The Journal of Sexual Medicine, 2024, **21**, 350–356
https://doi.org/10.1093/jsxmed/qdae014
Advance access publication date 1 March 2024
Original Research

A European Network for the Investigation of Gender Incongruence in adolescents

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Abstract

Background: Knowledge regarding the effects and side effects of gender-affirming hormone therapy (GAHT) in adults is rapidly growing, partly through international research networks such as the European Network for the Investigation of Gender Incongruence (ENIGI). However, data on the effects of puberty suppression (PS) and GAHT in transgender and gender diverse (TGD) youth are limited, although these data are of crucial importance, given the controversies surrounding this treatment.
Aim: We sought to present a detailed overview of the design of the ENIGI Adolescents study protocol, including the first baseline data.
Methods: The ENIGI Adolescents study is an ongoing multicenter prospective cohort study. This study protocol was developed by 3 European centers that provide endocrine care for TGD adolescents and were already part of the ENIGI collaboration: Amsterdam, Ghent, and Florence.
Outcomes: Study outcomes include physical effects and side effects, laboratory parameters, bone mineral density, anthropometric characteristics, attitudes toward fertility and fertility preservation, and psychological well-being, which are measured in the study participants during PS and GAHT, up to 3 years after the start of GAHT.
Results: Between November 2021 and May 2023, 172 TGD adolescents were included in the ENIGI Adolescents protocol, of whom 51 were assigned male at birth (AMAB) and 121 were assigned female at birth (AFAB); 3 AFAB participants reported a nonbinary gender identification. A total of 76 participants were included at the start of PS, at a median (IQR) age of 13.7 (12.9-16.5) years in AMAB and 13.5 (12.4-16.1) years in AFAB individuals. The remaining 96 participants were included at start of GAHT, at a median (IQR) age of 15.9 (15.1-17.4) years in AFAB and 16.0 (15.1-16.8) years in AMAB individuals. At the time of this report the study was open for inclusion and follow-up measurements were ongoing.
Clinical implications: In response to the rising demand for gender-affirming treatment among TGD youth, this ongoing study is fulfilling the need for prospective data on the effects and safety of PS and GAHT, thus providing a foundation for evidence-based healthcare decisions.
Strengths and Limitations: This study has a strong multicenter, prospective design that allows for systematic data collection. The use of clinical and self-reported data offers a broad range of outcomes to evaluate. Nevertheless, the burden of additional measurements and questionnaires may lead to withdrawal or lower response rates. Few participants with a non-binary gender identity have been included.
Conclusion: With the ENIGI Adolescents study we aim to create a comprehensive dataset that we can use for a wide range of studies to address current controversies and uncertainties and to improve healthcare for TGD adolescents.

Keywords: transgender; adolescent; puberty; GnRHa; estradiol; testosterone; GAH.

Received: October 10, 2023. **Revised:** December 27, 2023. **Accepted:** January 3, 2024

Introduction

Gender dysphoria is the distress caused by the incongruence between sex assigned at birth and experienced gender. For some transgender and gender diverse (TGD) individuals this distress is so severe that they seek medical treatment to induce physical changes matching their experienced gender identity. [1] Feminizing and demasculinizing treatment in people assigned male at birth (AMAB) consists of testosterone suppression and estradiol supplementation which, among other effects, induces breast development, changes in body shape, and decreases in body hair and facial hair growth.[2,3] Testosterone may be prescribed in people assigned female at birth (AFAB) to induce masculinization (eg, lowering of the voice, increase in muscle mass and strength, and increase in body and facial hair).[4-6] The most suitable therapy may vary between individuals depending on their gender identity, which may also be nonbinary. Some individuals also use additional nonmedical strategies to reduce their dysphoria, such as tucking of the male genitals in AMAB individuals and binding of the chest in AFAB individuals.[7]

Since the initiation of medical treatment in TGD individuals, multiple studies have been performed to assess the treatment effects and safety of gender-affirming hormone treatment (GAHT) in TGD adults.[8,9] In 2009, the European Network for the Investigation of Gender Incongruence (ENIGI) was founded by mental health professionals to obtain an extensive multicenter collection of data, including outcomes of diagnostic assessment and treatment in adults with gender dysphoria.[10] In 2010, this collaboration was expanded to include endocrine aspects of gender dysphoria elucidated through investigations of the clinical effects and side effects of GAHT in adults with gender dysphoria.[11] At the time of this report, 6 centers had participated in the ENIGI collaboration and more than 40 studies had been published with the acquired data.

In the past decade, the number of TGD adolescents seen at specialized gender identity clinics has rapidly increased.[12,13] In Amsterdam for example, the number of newly referred TGD individuals <18 years old seen per year increased from approximately 60 in 2005 to more than 200 in 2015 and predominantly included individuals AFAB.[12] After a diagnostic period, medical interventions in TGD adolescents, in contrast to interventions in adults, consist of 2 phases. In line with "The Dutch Protocol" introduced in the late 1990s[14,15], the initial phase involves initiating puberty suppression (PS) through the use of a GnRH analog (GnRHa). This treatment phase is initiated to prevent unwanted pubertal changes such as lowering of the voice in AMAB individuals and breast development in AFAB individuals. If the adolescent presents for treatment at a late pubertal stage, antiandrogens for individuals AMAB or progestins for individuals AFAB can be prescribed as an alternative to GnRHa to reduce endogenous sex steroid concentrations and their effects, such as erections in AMAB individuals and menstruation in AFAB individuals. During the period of PS, adolescents can further explore their gender identity before deciding if they wish to pursue GAHT. When adolescents show sufficient capacity to give informed consent for a partially irreversible treatment, generally from the age of 15 to 16 years, GAHT may be initiated to induce the secondary sex characteristics of the desired gender.[1]

Recently, this approach has been criticized because of the lack of long-term outcome data, the young age at which PS is started, and the strongly increased prevalence and changing needs of TGD youth, highlighting the need for structural, long-term, and large-scale data collection and research on treatment outcomes. In this article, the design of the ENIGI adolescents study is described and the first baseline data are presented.

Methods

Participating centers

The ENIGI Adolescents study is a multicenter prospective cohort study. Thus far, 3 European gender identity clinics that provide endocrine care for TGD adolescents have participated in this collaboration. The 3 gender identity clinics are affiliated with the Amsterdam University Medical Center, the Netherlands; Ghent University Hospital, Belgium; and Careggi University Hospital Florence, Italy, respectively. Inclusions of study participants started in November 2021 and are ongoing. Data presented in this article are those for participants who entered the study from its initiation until May 2023.

Study participants

Subjects are eligible for the ENIGI Adolescents study if they are younger than 18 years and if they have sufficient knowledge of the native languages: Dutch for participants from Ghent and Amsterdam, and Italian for participants from Florence. People who use self-medication with GAHT (estrogen or androgens) or medication by another transgender care service are excluded. Individuals can be included at the start of PS or at the start of GAHT.

Treatment protocol

Before medical treatment is initiated, all study participants are assessed by a mental health professional to confirm gender dysphoria according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, or the International Classification of Diseases, 11th Revision.[16,17] The puberty suppression phase can be initiated when gender dysphoria is confirmed and long-lasting, puberty has started (Tanner stage ≥G2/B2), the patient has sufficient social support from a parent or other person and the capacity for informed consent, the consequences for fertility have been discussed, and co-occurring mental health challenges have been addressed.[1] GAHT can be initiated when the adolescent has reached sufficient capacity to give informed consent for therapy that results in partially irreversible physical changes. This assessment is usually made around the age of 15-16 years. Parents need to provide informed consent for the use of GnRHa and/or GAHT in their children according to national legislation.

Puberty suppression

Puberty suppression consists of subcutaneous or intramuscular injection of GnRHa (see Table 1 for detailed treatment options per clinic). In Amsterdam and Ghent, lynestrenol 5-10 mg/d is also offered to AFAB adolescents in late puberty (Tanner stage B4-B5) as an alternative to GnRHa to stop menstrual bleeding. In Ghent, cyproterone acetate (CPA) 12.5-25 mg/d is used occasionally in late-pubertal AMAB individuals (Tanner stage G4-G5) to reduce facial and body hair growth and erections.

Table 1. Treatment options and common dosages per clinic.[a]

Treatment	Clinic location		
	Amsterdam	Ghent	Florence
PS	Triptorelin 11.25 mg every 10-12 wk Lynestrenol 5-10 mg/d	Triptorelin 11.25 mg every 10-12 wk Lynestrenol 5-10 mg/d Cyproterone acetate 12.5-25 mg/d	Triptorelin 3.75 mg every 4 wk
Feminizing GAHT[b]	17β-estradiol tablet 2-6 mg/d Estradiol patches 50-200 μg twice a wk	17β-estradiol tablet 2-4 mg/d Estradiol patches 50-200 μg twice a wk Estradiol gel 2-4 mg/d	17β-estradiol tablet 2-6 mg/d Estradiol patches 50-200 μg twice a wk Estradiol gel 2-4 mg/d
Masculinizing GAHT[b]	Testosterone esters 250 mg every 3 wk Testosterone gel 40.5-50 mg/d	Testosterone esters 125 mg every 2 wk Testosterone gel 46 mg/d	Testosterone undecanoate i.m. every 12 wk Testosterone gel 50 mg/d

Abbreviations: GAHT, gender-affirming hormone therapy; PS, puberty suppression. [a]Adjusted based on individual goals, effects and serum levels. [b]Gradual increase up to, starting dose is specified in methods section.

Gender affirming hormone therapy

Feminization

Estradiol treatment consists of oral 17β-estradiol tablets daily or transdermal 17β-estradiol patches or gel. Patients who start with estradiol tablets have a starting dose of 5 μg/kg/d, which is increased by 5 μg/kg/d every 6 months up to an adult dose of 2-6 mg/d, with variations on this schedule per center (Table 1). Individuals who prefer transdermal estrogen patches have a starting dose of 12.5 μg/d (replaced twice weekly) with gradual increase every 6 months up to an adult dose of 50-200 μg/d. If endogenous puberty is (nearly) completed before the start of treatment, the estradiol dose is increased more rapidly.[1]

Masculinization

Testosterone treatment consists of testosterone ester injections or testosterone gel applications. A mix of testosterone esters is prescribed with a starting dose of 37.5 mg/m^2 every 3 weeks, with variations in the protocol per center (Table 1). The dosage is gradually increased every 6 months, leading up to an adult dose of 125 mg every 2 weeks or 250 mg every 3 to 4 weeks. If patients wish to start with testosterone gel, a starting dose of 10 mg/d is prescribed, which is gradually increased according to testosterone concentrations up to the adult dose of 60 mg/d. If endogenous puberty is (nearly) completed before the start of treatment, the testosterone dose is increased more rapidly.[1]

Data collection

Clinical measurements

At baseline and during follow-up visits body weight and standing and sitting height of participants, wearing light clothing and no shoes, were measured using a stadiometer and a digital floor scale. Additionally, foot length of the right foot is determined using a foot measurement device, and waist, hip, breast, and chest circumference are measured. Waist circumference is measured midway between the lower end of the rib cage and the iliac crest and hip circumference is measured at the level of the greater trochanters. The breast circumference is measured at the level of the nipples and the chest circumference is measured at the inframammary fold, just below the breasts. Tanner stages are assessed by the pediatric endocrinologist at start of PS and after 6 months of PS.[18] Blood pressure is measured using an electronic blood pressure monitor. Average grip strength is calculated from 2 measurements at the dominant hand, using an adjustable hand-held standard grip device (JAMAR hand dynamometer, Sammons and Preston; Bolingbrook, IL, United States).[19] The Modified Ferriman-Gallwey score is used to evaluate and quantify body hair.[20] Acne is assessed using the Global Acne Grading Scale.[21] Voice frequency is measured when the participant is reading "*The Northwind and the sun*" in Dutch or 5 phonetically balanced sentences of the Italian version of the Consensus Auditory-Perceptual Evaluation of Voice [22] out loud using an smartphone with the application Voice Tools which has been validated for this purpose.[23] In AMAB subjects from Amsterdam who are in the GAHT phase, a 3D picture is generated of the chest using the Artec Leo scanner to assess breast development.

Bone measurements and body composition

Body fat and lean mass, bone mineral content, bone area, and areal bone mineral density at the lumbar spine and proximal femur (total hip and femoral neck region) are measured by DXA using a Hologic Horizon scanner (Hologic Inc, Bedford, MA, United States) in Amsterdam and a Hologic Discovery A scanner (Hologic QDR Discovery A device) (APEX Software v2.3.1, Hologic Inc, Bedford, MA, United States) in Ghent and Florence. The DXA scans are performed according to the clinical protocol of the center, usually before the start of PS, after 2 years of PS, before the start of GAHT, and after 2 years of GAHT.

X-rays of the left hand are usually performed every 1 to 2 years until near–adult height is reached (based on bone age or a growth rate of <2 cm/y). Bone age is determined automatically by BoneXpert[24] in Amsterdam. In Ghent and Florence, bone age is determined manually according to Greulich and Pyle.[25] Predicted adult height is calculated with BoneXpert using age, bone age, and current height.[26]

Laboratory investigations

Venous blood samples are obtained at start of PS, at start of GAHT, and annually during GAHT. Since samples are used for clinical purposes, they are analyzed at the local laboratory. Serum measurements in all centers include at least 17β-estradiol, testosterone, sex hormone–binding globulin, luteinizing hormone (LH), follicle stimulating hormone (FSH), total cholesterol, high-density lipoprotein cholesterol, triglycerides, and 25 hydroxy-vitamin D in all participants and

hematocrit in AFAB adolescents with local variations in extent and frequency of the measurements.

Questionnaires

A subset of the following questionnaires, depending on the time point, are sent by email at 0, 6, 12, and 24 months after start of PS and GAHT. The questionnaires are distributed via email and completed online, a reminder is sent after 7 days. Participants have the option to skip specific questions or questionnaires if they do not wish to answer certain questions. *Background questionnaire:* A questionnaire developed to assess demographic characteristics, medical and family history, and lifestyle.

Side effects questionnaire

A questionnaire was constructed to evaluate side effects of the treatment based on the questionnaire by Gallagher et al.[27]

Vaginal bleeding

For AFAB individuals, a questionnaire was developed to evaluate vaginal bleeding in the past 6 months. For all episodes with vaginal blood loss, the duration and the amount of blood loss is assessed.

Physical Activity

Physical activity is measured using the Physical Activity Questionnaire for Adolescents (PAQ-A), a self-reported 7-day activity measurement tool that assesses the general level of physical activity.[28,29]

Experienced vocal performance

The Voice-related Experiences of Nonbinary Individuals tool was used to measure self-perception of voice and voice-related experiences.[30]

Quality of Life

The Pediatric Quality of Life Inventory 4.0 Generic Core Scales is a reliable and validated questionnaire to measure health-related quality of life in children and adolescents.[31] The questionnaire consists of 23 items covering physical, emotional, social, and school functioning.

Body image

Body image is evaluated using the Body Image Scale developed by Lindgren et al. for individuals with gender dysphoria.[32] With this questionnaire, participants can rate 30 items representing various body parts on a 5-point scale of satisfaction.

Binding and tucking

Two separate questionnaires have been constructed to evaluate binding and tucking behavior such as the type of binders used, the way individuals tuck, and the amount of time spent on these behaviors.

Fertility

The Transgender Youth Fertility Attitudes Questionnaire is an instrument to assess the attitude of transgender adolescents and their parents toward the potential impact of GAHT on fertility and fertility preservation.[33] The decision conflict scale has been added to the questionnaire to evaluate the decision making process.[34]

Follow-up and data management

The follow-up consists of 2 phases, PS and GAHT. The PS phase compromises visits at baseline and after 6, 12, 18, 24, 36, and 48 months from the start of PS as long as GAHT has not yet been initiated. The GAHT phase consist of visits at the start of GAHT and after 6, 12, 18, 24, and 36 months from the start of GAHT.

Statistical analysis

Statistical analyses were performed using STATA 15.1 (StataCorp). Data are presented as number (%) or median (IQR). SD scores (SDS) for height and BMI were calculated using sex registered at birth.[35,36] For future studies with the ENIGI Adolescents data, we plan to use linear regression for cross-sectional analyses for continuous outcome measures and logistic regression for dichotomous outcome measures. Mixed-model analyses will be used to analyze changes over time with measurements clustered within participants within centers, performed separately for sex assigned at birth and stratified for age at start of treatment and Tanner stage at start of PS. Because the scanners are not identical, outcomes of the DXA scans will be analyzed separately for each center.

Ethics

The study protocol was approved by the local ethical committee of each participating clinical center (reference numbers: Amsterdam, 2021.0431; Ghent, B6702022000187; Florence, CEAVC Em. 2021-502 del 22/03/2022). Written informed consent was obtained from adolescents and both parents/caregivers according to national legislation.

Results

From November 2021 until May 2023, 51 (29%) AMAB and 121 (70%) AFAB individuals have been included in the the ENIGI Adolescents protocol. Three AFAB individuals (2%) identified as nonbinary. The majority of the participants (70%) were included in Amsterdam since inclusions in Florence and Ghent started later, in July 2022 and September 2022, respectively. A total of 72 subjects who were eligible (29%) declined participation, in most cases because participation was considered too time-consuming or burdensome. This group was comparable to the included individuals regarding transition type (30% AMAB included vs 29% AMAB declined) and transition stage at the time of approach (44% PS included vs 42% PS declined). The baseline characteristics of our study population are presented in Table 2. Almost 45% of the adolescents were included at the start of PS, of whom the majority (90%) were treated with GnRHa. The other 55% of participants were included at the start of GAHT. Feminizing treatment usually consisted of oral estradiol (75%). Masculinizing treatment most often consisted of injections with testosterone esters (77%).

Discussion

The ENIGI Adolescents protocol aims to investigate the clinical effects and side effects of PS and GAHT in TGD youth. Data collection started in November 2021 in Amsterdam and in May 2023 a total of 172 TGD adolescents had been included from 3 different European expert centers. In this cohort, there is a higher representation of individuals AFAB

Table 2. Baseline characteristics of the study population.[a]

	AFAB		AMAB	
Total number of participants, No.	121		51	
Gender identity, No. (%)	Trans boy 118 (98)		Trans girl 51 (100)	
	Nonbinary 3 (2)		Nonbinary 0 (0)	
Included at start of PS, No.	47		29	
Age at start of PS, y	13.7 (12.4 to 16.1)		13.7 (12.9 to 16.5)	
Tanner stage at start of PS, No. (%)	B2, 7 (15)		G2, 8 (28)	
	B3, 12 (26)		G3, 5 (17)	
	B4, 6 (13)		G4, 3 (10)	
	B5, 19 (40)		G5, 10 (35)	
	Missing, 3 (6)		Missing, 3 (10)	
Body weight, kg	51.9 (44.2 to 57.0)		54.3 (47.0 to 66.0)	
Height, cm	160.2 (154.0 to 167.0)		173.0 (160.0 to 177.9)	
Height SDS	−0.47 (−1.31 to 0.03)		−0.15 (−0.67 to 0.27)	
BMI, kg/m^2	19.1 (17.7 to 21.6)		17.8 (16.5 to 22.2)	
BMI SDS	0.19 (−0.67 to 0.66)		−0.08 (−1.60 to 1.06)	
Medication type, No. (%)	GnRHa Lynestrenol	37 (79)	GnRHa	27 (93)
		10 (21)	CPA	2 (7)
LH, U/L	4.0 (2.1 to 6.5)		1.5 (1.0 to 4.5)	
FSH, U/L	5.2 (3.3 to 5.8)		2.8 (2.0 to 3.2)	
Estradiol, pmol/L	120 (54 to 201)		55 (22 to 72)	
Testosterone, nmol/L	0.6 (0.5 to 0.9)		8.2 (1.5 to 14.3)	
Included at start of GAHT, No.	74		22	
PS prior to GAHT, No. (%)	48 (65)		17 (77)	
PS age at start, y	12.9 (11.7 to 14.3)		13.1 (12.3 to 13.9)	
PS duration, y	2.8 (1.8 to 3.5)		2.2 (1.7 to 3.2)	
Age at start of GAHT, y	16.0 (15.1 to 16.8)		15.9 (15.1 to 17.4)	
Body weight, kg	63.9 (56.9 to 71.4)		63.5 (58.0 to 71.0)	
Height, cm	167.4 (164.1 to 171.5)		170.7 (167.0 to 174.0)	
Height SDS	−0.04 (−0.63 to 0.53)		−0.73 (−1.80 to −0.54)	
BMI, kg/m^2	23.4 (20.4 to25.6)		22.3 (20.4 to 24.0)	
BMI SDS	1.18 (0.23 to 1.87)		1.44 (0.36 to 1.62)	
Medication type, No. (%)	Testosterone esters	55 (75)	Estradiol tablets	17 (77)
	Testosterone undecanoate	2 (3)	Estradiol patches	4 (18)
	Testosterone gel	16 (22)	Estradiol gel	1 (5)
LH,[b] U/L	1.0 (0.3 to 3.6)		0.6 (0.4 to 1.9)	
FSH,[b] U/L	2.0 (1.0 to 4.5)		0.7 (0.4 to 2.3)	
Estradiol,[b] pmol/L	33 (20 to 81)		20 (20 to 30)	
Testosterone,[b] nmol/L	0.5 (0.4 to 0.7)		0.5 (0.3 to 13.0)	

Abbreviations: AFAB, assigned female at birth; AMAB, assigned male at birth; FSH, follicle-stimulating hormone; GAHT, gender-affirming hormone therapy; LH, luteinizing hormone; PS, puberty suppression; SDS, SD scores; [a]Data are presented as median (IQR) unless stated otherwise. [b]Some individuals used a GnRH analogue or progestin at the time of blood withdrawal.

than of those AMAB, which is in line with demographic distributions observed in previous studies.[12,37] However, there is no definitive explanation for this trend. At the time of this report thestudy included a relatively small number of nonbinary individuals, potentially due to our specific focus on those initiating hormonal treatment. Nonbinary individuals may have alternative treatment preferences that render them ineligible for inclusion in our study. We hope to include more nonbinary participants to assess their needs and outcomes. To our knowledge, the ENIGI Adolescents collaboration is the first multicenter European cohort study of TGD adolescents. The Trans Youth Care Study and the Trans Youth CAN! are similar multicenter initiatives from the United States and Canada, respectively; participant enrollment was completed after 2 years in both these studies.[38-40]) Furthermore, the Trans20 is a monocenter prospective study from Australia that included over 600 transgender adolescents between 2017 and 2020.[41] A European multicenter study is of added value since it increases the knowledge on transgender adolescents from Europe, with different ethnic and cultural backgrounds and different treatment strategies. This heterogeneity offers the opportunity to compare results between European centers within the ENIGI Adolescents study but also between different continents.

A strength of this study is the prospective design, which provides the opportunity for systematic collection of data. The combination of objective clinical data and subjective, self-reported data collected via questionnaires provides a broad range of outcomes we can use to evaluate and ultimately to improve care for adolescents. Despite the sensitive nature of the topic, the majority (71%) of eligible individuals agreed to take part in the study, which is encouraging, indicating a high willingness of TGD youth to participate in clinical studies that aim to improve medicinal treatment strategies for TGD adolescents. A potential weakness of this study is the fact that the additional measurements and questionnaires might be burdensome for some adolescents, which may lead to withdrawal from the study over time or a lower response rate for questionnaires. This potential weakness motivates the research team to try to understand the hurdles and design creative solutions. Questionnaires can be filled out online and at home, so that adolescents can choose to complete them at a convenient time, which will hopefully maximize the response rate. Furthermore, in future studies the baseline characteristics

of nonresponders will be compared to those of responders to explore possible bias.

Conclusion

The ENIGI adolescents study was designed to improve knowledge regarding clinical effects and side effects of PS and GAHT, binding and tucking, and attitudes toward fertility in TGD adolescents. International collaboration provides the opportunity to form a large cohort and offers the chance to compare different treatment strategies. The outcome data that will be collected should help to improve healthcare for TGD adolescents.

Acknowledgments

We thank all of the collaborators and study participants for their contribution.

Author contributions

L.S.B., Conceptualization, Investigation, Methodology, Project administration, Writing – original draft; C.M.W., Conceptualization, Supervision, Writing – review & editing; A.S.S., Conceptualization, Data curation, Writing – review & editing; D.T.K., Conceptualization, Investigation, Methodology, Writing – review & editing; S.C., Conceptualization, Investigation, Methodology, Writing – review & editing; A.R., Conceptualization, Investigation, Methodology, Writing – review & editing; T.H.R.S., Investigation, Methodology, Writing – review & editing; E.V.D.B., Methodology, Writing – review & editing; T.D.S., Methodology, Writing – review & editing; A.L.C.D.V., Methodology, Writing – review & editing; A.S.P.V.T., Methodology, Writing – review & editing; M.D.H., Methodology, Writing – review & editing; A.D.F., Conceptualization, Investigation, Methodology, Project administration, Writing – review & editing; M.C., Conceptualization, Investigation, Methodology, Project administration, Writing – review & editing; S.E.H., Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Conflicts of interest

None declared.

Funding

None declared.

References

1. Hembree WC, Cohen-Kettenis PT, Gooren L, *et al.* Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;**102**(11):3869–3903. https://doi.org/10.1210/jc.2017-01658
2. de Blok CJM, Dijkman BAM, Wiepjes CM, *et al.* Sustained breast development and breast anthropometric changes in 3 years of gender-affirming hormone treatment. *J Clin Endocrinol Metab*. 2021;**106**(2):e782–e790. https://doi.org/10.1210/clinem/dgaa841
3. Klaver M, van Velzen D, de Blok C, *et al.* Change in visceral fat and Total body fat and the effect on Cardiometabolic risk factors during transgender hormone therapy. *J Clin Endocrinol Metab*. 2022;**107**(1):e153–e164. https://doi.org/10.1210/clinem/dgab616
4. Van Caenegem E, Wierckx K, Taes Y, *et al.* Body composition, bone turnover, and bone mass in trans men during testosterone treatment: 1-year follow-up data from a prospective case-controlled study (ENIGI). *Eur J Endocrinol*. 2015;**172**(2):163–171. https://doi.org/10.1530/EJE-14-0586
5. Nygren U, Nordenskjöld A, Arver S, Södersten M. Effects on voice fundamental frequency and satisfaction with voice in trans men during testosterone treatment-a longitudinal study. *J Voice*. 2016;**30**(6):766.e23–766.e34. https://doi.org/10.1016/j.jvoice.2015.10.016
6. Wierckx K, Van de Peer F, Verhaeghe E, *et al.* Short- and long-term clinical skin effects of testosterone treatment in trans men. *J Sex Med*. 2014;**11**(1):222–229. https://doi.org/10.1111/jsm.12366
7. Coleman E, Radix A, Bouman W, *et al.* Standards of care for the health of transgender and gender diverse people, version 8. *Int J Transgender Health*. 2022;**23**(sup1):S1–S259. https://doi.org/10.1080/26895269.2022.2100644
8. Heinemann LA, Will-Shahab L, van Kesteren P, Gooren LJ. Safety of cyproterone acetate: report of active surveillance. *Pharmacoepidemiol Drug Saf*. 1997;**6**(3):169–178. https://doi.org/10.1002/(SICI)1099-1557(199705)6:3<169::AID-PDS263>3.0.CO;2-3
9. Spinder T, Spijkstra JJ, Gooren LJ, Hompes PG, van Kessel H. Effects of long-term testosterone administration on gonadotropin secretion in agonadal female to male transsexuals compared with hypogonadal and normal women. *J Clin Endocrinol Metab*. 1989;**68**(1):200–207. https://doi.org/10.1210/jcem-68-1-200
10. Kreukels BP, Haraldsen IR, De Cuypere G, Richter-Appelt H, Gijs L, Cohen-Kettenis PT. A European network for the investigation of gender incongruence: the ENIGI initiative. *Eur Psychiatry*. 2012;**27**(6):445–450. https://doi.org/10.1016/j.eurpsy.2010.04.009
11. Dekker MJ, Wierckx K, Van Caenegem E, *et al.* A European network for the investigation of gender incongruence: endocrine part. *J Sex Med*. 2016;**13**(6):994–999. https://doi.org/10.1016/j.jsxm.2016.03.371
12. Wiepjes CM, Nota NM, de Blok CJM, *et al.* The Amsterdam cohort of gender dysphoria study (1972-2015): trends in prevalence, treatment, and regrets. *J Sex Med*. 2018;**15**(4):582–590. https://doi.org/10.1016/j.jsxm.2018.01.016
13. Butler G, De Graaf N, Wren B, Carmichael P. Assessment and support of children and adolescents with gender dysphoria. *Arch Dis Child*. 2018;**103**(7):631–636. https://doi.org/10.1136/archdischild-2018-314992
14. Cohen-Kettenis PT, van Goozen SH. Pubertal delay as an aid in diagnosis and treatment of a transsexual adolescent. *Eur Child Adolesc Psychiatry*. 1998;**7**(4):246–248. https://doi.org/10.1007/s007870050073
15. van der Loos M, Klink DT, Hannema SE, *et al.* Children and adolescents in the Amsterdam cohort of gender dysphoria: trends in diagnostic- and treatment trajectories during the first 20 years of the Dutch protocol. *J Sex Med*. 2023;**20**(3):398–409. https://doi.org/10.1093/jsxmed/qdac029
16. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington, DC:American Psychiatric Association; 2018.
17. *International Classification of Diseases, Eleventh Revision* (ICD-11). WHO 2019/2021 https://icd.who.int/browse11.
18. Tanner JM. Growth at Adolescence. *1st ed. Thomas: Springfield, IL*. 1955;
19. Schmidt RT, Toews JV. Grip strength as measured by the Jamar dynamometer. *Arch Phys Med Rehabil*. 1970;**51**(6):321–327
20. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol*. 1981;**140**(7):815–830. https://doi.org/10.1016/0002-9378(81)90746-8
21. Doshi A, Zaheer A, Stiller MJ. A comparison of current acne grading systems and proposal of a novel system. *Int J Dermatol*. 1997;**36**(6):416–418. https://doi.org/10.1046/j.1365-4362.1997.00099.x
22. Schindler A, Ginocchio D, Maccarini AR, Bisetti M, Ruoppolo G, Accordi M. CAPE-V (consensus auditory-perceptual evaluation of voice): versione italiana. *Acta Phoniatrica Latina*. 2006;**28**(4):383

23. Boogers LS, Chen BSJ, Coerts MJ, Rinkel R, Hannema SE. Mobile phone applications voice tools and voice pitch Analyzer validated with LingWAVES to measure voice frequency. *J Voice*. 2022;**S0892-1997**(22):00318–00316. https://doi.org/10.1016/j.jvoice.2022.10.013
24. Thodberg HH. Clinical review: an automated method for determination of bone age. *J Clin Endocrinol Metab*. 2009;**94**(7):2239–2244. https://doi.org/10.1210/jc.2008-2474
25. Greulich WW, Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. 2nd ed. Stanford:Stanford University Press; 1959.
26. Thodberg HH, Neuhof J, Ranke MB, Jenni OG, Martin DD. Validation of bone age methods by their ability to predict adult height. *Hormone Res Paediatr*. 2010;**74**(1):15–22. https://doi.org/10.1159/000313592
27. Gallagher JS, Missmer SA, Hornstein MD, Laufer MR, Gordon CM, DiVasta AD. Long-term effects of gonadotropin-releasing hormone agonists and add-back in adolescent endometriosis. *J Pediatr Adolesc Gynecol*. 2018;**31**(4):376–381. https://doi.org/10.1016/j.jpag.2018.03.004
28. Kowalski KC, Crocker PR, Kowalski NP. Convergent validity of the physical activity questionnaire for adolescents. *Pediatr Exerc Sci*. 1997;**9**(4):342–352. https://doi.org/10.1123/pes.9.4.342
29. Janz KF, Lutuchy EM, Wenthe P, Levy SM. Measuring activity in children and adolescents using self-report: PAQ-C and PAQ-A. *Med Sci Sports Exerc*. 2008;**40**(4):767–772. https://doi.org/10.1249/MSS.0b013e3181620ed1
30. Shefcik G, Tsai PT. Voice-related experiences of nonbinary individuals (VENI) development and content validity. *J Voice*. 2023;37(2):294.e5-294.e13.
31. Varni JW, Seid M, Kurtin PS. PedsQL 4.0: reliability and validity of the Pediatric quality of life inventory version 4.0 generic corescales in healthy and patient populations. *Med Care*. 2001;**39**(8):800–812. https://doi.org/10.1097/00005650-200108000-00006
32. Lindgren TW, Pauly IB. A body image scale for evaluating transsexuals. *Arch Sex Behav*. 1975;**4**(6):639–656. https://doi.org/10.1007/BF01544272
33. Strang JF, Jarin J, Call D, *et al*. Transgender youth fertility attitudes questionnaire: measure development in nonautistic and autistic transgender youth and their parents. *J Adolesc Health*. 2018;**62**(2):128–135. https://doi.org/10.1016/j.jadohealth.2017.07.022
34. O'Connor AM. Validation of a decisional conflict scale. *Med Decis Mak*. 1995;**15**(1):25–30. https://doi.org/10.1177/0272989X9501500105
35. Schönbeck Y, Talma H, van Dommelen P, *et al*. The world's tallest nation has stopped growing taller: the height of Dutch children from 1955 to 2009. *Pediatr Res*. 2013;**73**(3):371–377. https://doi.org/10.1038/pr.2012.189
36. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;**320**(7244):1240–1243. https://doi.org/10.1136/bmj.320.7244.1240
37. Expósito-Campos P, Gómez-Balaguer M, Hurtado-Murillo F, Morillas-Ariño C. Evolution and trends in referrals to a specialist gender identity unit in Spain over 10 years (2012-2021). *J Sex Med*. 2023;**20**(3):377–387. https://doi.org/10.1093/jsxmed/qdac034
38. Olson-Kennedy J, Chan YM, Garofalo R, *et al*. Impact of early medical treatment for transgender youth: protocol for the longitudinal, observational trans youth care study. *JMIR Res Protoc*. 2019;**8**(7):e14434. https://doi.org/10.2196/14434
39. Olson-Kennedy J, Chan YM, Rosenthal S, *et al*. Creating the trans youth research network: a collaborative research endeavor. *Transgend Health*. 2019;**4**(1):304–312. https://doi.org/10.1089/trgh.2019.0024
40. Bauer GR, Pacaud D, Couch R, *et al*. Transgender youth referred to clinics for gender-affirming medical care in Canada. *Pediatrics*. 2021;**148**(5):e2020047266. https://doi.org/10.1542/peds.2020-047266
41. Tollit MA, Pace CC, Telfer M, *et al*. What are the health outcomes of trans and gender diverse young people in Australia? Study protocol for the Trans20 longitudinal cohort study. *BMJ Open*. 2019;**9**(11):e032151. https://doi.org/10.1136/bmjopen-2019-032151