is used to promote the secondary sexual characteristics of the sex most compatible with the individual's declared gender identity. These interventions also suppress the effects of an individual's endogenous hormones. Eligibility and readiness criteria described in the WPATH SOC7 (Coleman et al., 2011) include the following: (1) persistent, well-documented GD, (2) capacity to make a fully informed decision and consent to treatment, (3) age of majority in a given country, and (4) reasonably well- controlled significant medical or mental health concerns, if present. Specific hormone regimens appropriate for adolescents are detailed in Endocrine Society guidelines (Hembree et al., 2009).

The effects of oestrogen and testosterone are partially irreversible and therefore widespread consensus is that the potential effects that these treatments may have on the reproductive system (among other body systems) should be explored within therapy prior to initiation. For adolescents already being treated with GnRH agonists it has been asserted that continuing this treatment can allow for reduced doses of testosterone or oestrogen needed to achieve appropriate masculinizing or feminizing effects (Coleman et al., 2011). This may minimize the side effect burden that the sex hormone therapies may have.

Recently, the age of initiation of oestrogen or testosterone has become somewhat controversial, especially for

adolescents on pubertal suppression (Edwards-Leeper, Leibowitz, Sangganjanavanich, unpublished; Steever, 2014, de Vries et al., 2014). Historically, this intervention has been recommended at approximately 16 years of age (Hembree et al., 2009) for adolescents with GD, and evaluation studies on starting cross-gender hormones at this age have revealed positive effects (Cohen-Kettenis & van Goozen, 1997; Smith, van Goozen, & Cohen-Kettenis, 2001). When initiating treatment prior to the age of majority, however, parental consent is often required unless the individual is an emancipated minor in certain countries where this applies. The age of majority may differ by country or state, and therefore requires a mental health clinician to know the appropriate legal considerations in the location where they practice. Some clinicians have recommended initiating testosterone or oestrogen in young people during mid-adolescence at approximately 14 years of age (Steever, 2014). The philosophical premise of doing so is to avoid a perceived excessive wait to initiate the physical changes that the adolescent may seek. Additionally, for some providers who are treating peri-pubertal adolescents with GnRHa at younger ages then, depending on the age, waiting until 16 years of age could mean suppressing puberty for many years without exposing the body to any sex hormones. No research has demonstrated the long-term safety or benefit to starting cross-gender hormones earlier than age 16, yet anecdotal experience suggests positive psychological outcomes in some of these adolescents.

Co-occurring mental health issues should be 'stable' such that they present no significant barriers when an adolescent provides informed consent (Coleman et al., 2011). The presence of a co-occurring psychiatric illness itself, should not be an absolute contraindication to initiating cross-gender hormones. However, when co-occurring mental health disorders complicate the diagnostic understanding of GD, experts largely agree that conservative management and treatment of the co-occurring illness should be prioritized. This may help to further clarify the adolescent's assertion over time.

Irreversible surgical interventions

Various irreversible surgical interventions are described in the WPATH SOC7 as appropriate treatments for individuals with GD who are the age of majority in a given country. In adolescents, the most common surgical intervention used is chest surgery for natal girls. The standards recommend 1 year of testosterone therapy prior to undergoing chest surgery to allow the adolescent time to adjust to the masculine changes experienced with hormonal intervention, although this is not an absolute requirement (Coleman et al., 2011). Genital surgeries are typically performed over the age of majority in appropriately screened individuals who meet the eligibility and readiness requirements specified in the standards of care.

Conclusions

Treating adolescents presenting with gender-related issues is a quickly evolving field. With rising visibility in the media and Internet, mental health professionals may find themselves at crossroads of understanding best ethical practices given the evolution of understanding of aetiological, phenomenological, and philosophical concepts that continue to develop in guiding best practice. While the research is growing in these areas, helping families and young people often requires detailed and nuanced understanding of both the empirical evidence, and the deficits in scientific knowledge, when framing the pros and cons of various interventions – some of which are irreversible. Addressing these issues across multidisciplinary perspectives in clinics that are being newly formed across the world will help bridge the gaps in treatment that have historically been faced by this underserved population.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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the choice of aspirin or heparin for venous thromboembolism prophylaxis among patients with operatively treated extremity fractures (or any pelvic or acetabular fracture), this is by far the largest trial to date and provides compelling evidence that a readily available, inexpensive drug, taken orally, is a viable alternative to an injectable pharmacologic prophylaxis.

Are there any caveats to this message? The trial shows several secondary outcomes that support the main conclusion of the trial, including a similar risk of pulmonary embolism in the two groups and, in terms of safety outcomes, no evidence of a difference in the incidence of bleeding events, which occurred in 13.72% of patients in the aspirin group and 14.27% in the low-molecular-weight–heparin group. However, in keeping with previous trials, the authors noted that deep-vein thrombosis was more frequent in patients who had received aspirin than in those who had received heparin (2.51% vs. 1.71%), although the absolute difference was small (0.80 percentage points). Although deep-vein thrombosis is clearly not as serious as a fatal pulmonary embolism, it is not an inconsequential problem. Post-thrombotic syndrome affects some people who have had a deep-vein thrombosis of the leg, and this condition can cause chronic pain and swelling.[9]

The findings in this trial clearly indicate that guidelines for the prevention of hospital-acquired venous thromboembolism will need to be rewritten to include the option of aspirin in patients with traumatic injuries. More work is needed to determine whether aspirin should also be considered for venous thromboembolism prophylaxis after other types of surgeries and for nonsurgical patients who have risk factors for venous thromboembolism.

Disclosure forms provided by the author are available with the full text of this editorial at NEJM.org.

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Growing Evidence and Remaining Questions in Adolescent Transgender Care

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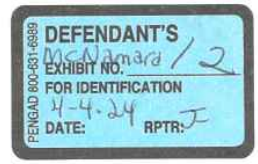

This week in the *Journal*, a much-awaited primary report from Chen et al.[1] on 2 years of gender-affirming hormones (GAH) in transgender adolescents appears. The approach to adolescent transgender care with early treatment with puberty blockers, and GAH in youth from 16 years of age, originated in the Netherlands ("the Dutch model") and became the dominant medical care model for transgender adolescents.[2] Especially over the past decade, marked increases in referrals but limited evidence as to long-term outcomes have led to controversies and debate regarding this approach. Indeed, some European countries are adapting their guidelines and re-

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stricting access to care for transgender youth, and some states in the United States have introduced laws to ban such care.[3] Therefore, rigorous longitudinal outcome studies that provide evidence about whether this approach is effective and safe are needed.

The results of the current study — involving a large, multisite sample of 315 participants — provide such evidence. During 24 months of GAH treatment, participant-reported appearance congruence (alignment between gender identity and physical appearance), positive affect, and life satisfaction increased and depression and anxiety decreased. In addition, initial levels and rates of change in appearance congruence correlated with the psychosocial outcomes. These results corroborate the positive effects in several earlier studies of smaller samples of adolescents and add to the evidence base that GAH can have a positive effect on mental health.[4]

Yet the study leaves some concerns unanswered. Although overall psychological functioning in the study participants improved, there was substantial variation among participants; a considerable number still had depression, anxiety, or both at 24 months, and two died by suicide. The correlation between appearance congruence and various psychological-outcome variables suggests an important mediating role of GAH and consequent bodily changes. However, other possible determinants of outcomes were not reported, particularly the extent of mental health care provided throughout GAH treatment. To date, international guidelines for transgender adolescent care recommend a psychosocial assessment and involvement of mental health professionals in a multidisciplinary care model.[5] Whether participating centers in the current study followed that approach is unfortunately unclear. Future studies that compare outcomes with different care models are needed, preferably using similar measures.

In addition, some are concerned that young persons may not be capable of making decisions regarding medical treatments that have irreversible effects that they might regret later in life. In the 2-year study by Chen et al., 9 of 314 adolescents (2.9%) stopped GAH, but it is unclear whether they detransitioned or regretted their treatment or whether they stopped because they were satisfied with treatment-related changes. Despite concerns about detransitioning, few studies have provided data on the incidence of detransitioning, and available results are inconsistent. Although one U.S. study showed that 74% of adolescents who started GAH treatment were still receiving it 4 years later, 98% of 720 Dutch adolescents who began such therapy were receiving it after a median of 2.7 years (range, 0.0 to 20.0).[6,7] Similar studies in other centers, regions, and countries are necessary to learn whether the incidence of detransitioning differs between settings and what factors are associated with these differences. It will be especially important to evaluate outcomes in adolescents starting GAH before 16 years of age, the age limit in the initial Dutch protocol.[2]

Furthermore, although Chen et al. investigated relevant psychological and gender outcome measures (e.g., depression, appearance congruence, and life satisfaction), additional factors such as autism spectrum disorder and the quality of peer relations and family support are also of interest. Social support has been hypothesized as explaining why Dutch transgender adolescents have better psychological function than those in other countries.[8] Understanding additional factors that influence outcomes should help to determine which components of care and support other than GAH might improve the lives of transgender adolescents.

Finally, benefits of early medical intervention, including puberty suppression, need to be weighed against possible adverse effects — for example, with regard to bone and brain development and fertility. At present, studies involving young adults from the Dutch adolescent transgender cohort show that accrual of bone mineral decelerates during puberty suppression but increases during GAH treatment and also that adolescents' educational achievements are as expected given their pretreatment status, which is reassuring.[9,10] However, those results from a single Dutch center should be replicated and validated in other contexts, as in a sample followed in the current study.

Despite uncertainties that call for further study, current information shows that mental health improves with GAH, whereas withholding treatment may lead to increased gender dysphoria and adversely affect psychological functioning. The study by Chen et al. adds to the

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evidence of the effectiveness of the current care model that includes hormonal treatment for transgender adolescents.

Disclosure forms provided by the authors are available with the full text of this editorial at NEJM.org.

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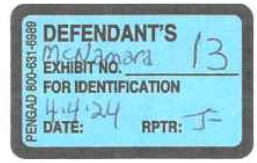
ACTA PÆDIATRICA NURTURING THE CHILD WILEY

REVIEW ARTICLE

The impact of suppressing puberty on neuropsychological function: A review



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Abstract

Aim: Concerns have been raised regarding the impact of medications that interrupt puberty, given the magnitude and complexity of changes that occur in brain function and structure during this sensitive window of neurodevelopment. This review examines the literature on the impact of pubertal suppression on cognitive and behavioural function in animals and humans.

Methods: All studies reporting cognitive impacts of treatment with GnRH agonists/antagonists for pubertal suppression in animals or humans were sought via a systematic search strategy across the PubMed, Embase, Web of Science and PsycINFO databases.

Results: Sixteen studies were identified. In mammals, the neuropsychological impacts of puberty blockers are complex and often sex specific ($n=11$ studies). There is no evidence that cognitive effects are fully reversible following discontinuation of treatment. No human studies have systematically explored the impact of these treatments on neuropsychological function with an adequate baseline and follow-up. There is some evidence of a detrimental impact of pubertal suppression on IQ in children.

Conclusion: Critical questions remain unanswered regarding the nature, extent and permanence of any arrested development of cognitive function associated with puberty blockers. The impact of puberal suppression on measures of neuropsychological function is an urgent research priority.

KEYWORDS
gonadotropin-releasing hormone (GnRH), intelligence, memory, puberty, cognition, neurodevelopment, review

1 | INTRODUCTION

Puberty blockers and cross sex hormones are prescribed to transgender and gender diverse (TGD) young people with the aim of aligning physical appearance with gender identity, as part of a gender-affirming model of care.[1] The medications most commonly used to suppress puberty are gonadotropin-releasing hormone (GnRH) agonists or antagonists. The number of young people seeking gender-affirming treatments has grown significantly over the past 10 years.[2,3] Data from the Gender Identity Development Service

Abbreviations: GnRH, Gonadotropin hormone-releasing hormone; GnRHa, GNRH agonists/antagonists; IQ, Intelligence Quotient; TGD, Transgender and Gender Diverse; GIDS, Gender Identity Development Service; MRI, Magnetic Resonance Imaging; fMRI, Functional Magnetic Resonance Imaging; CPP, Central Precocious Puberty.

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(GIDS) in the United Kingdom indicate an over 3000% increase in referrals to the service over an 8-year period from 2009 to 2016. This increase was most marked in females and adolescent females in particular, where the numbers increased by more than 7000% over the 7-year period.

Given the magnitude and complexity of changes that occur in brain function and structure during puberty,[4,5] concerns have been raised regarding the impact of medications that interrupt and interfere with this process during this important period of neurodevelopment.[6] In a statement of expert consensus from 24 international specialists (in neurodevelopment, gender development, puberty, neuroendocrinology and research methods), the impact of pubertal suppression on different aspects of neuropsychological function comprised the majority of research priorities identified, with nine of the 17 priorities related to possible neuropsychological impacts, namely, effects on executive function, social awareness, functional connectivity, brain structure/volume, emotional awareness, IQ, risk-taking, processing speed and memory.[6]

Unsurprisingly, given the critical role of puberty in the development of the brain's anterior regions including the prefrontal cortex,[4] the study of executive functions/control and attention topped the list of neuropsychological priorities for future research. The expression of GnRH receptors outside the reproductive axis in brain areas such as the hippocampus and amygdala also highlights learning, memory and emotional processing as relevant areas of neuropsychological interest in outcome studies in these patients.[7–9]

The first part of this paper summarises our contemporary understanding of puberty from a neuropsychological perspective as the driver of a sensitive 'window of opportunity' for the development of executive functions and social cognition. A brief overview of our current state of knowledge regarding of the role of pubertal hormones in the functional and structural brain changes that occur during adolescence is presented. This literature provides the medical and scientific rationale for neuropsychological outcomes to be included as an essential component of any evaluation of outcome following pharmacological interventions that suppress or delay puberty in adolescents.

Since the current neuropsychological literature is not sufficient to allow for a more precise systematic review,[3] the second part of the manuscript presents a scoping review of the literature that has examined the impact of pubertal suppression on cognitive/neuropsychological function in both animal and human studies. For clarity and consistency, in this review, trans women/girls are referred to as male-to-female and trans men/boys as female-to-male.

1.1 | Puberty as a critical window in neurodevelopment

The concept of critical 'windows' of plasticity during neurodevelopment refers to specific periods in infancy, childhood and adolescence when the developing brain is programmed to generate dedicated neuronal networks in response to environmental inputs.[10] A period is defined as a 'critical window' if the brain requires a specific input to allow for the optimal development of a particular function (e.g. exposure to language or visual stimuli). If the neural network is left without the correct input or stimulation, the functions served by that circuit will be permanently compromised.[11] Essential inputs may be internal, for example, hormonal or nutritional state,[12] and external, for example, the presence/absence of environmental stimuli.[13] Neural networks that develop in impoverished environments during sensitive periods can sometimes be remoulded by subsequent experiences in later life, although function may always remain suboptimal.[13,14] Windows of plasticity for neurodevelopment are staggered throughout development (from birth to the third decade of life) and follow a set pattern with sensory pathways (vision, hearing) prioritised in infancy, followed by motor and language functions in early childhood. Adolescence is a critical window of development for executive functions (behavioural and cognitive) and social cognition.[15]

Key notes

- Adolescence is a critical window of neurodevelopment and puberty plays a critical role in these neurodevelopmental processes.
- The suppression of puberty impacts brain structure and the development of social and cognitive functions in mammals, the effects are complex and often sex specific.
- No human studies have systematically explored the neuropsychological impact of pubertal suppression in transgender adolescents with an adequate baseline and follow-up, this is an urgent research priority.

1.2 | Adolescence: A critical period for synaptic pruning & myelination

The approximate 100 trillion synaptic connections that subserve normal adult function do not develop in a linear fashion. Brain development involves both progressive (proliferation, neurite outgrowth, synapse connectivity) and regressive events (cell death, axon pruning, synapse elimination).[16] The regressive events are just as much an integral part of the brain maturation process as the progressive processes. Approximately half of the neurons formed during brain development do not survive into adulthood, with most eliminated via apoptosis or other forms of programmed cell death in utero or early childhood. Just as some cells are programmed to die once they have served their purpose in neurodevelopment, similarly the brain is programmed to eliminate initially over-produced synapses,[17] a process known as pruning. During childhood, neurons enthusiastically establish trillions of synaptic connections as the individual learns how the world works and their place and agency

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within it. Dendritic spine density in childhood is three times greater than that seen in adults prior to puberty.[18] It is now recognised that substantial pruning continues well beyond adolescence and into the third decade of life before stabilising at the adult level.[18] However, not all changes in the adolescent brain are regressive. Although myelination begins in utero and continues into adulthood, myelin production escalates significantly during adolescence, with biological sex being a significant determinant, particularly in females,[19] resulting in significant increases in both the speed of electrical transmission along axons and the energy efficiency of this process.

Biological sex is not just a significant determinant of myelin distribution. A review of MRI studies of male and female brain structure found that adolescence was a time of divergence in the structural characteristics of the brain.[20] Unsurprisingly, sex differences in structures with a high density of sex steroid receptors such as the caudate nucleus, amygdala, hippocampus, and cerebellum have been reported. These differences are dynamic and change over the course of development during adolescence. Regional cortical grey matter volumes follow an inverted U-shaped developmental trajectory with peak size occurring 1–3 years earlier in females compared to males. While white matter volumes increase throughout adolescence in both sexes, this process occurs more rapidly in adolescent males resulting in an increasing magnitude of sex differences.[20]

1.3 | The role of puberty versus chronological age in neurodevelopment in adolescence

Hormonal changes in puberty are not just responsible for the development of physical secondary sex characteristics; they also drive many of the neurodevelopmental changes in the adolescent brain described above, particularly with respect to the development of frontal cortical circuits, and hippocampal and amygdala connectivity.[21–24] In a functional MRI study of 105, 8–19 year olds, Ravindranath et al. found that while chronological age was associated with activations in the right dorsolateral prefrontal cortex on a task requiring inhibitory control, puberty stage was associated with activation in the right ventrolateral prefrontal cortex. Metrics of broader connectivity between the ventrolateral prefrontal cortex and cingulate were also associated with puberty stage. The authors conclude that while age-related developmental processes may support maturation of brain systems underlying the ability to inhibit a response, processes associated with puberty may play a larger role in the effectiveness of generating cognitive control responses.[22]

In summary, puberty is characterised by both regressive and progressive stages of brain development. Unlike earlier developmental milestones, many of these processes are associated with pubertal stage rather than chronological age[22,25–27] and hormonal regulation plays an important part in these developments. The prefrontal cortex undergoes significant rewiring during puberty, with corresponding behavioural changes in associated executive functions including impulse control, decision-making and goal-directed behaviours. Other behavioural manifestations of the rewiring process in puberty include enhanced reactivity to social and emotional stimuli, especially in relation to peers, and changes in the evaluation of potential rewards.[4,15,28–33] The male and female brain develops differently during adolescence both in terms of structural connectivity and developmental trajectory.

Completely reversible neuropsychological effects would not be predicted given our current understanding of the 'windows of opportunity' model of neurodevelopment. If neuropsychological deficits associated with puberty blockers were completely reversible, it would mean that puberty is very different from the other pre-programmed windows of opportunity in neuropsychological development and any literature supporting this would present a significant challenge to our current understanding of neurodevelopment. It was the apparent incongruity between claims of full reversibility in the TDG literature and the neuropsychological puberty literature that prompted this review.

2 | LITERATURE REVIEW

2.1 | Methods

2.1.1 | Search strategy and selection criteria

All studies reporting neuropsychological, neurobehavioral or cognitive impacts of GnRH analogues in pubertal suppression in animals or humans were sought in the initial search. Searches were conducted on PubMed, Embase, Web of Science and PsycINFO in April 2023 using the following terms: 'GnRH*' or 'Lupron' AND 'Pubert*' and any of the following neuropsychological terms: Cogniti*, OR Neuropsychol*, OR 'Executive', OR 'Language', OR 'Memory', OR 'Learning', OR 'Spatial', OR 'Intelligence', OR 'IQ', OR 'Processing', OR 'Attention', OR 'Social'. The search was limited to English language publications.

Excluding duplicates, the search strategy returned a total of 646 papers across the four search engines for initial review: See Figure 1 for PRISMA flow diagram.

Review articles, book chapters and conference proceedings were excluded from the review. The remaining abstracts ($n = 498$) were reviewed for reports of measures of cognitive, neurobehavioural or neuropsychological function assessed on standardised tests and described in relation to the administration of GnRH analogues for puberty suppression in either clinical or experimental settings. Forty-two records met these criteria and the full text was reviewed. Citation searching in these publications revealed a further possible 10 citations for review.

Since the focus of the review was on neuropsychological function, papers were included if they reported any quantified measure of cognitive function assessed on standardised neuropsychological measures or psychometric tests (or the equivalent in animal studies). Papers that reported outcomes measured via questionnaires or checklists (in humans) or self-reported measures of function were

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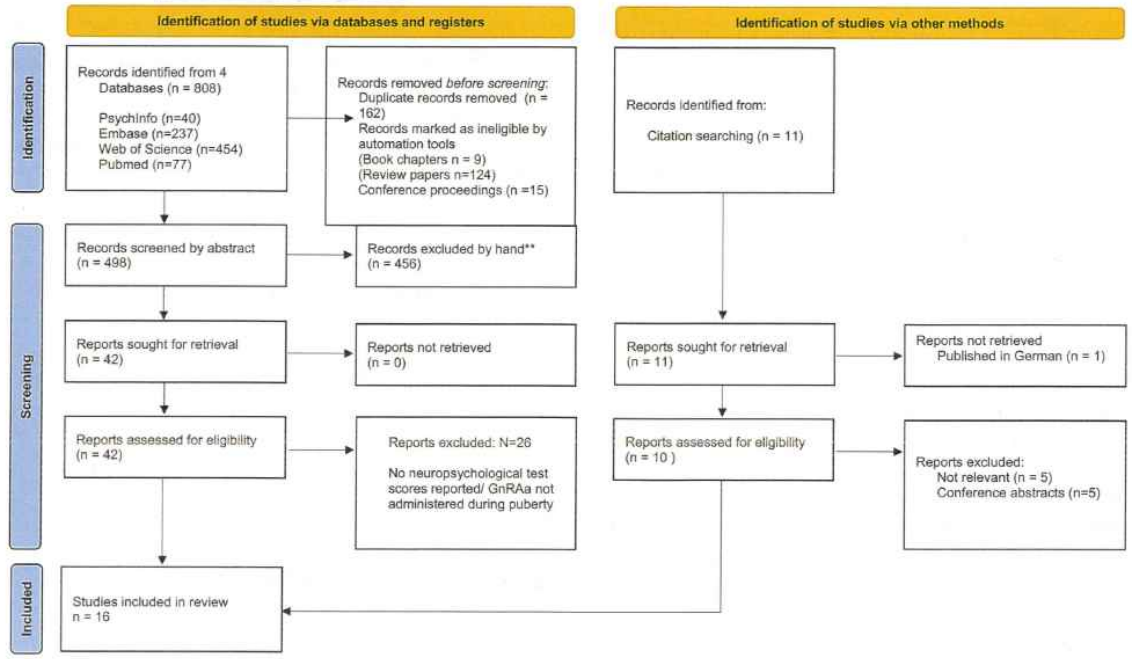

FIGURE 1 PRISMA flow diagram for systematic reviews which included searches of databases, registers and other sources: Search terms: GnRH*' or 'Lupron' AND 'Pubert*' and any of the following Neuropsychological Terms: Cogniti*, OR Neuropsychol*, OR 'Executive', OR 'Language', OR 'Memory', OR 'Learning', OR 'Spatial', OR 'Intelligence', OR 'IQ', OR 'Processing', OR 'Attention', OR 'Social'.

excluded. Papers that described psychological, psychosocial or psychiatric outcomes were also excluded.

3 | RESULTS

A number of relevant studies have been presented at conferences but have not subsequently been published in peer-reviewed journal articles. Sixteen peer-reviewed studies that have examined the impact of suppressing puberty with GnRH analogues on cognitive, neurobehavioral (animals) or neuropsychological function were identified with the search strategy described. The majority of these studies ($n=11$) have been conducted in animals.

3.1 | Animal studies

The wider search strategy identified experimental studies on the physiological impacts of GnRH blockade in 17 species of animals (including hyenas, sheep, goats, rats, naked mole rats, giant pouched rats, mice, hamsters, macaques, rhesus monkeys, marmoset monkeys, carp, gilt, chicken, pigs, cows and dogs). Eleven of these studies reported the impact of pharmacological puberty suppression on indices of behavioural function in the animal. These studies are summarised in Table 1. The majority of these studies ($n=8$) have been conducted in the same flock of sheep using twin controls.[7,8,34–39] Two studies in monkeys[40,41] and one mouse study[42] were also identified. Measures of brain structure were reported in five studies and included structural MRI, resting state functional MRI and histopathology (see Table 1).

The behavioural and cognitive measures used in these animal studies can be broadly divided into three categories:

1. Positive interactions with the environment (e.g. locomotion, food acquisition, preferences for novel objects, hyponeophagia, social preferences);
2. Responses to stress (responses to social isolation, vocalisations, emotional reactivity, forced swim test, human intruder test, manifestations of social status);
3. Performance on cognitive tasks (maze tasks).

As can be seen in Table 1, the results from these studies indicate that treatment with a GnRH antagonist/agonist has a detrimental impact on learning and the development of social behaviours and responses to stress in mammals.[7,8,34–36,38,39,41,42] Sex-specific effects were observed in multiple studies.[8,37,42] In male sheep, impairments in spatial memory associated with the treatment were not fully reversed following discontinuation of treatment.[39] Significant effects of treatment were also evident on measures of brain structure including overall volume,[41] functional connectivity[40] and neuronal density.[42]

The results from these studies are broadly consistent and indicate that the suppression of puberty impacts brain structure and the development of social and cognitive functions in mammals, but the

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TABLE 1 Animal studies examining the impact of GnRHa treatment on neuropsychological function or brain–behaviour relationships.

		Animal Model	Study design	Behavioural/cognitive domain assessed	Structural brain analyses	Main finding
1	Wojnius et al. (2011)	Male & female sheep	*N* = 48 same sex twin pairs GnRHa treated group (twin 1) vs. untreated controls (twin 2)	Food acquisition task	n/a	Results: Significant sex vs. treatment effects Treated males were more likely to leave their companions to acquire food than untreated males, while the opposite effect was observed in females Conclusion: Long-term prepubertal GnRHa treatment significantly affected sex-specific brain development, which impacted emotion and behaviour regulation in sheep. These results suggest that GnRH is a modulator of cognitive function in the developing brain and that the sexes are differentially affected by GnRH modulation
2	Evans et al. (2012)	Male & female sheep	*N* = 46 same sex twin pairs GnRHa treated group (twin 1) vs. untreated controls (twin 2)	Vocalisation Response to social isolation Tested at 8, 28 and 40 weeks	n/a	Results: Response to social isolation and vocalisation was significantly higher in females than males at all ages Conclusion: Development of responses to social isolation is sexually dimorphic and cortisol dependant. Treatment with a GnRH agonist results in changes in age-dependent development of this social function
3	Nuruddin et al. (2013)	Male & female sheep	*N* = 30 same sex twin pairs (14 female/16 male) GnRHa treated group (twin 1) vs. untreated controls (twin 2)	Test of spatial orientation 48 weeks of age	Hippocampal gene expression	Results: GnRHa treatment was associated with significant changes within the hippocampus, of levels of expression of mRNA transcripts known to be involved in endocrine signalling and synaptic plasticity. Expression of 12 out of the 16 genes was altered in GnRHa treated sheep compared to controls. These changes were not related to performance on a spatial maze test. Although there were no significant effects of treatment on performance in spatial maze, in males, there was a tendency that T animals were slower in completing the spatial maze than the controls during every trial. The author speculate that treated males might have been less motivated than control males to complete the maze in fastest possible manner Conclusion: GnRH1 mRNA expression in females might be more sensitive to GnRHa treatment
4	Nuruddin et al. (2013)	Male and female sheep	41 brains of sheep from the experiment described above 17 treated (10 females, 7 males) 24 controls (11 females, 13 males)	n/a	MRI volumes 1. Total brain 2. Amygdala 3. Hippocampus	Results: Highly significant GnRHa treatment effects were found in the volume of the right and left amygdala in treated animals, with larger amygdale in treated animals. Significant sex differences were found for total grey matter and right amygdala with larger volumes in males Conclusions: The effects of GnRHa treatment on amygdala volumes indicate that increasing GnRH concentrations during puberty may have an impact on normal brain development in mammals
5	Wojniusz et al. (2013)	Male and female Sheep	*N* = 46 twin pairs GnRHa treated group (twin 1) vs. untreated controls (twin 2)	Spatial orientation maze task 8 weeks 28 weeks 48 weeks	n/a	Results: GnRHa treatment did not affect spatial maze performance. No significant differences in traverse time between treated and untreated animals were observed at any time point prior to or following treatment. Adolescent females (48 weeks) outperformed the males in both groups Conclsuions: Development of sex differences in spatial orientation is independent from exposure to pubertal hormones

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	Animal Model	Study design	Behavioural/cognitive domain assessed	Structural brain analyses	Main finding
6 Hough et al. (2017a)	Male Sheep	Group 1. GnRH and testosterone blocked $n=49$ Group 2. GnRH blocked. with testosterone replacement $n=22$ Group 3: Controls $n=56$	Spatial maze task 1. Traverse time 2. Long-term memory 3. Emotional reactivity 8 weeks 27 weeks 41 weeks	n/a	Results: Emotional reactivity was compromised by blockade of testosterone signalling, but was restored in the testosterone replacement group. The blockade of GnRH signalling alone was associated with impaired retention of long-term spatial memory and this effect was not restored with the replacement of testosterone signalling. The GnRHa + T group required fewer training sessions than the GnRHa group Conclusion: These results indicate that GnRH signalling is involved in the retention and recollection of spatial information, potentially via alterations to spatial reference memory. Therapeutic medical treatments using chronic GnRHa may have effects on this aspect of cognitive function
7 Hough et al. (2017b)	Male Sheep (as above)	Group 1. GnRHa treated until 44 weeks of age $n=25$ (Twin 1) Group 2. Controls $n=30$ (Twin 2)	Spatial memory task (as above) 83 weeks 95 weeks	n/a	Results: The long-term spatial memory performance of GnRHa-Recovery rams remained reduced ($p<0.05$, 1.5-fold slower) after discontinuation of GnRHa, compared to controls Conclusions: The time at which puberty normally occurs may represent a critical period of hippocampal plasticity. Perturbing normal hippocampal formation in this peripubertal period may also have long-lasting effects on other brain areas and aspects of cognitive function
8 Hough et al. (2019)	Male Sheep	Group 1. GnRH and testosterone blocked ($n=55$) Group 2. GnRH blocked. with testosterone replacement ($n=24$) Group 3: Controls $n=60$	Preference for novel vs. familiar objects Approach/avoidance behaviours Emotional reactivity 8 weeks 28 weeks 46 weeks	n/a	Results: Specific suppression of testosterone during a developmental window in late puberty may reduce emotional reactivity and hamper learning a flexible adjustment to environmental change Conclusion: Disruption of either endogenous testosterone signalling or a synergistic action between GnRH and testosterone signalling, may delay maturation of cognitive processes (e.g. information processing) that affects the motivation of rams to approach and avoid objects
9 Anacker et al. (2021)	Male and female mice	Control vs. GnRA injected mice	Locomotion Social preference Hyponeophagia Forced swim test	Brain immunohistochemistry	Results: *Sex-specific effects:* Males: GnRHa treatment altered locomotion and social preference and increased the corticosterone response to novelty exposure in the male but not female mice. Females. Treatment was associated with increased hyponeophagia and despair-like behaviour and neural activity in the dentate gyrus in female mice without an effect in male mice. No treatment effects were observed on measures of avoidance behaviour or contextual fear discrimination in either sex Conclusion: GnRHa treatment is associated with sex-specific effects on measures of social and affective behaviour, stress regulation and neural activity

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	Animal Model	Study design	Behavioural/cognitive domain assessed	Structural brain analyses	Main finding
10	Pincus et al. (2021) Female Macaque Monkeys	GnRHa treated $n = 34$ Controls $n = 36$	Indices of social rank and social behaviour Responses to the human intruder task Tested at 43–46 months of age	Resting state MRI and T1 images	Results: GnRHa treated monkeys were more submissive and less affiliative than controls. They were less anxious and exhibited less displacement activity in the human intruder task Imaging revealed stronger functional connectivity between the left amygdala and left orbital frontal cortex in the treated group compared to controls Conclusion: Delayed puberty and subordination stress had separable effects, suggesting that the overlapping socioemotional outcomes may be mediated by distinct neuroplastic mechanisms
11	Godfrey et al. (2023) Rhesus Macque monkeys	GnRHa treated $N = 23$ Controls $n = 22$	Measures of emotionality Response to acute stress	Structural MRI	Results: Treated animals differed from controls in intracranial volume (control volume < treated volume) however, hippocampal volume was larger in controls Conclusion: There are region-specific effects of Estradiol on structural brain development during adolescence

impacts are complex and often sex specific, consistent with the MRI evidence of sex-specific differences in neurodevelopment in human adolescence.[20] There is no evidence in the animal literature that these effects are reversible following discontinuation of treatment.

3.2 | Human studies

The search strategy identified just five studies that have reported some aspect of neuropsychological function following the administration of medications to suppress puberty in young people. Two studies reported the impact of treatment with a puberty blocker in young people with precocious puberty (CPP) and three reported neuropsychological test performance in people treated for gender dysphoria. One of these studies was a single case study.

3.3 | Central precocious puberty

In the only human study that established a baseline prior to treatment, Mul et al.[43] examined the response to treatment with puberty blockers on a number of psychosocial outcomes including the Child Behaviour Checklist and performance on the shortened version of the Wechsler Intelligence Scales for Children in a group of 25 girls treated for early puberty. Three years after treatment commenced, the group as a whole had experienced a loss in both performance IQ and full scale IQ, with a decline of 7 points in the latter. While statistically significant at $p < 0.01$, the authors state that the decrease in IQ was not 'clinically relevant', a conclusion repeated in a later citation of the study.[44] While the average loss of IQ points was 7, it is noteworthy that at least one patient in this study experienced a significant loss of 15 points or more, since the highest IQ score in the group was 138 at baseline and this dropped to 123 following treatment.

The Wechsler Intelligence Scales are well designed to measure the impact of treatments on IQ in children. The norms are very robust and are provided for children at 3-month intervals, from age 6 to 17 years. Different abilities develop at different times and at different rates but at any point during their development, a child's scores on the tests that comprise the IQ battery can be compared to that of their age-matched peers. In order to maintain a stable IQ, the child will need to keep pace with the development of that seen in their peers. Of course, some children are very able, others less so. But the key characteristic of IQ is that it should remain stable throughout a child's development. Regardless of whether an individual performs at the 10th, 50th or 90th percentile when they are 8, they should continue to do so when they are 16. Any loss of IQ associated with treatment with puberty blockers indicates that the child's cognitive development is not keeping pace with that of his/her peers.

The Galatzer et al.[46] and Ehrhardt et al.[47] studies did not report the impact of puberty blockers on IQ but rather reported the IQ of girls with CPP. It is noteworthy that only three of the 12 girls in the

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Ehrhardt study with idiopathic precocious puberty had been treated with Provera (medroxyprogesterone acetate). Galatzer et al. found that the verbal IQ distribution in 52 girls with precocious puberty was two or more times the expected theoretical percentile in the above average area (greater than 110, 56.9% vs 25%), and five times more in the very superior area (greater than 130, 10.1% vs 2.2%). However, the treatment status of the sample is not reported, other than in the final paragraph of the discussion where the authors note that 'Another aspect that requires further delineation is the effect of medical treatment of these patients. At present, it is common practice to postpone physiologic development with the use of antiandrogen or gonadotrophin-releasing hormone analogues. The impact of these drugs on the intellectual and possibly emotional development of girls with precocious puberty remains to be evaluated'. Galatzer et al. interpreted their findings as possible evidence of an effect of sex hormones on brain development, especially on the left hemisphere, during the prepubertal period.

Wojniusz et al.[45] compared the neuropsychological function of 15 girls with central precocious puberty (CPP) (mean age 10.4 years; range 9.2–11.8) and age-matched controls on a very comprehensive battery of neuropsychological tests which yielded 44 scores of function across multiple cognitive domains. All of the girls in the CPP group had been on GnRH analogue treatment for at least 6 months. The authors found no statistically significant differences between the CCP group and controls on any measures with the exception of the Trail Making Number Sequencing Task score. Given that the authors did not control for multiple comparisons (over 40) and that the groups did not differ on other tests of processing speed, the authors speculate that this finding is 'accidental'. In their discussion, the authors note that in contrast to previous reports of elevated verbal IQ scores and accelerated school performance in CPP girls (studies from Galatzer et al. and Ehrhardt et al.),[46,47] the IQ in their CCP group was somewhat lower than the controls, although the difference was not statistically significant.

Wojniusz et al. state '*both groups (CPP and controls) showed* ***very similar*** (my emphasis) *scores with regard to cognitive performance*'.[45] This conclusion was questioned by Hayes (2017) who noted that the authors discussion of their findings minimised the substantial difference in IQ scores between the groups (7 points) by overemphasising the lack of statistical significance in the small sample ($p=0.09$) and ignoring the clinical difference between someone functioning at the 55th centile and someone at the 34th centile.[48]

3.4 | GnRH analogues and transgender and gender diverse young people

Three studies were identified that examined the neuropsychological impact of GnRH analogue treatments in transgender and gender diverse young people. In a single case study, Schneider et al. (2017) examined the impact of pubertal suppression on brain white matter and (white matter fractional anisotropy) and cognitive function (Wechsler Intelligence Scale for Children-IV) in an 11-year-old treated for gender dysphoria (male to -female). On admission, at the age of 11 years and 10 months, the patient was assessed to have a global IQ of 80. Treatment with GnRHa was instigated at age 11 years, 11 months. The patient was reassessed age 13 and 3 months, at which time, a loss of 9 IQ points had occurred, and the IQ had dropped to 71. A loss of 15 points was evident in working memory. At 14 years and 2 months, a loss of 10 global IQ points and 9 points in working memory remained apparent. The verbal comprehension index (a measure which depends on the expansion of vocabulary and conceptual thinking in adolescence, for the standardised score to remain stable) deteriorated progressively over the follow-up, falling from the initial baseline of 101, to 91 (age 13) and 86 (age 14), a loss of 15 points over 3 years.[49] (See Figure 2).

In a cross-sectional design, Staphorsius et al.[50] compared the performance of GnRH treated (8 male to female; 12 female to male) and untreated transgender adolescents (10 male to female; 10 female to male) on the Tower of London Test (a test of executive function tapping the ability to strategise). No baseline measure of function was taken. The subjects also completed four subscales of the Wechsler Intelligence Scales (arithmetic, vocabulary picture arrangement and block design) and tests of mental rotation and face recognition. Only IQ, and accuracy and timed scores from the Tower of London Test are reported. The groups were not matched for IQ, with control males functioning at a significantly higher level than the suppressed male to female group. No results for the tests of mental rotation or face recognition are reported (but are promised in a later publication). While the groups did not differ with respect to reaction time on the Tower of London Test, suppressed male to females had significantly lower accuracy scores compared to the control groups. This pattern remained significant after controlling for IQ. Despite this, the reaction time finding has been subsequently been reported as evidence for no detrimental effects on performance in citations in the subsequent literature[44] and in policy documents.[51]

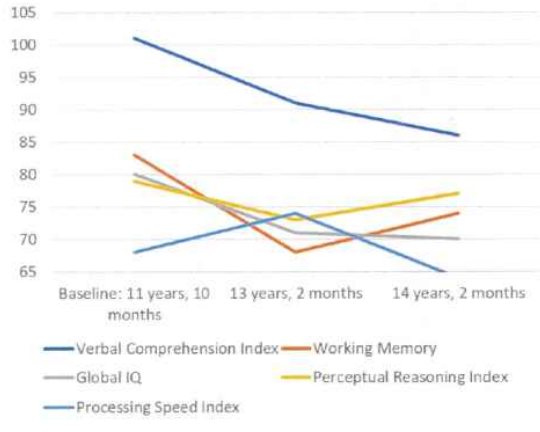

FIGURE 2 Longitudinal IQ scores following pubertal suppression in a single case study (adapted from Schneider et al.[49]).

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Arnoldussen et al.[52] reported the results of an assessment of IQ, before the commencement of GnRH analogue treatment in 72 children and examined the relationship between this measure and a highly simplified, dichotomised index of educational progress/achievement ('vocational educated' vs. 'higher vocational educated/academic educated'). Prior to treatment, the mean and standard deviation of the IQ score in the group was comparable to the general population (mean = 100, standard deviation = 15). Forty per cent of the eligible subjects declined to participate in the follow-up. No conclusions can be drawn from this study with respect to the impact of puberty suppression on the development of cognitive function.

4 | DISCUSSION

The synthesis of findings from multiple fields of study (neurodevelopment, neuroimaging, neuroendocrinology) indicates an association between GnRH expression and brain function and structure. Despite the broad and multidisciplinary knowledge base which indicates disruption of GnRH expression is likely to have an impact on cognitive function, and explicit calls in the literature for this to be studied that date back three decades,[46] there have been no human studies to date that have systematically explored the impact of these treatments on neuropsychological function with an adequate baseline and follow-up.

While no means conclusive due to the poor quality of evidence, studies examining the impact of puberty suppression in young people indicate a possible detrimental impact on IQ.[43,48,49] These findings accord with the wider literature on GnRH expression and brain structure and function. Studies in mice, sheep and primates indicate an impact of GnRH suppression on behavioural analogues of cognitive function, effects that are often sex specific. While there is some evidence that indicates pubertal suppression may impact cognitive function, there is no evidence to date to support the oft cited assertion that the effects of puberty blockers are fully reversible.[51,53] Indeed, the only study to date that has addressed this in sheep suggests that this is not the case.[39]

Vague hints from poor quality studies are insufficient to allow people considering these treatments to make an informed decision regarding the possible impact on their neuropsychological function. Critical questions remain unanswered regarding the nature, extent and permanence of any arrested development of cognitive function that may be associated with pharmacological blocking of puberty. If cognitive development 'catches up' following the discontinuation of puberty suppression, how long does this take and is the recovery complete? Several animal studies indicate that some cognitive effects may be sex specific[19,34,42] consistent with imaging studies in adolescents which indicate different trajectories of neurodevelopment in males and females.[20] Natal sex must therefore be a critical variable of interest in future research designs. How does subsequent treatment with cross sex hormones influence neuropsychological development following puberty suppression? Given the very high proportion of patients who proceed to treatment with cross sex hormone following treatment with puberty blockers,[54] it is critical that research designs utilise the narrow window before introducing same sex hormone to assess impact. What impact does any delay in cognitive development have on an individual's educational trajectory and subsequent life opportunities given the critical educational window in which these treatments are typically prescribed? Longitudinal studies are urgently needed to study the educational and vocational trajectories of people undergoing these treatments.

The importance of an adequate baseline prior to treatment when assessing the impact of puberty blocking agents on neuropsychological function cannot be overstated given the multiple vulnerabilities associated with gender identity disorder. Many conditions which are likely to compromise cognitive function are overrepresented in this population.[55,56] Neurodiversity is overrepresented in TGD people, who are three to six times more likely to have a diagnosis of autism than their peers.[55] Attention deficit hyperactivity disorder is also overrepresented in this group. In addition to increased representation of neurodiverse conditions, the rates of mental health difficulties in this population are high, with adolescents seeking gender-affirming treatments presenting with psychiatric symptoms and disorders comparable to those seen among adolescent psychiatric patients.[56] All of these conditions are known to compromise neuropsychological function and future study designs must take this into consideration. Even without a psychiatric comorbidity, the psychosocial stresses associated with living with gender dysphoria as a young person can be very significant and would be expected to have a substantial impact on cognitive reserve. This would be consistent with the findings of Haraldsen[57] who in a conference presentation reported highly significant differences between gender identity disorder patients and controls on measures of verbal and executive function with significantly atrophic hippocampal and cerebellum tissue *prior* to any treatment with puberty-blocking agents. A recent study from Turkey reported significantly worse performance on tests of response inhibition and verbal fluency in 22 adolescents with gender dysphoria compared to controls, with no group differences in set shifting. None of the patients in the gender dysphoria group had taken gender-affirming treatment at the time of the assessment, but levels of comorbid psychiatric disturbance were high with 72.7% having at least one psychiatric diagnosis.[58] This is consistent with earlier findings from the same group indicating more disturbed behaviour related to executive function and social impairment in children with gender dysphoria compared to controls.[59] The impact of blocking puberty in a brain that may already be developing in an atypical trajectory is unknown.

Subsequent follow-up should monitor development not just during and at the end of treatment, but to at least age 25, when neurodevelopment begins to complete.[60] Scores from single tests, in single domains tell us very little when they are presented and examined in isolation from the wider neuropsychological profile of the patient. Given that the impact of pubertal suppression on cognitive function is very likely to be governed to some extent by the pubertal stage at which it is commenced, broader indices of abnormality across a neuropsychological profile may be more illuminating than

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multiple individual comparisons between tests in specific cognitive domains. This will require administering a comprehensive test battery and indices such as the number of test scores outside the expected range, and indices of consistency across domains and other patterns indicative of wider abnormalities may be illuminating. As recommended by Ludvigsson et al. (2023), analyses which include measures of intra-individual change may be more useful than group level analyses, particularly given the selection bias and high dropout rates of participants in these studies. While randomised control trials may be difficult to conduct, controls should nevertheless be an integral part of a research protocol, with some thought given to the significant mental health comorbidities often reported by patients seeking these treatments and the independent impacts these exert on cognitive function (see above).

Despite the evidence base that indicates cognition is an important area to consider in the study of outcomes following pubertal suppression, it is an area that clinical neuropsychologists have largely neglected to date. The reasons for this are likely to be multifactorial and reflect to some degree the historical factors related to the introduction of this 'off label' treatment for TDG adolescents. The current, highly polarised socio-political atmosphere that surrounds much of the research published in this area may also make some academics wary about conducting and publishing research in this field. Whatever the reasons, the evidence base has not kept pace with the growth of the treatment[3] and TGD people have been poorly served by the absence of research in this area, which is urgently needed given the increasing numbers of young people seeking these treatments.

From a clinical perspective, a multidisciplinary approach is recognised as the gold standard in the assessment and monitoring pharmacological treatments for TGD young people. The results from this scoping review indicate that clinical neuropsychologists should be an integral members of this clinical team, providing a comprehensive neuropsychological baseline against which change can be measured in the future, monitoring change over time and providing clinical input to address any neuropsychological concerns, if and when they arise.

AUTHOR CONTRIBUTIONS

Sallie Baxendale: Conceptualization; data curation; formal analysis; visualization; writing – original draft; methodology; investigation; project administration; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

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