Research in Transgender Healthcare

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RESEARCH IN TRANSGENDER HEALTHCARE: WHAT HAVE WE LEARNED AND WHERE ARE WE GOING?

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RESEARCH IN TRANSGENDER HEALTHCARE: WHAT HAVE WE LEARNED AND WHERE ARE WE GOING?

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Table of Contents

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EDITORIAL
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Editorial: Research in Transgender Healthcare: What Have We Learned and Where Are We Going?

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Editorial on the Research Topic

Research in Transgender Healthcare: What Have We Learned and Where are We Going?

Gender incongruence (GI) is defined as "an individual's discontent with their assigned gender and their identification with a gender other than that associated with their birth sex based on physical sex characteristics" (1).

The origin of GI appears to be complex and multifactorial. From the extensive research that has been conducted over the past few years, three main factors have been identified as key mechanisms for understanding GI: genes, hormones, and the environment.

Accordingly, our Frontiers Research Topic includes twelve articles that cover very varied topics about GI, including cardiovascular effects of treatment, surgical outcomes, new treatment options and healthcare quality in a broader sense. This research involved the hard work of sixty-six authors that was carried out mainly in Europe (Austria, Belgium, Germany, Italy, Serbia, and Spain), Australia, and also the United States.

An interesting study about the prevalence of cardiometabolic risk factors in transgender individuals receiving feminizing therapy for ≥6 months was carried out by Balcerek et al. in a sample of 296 transgender individuals. The authors found that a greater proportion of trans individuals ≥45 years of age were treated with transdermal estradiol. Of those who received oral estradiol, the median dose was lower. Importantly, relatively higher doses of estradiol put people at risk of cardiometabolic diseases, especially in combination with older age. The authors point out that the most prevalent cardiometabolic risk factor in the ≥45 years group was hypertension (29%), followed by current smoking (24%), obesity (20%), dyslipidaemia (16%) and diabetes (9%).

On a related topic, Totaro et al., in a meta-analytic study, analyzed the risk of venous thromboembolism (VTE) in transgender people undergoing feminizing hormone treatment. The overall pooled prevalence estimate for VTE was 2%, but with a large heterogeneity across studies. A number of factors could contribute to the variable VTE risk in transgender people undergoing gender-affirming hormone treatment (GAHT), including the type of estrogen and the route of administration, age at the estrogen therapy onset, treatment duration, concomitant conditions such as smoking, obesity, thrombophilia and other comorbidities.

This topic of cardiovascular health outcomes due to GAHT was also examined by Aranda et al. The authors present a review of the current literature on the effects of GAHT, in both transgender men and transgender women, on various cardiovascular health outcomes.

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An opinion paper included in our Research Topic addresses a clever approach on increasing inclusiveness in studies on Traumatic Brain Injury (TBI). The authors, Duncan and Garijo-Garde emphasize the importance of studying the influence of sex, gender and sex hormones in the TBI population. They highlight the need for evidence-based guidelines, and clinically translatable diagnostic and prognostic models.

The current endocrinological treatment guidelines serve primarily those who wish to transition from male to female or vice versa. But non-binary individuals currently make up more than 25% of the transgender population (2). Thus, there is a dearth of hormonal options for those who identify as non-binary and seek an androgynous appearance that is neither overtly masculine nor feminine. Regarding this topic, a potential option for non-binary GAHT was presented by Xu et al. In this article, the authors discuss the theoretical use of selective estrogen receptor modulators (SERMs) for non-binary persons assigned male at birth seeking an androgynous appearance through partial feminization without breast growth. The authors concluded that an individualized decision is needed when considering the use of SERMs in non-binary people, with recognition of their experimental nature, significant potential risks, and the urgent need for more research.

In our Research Topic, we also include a case report by Pang et al. who present the use of minoxidil as an alternative treatment option to testosterone administration for transgender men who desire an increase in facial hair growth.

Bordas et al. report on a follow-up study of surgical outcomes in 813 transgender men. The authors evaluated outcomes of metoidioplasty after on average 94 months post-operation. Urethroplasty was found to be without complications in 81% to 90% of the cases, and 99% of the patients who answered the questionnaire were mainly to fully satisfied with the surgical result.

GAHT may be recommended in many cases and it has been shown to improve many facets of transgender individuals' functioning. In a prospective longitudinal controlled study, Foster Skewis et al. examined the effects of GAHT on quality of life of a group of transgender individuals over a 6-month period. The authors concluded that in transgender people initiating masculinizing or feminizing treatments, there was a decrease in gender dysphoria experienced and a clinically significant improvement in emotional well-being and social functioning aspects of quality of life, relative to a cisgender comparison group.

A systematic review of the barriers to accessing transgender healthcare in rural regions was carried out by Renner et al. The authors found an overrepresentation of transgender people in vulnerable socioeconomic situations, primarily due to experiences of discrimination. At the same time, rural or suburban living areas often lack specialized trans-related healthcare. Together, the lack of both socioeconomic resources and access to transgender healthcare can exacerbate health-related distress and impairment for transgender individuals.

A systematic review and a global expert survey in 39 countries about centralized (i.e., one interdisciplinary team) and decentralized (different medical institutions) delivery of transgender healthcare services was carried out by Koehler et al. The authors shed light on this important aspect of transgender healthcare and gained valuable knowledge for the further improvement of transgender healthcare quality.

Finally, in the work of Guethlein et al., the authors compare issues related to health and healthcare of transgender people in Germany with those in other European countries. The authors review the care offered by specialized centers with regard to treatment of and support for transgender people.

Approaching the topic of GI from a much different angle, Ramirez et al. address the question of the origins of GI with an epigenetics study. Both genetic, as well as (social and physical) environmental factors, have been found to play a role in the development of human gender, including transgender identity by directly or indirectly altering gene expression and behavior. In this respect, epigenetics offers many research opportunities, especially with regard to GI, as they reflect the interconnection between genes, hormones, and the environment.

The main finding of that study was that, prior to GAHT, the transgender group had a different global CpG methylome compared to the cisgender sample. This suggests that there are differences at the methylation level between cis- and transgender individuals even before GAHT. Furthermore, these findings indicate that not only genetics, but also epigenetics could be implicated in the biological basis of GI.

When the authors compared individuals assigned male at birth (cis vs. trans), they found significant differences in the methylation level of 22 CpGs. However, with respect to individuals assigned female at birth, significant differences in methylation of only 2 CpGs were found. Thus, there were larger differences in methylation level between the male-assigned groups (cis vs. trans) than between the female-assigned groups (cis vs trans). Furthermore, one of these CpGs, related to the *MPPED2* gene, was shared by both transgender men and women, suggesting a similar underlying mechanism for both sexes. Among the CpGs differing between the cis- and transgender samples, at least four genes were clearly involved in brain development and neurogenesis. These findings support the view that combining genetic and epigenetic approaches in parallel may be a successful way to better understand the mechanisms underlying sex- and gender identity related brain development.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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OPINION
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Sex, Genes, and Traumatic Brain Injury (TBI): A Call for a Gender Inclusive Approach to the Study of TBI in the Lab

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INTRODUCTION

Traumatic Brain Injury (TBI) is a leading cause of morbidity and moribundity in the United States (Bruns and Hauser, 2003; Corrigan et al., 2010). A number of factors including sex influence TBI clinical outcome. Both clinical and lab studies show a clear influence of sex on TBI outcome. However, whether this is mediated by hormones, genes, or both is still under debate (Gupte et al., 2019; Ma et al., 2019; Mikolić et al., 2020). The majority of research focuses on factors of endogenous hormone signaling (release and reception) in natal males (Slewa-Younan et al., 2004; Dubal et al., 2006; Herson et al., 2009; Griesbach et al., 2015; Clevenger et al., 2018; Mollayeva et al., 2018; Späni et al., 2018; Ma et al., 2019). This excludes natal females and both males and females taking exogenous hormones for hormone replacement (HRT) or cross sex hormone therapy (CSHT) as part of a gender confirming therapy (Mollayeva et al., 2018; Späni et al., 2018; Ma et al., 2019; Giordano et al., 2020; Biegon, 2021). While transgender and gender non-conforming (TGNC) individuals make up 0.3–0.5% of the global population, they are affected by violence at higher rates compared to cisgender individuals (Jauk, 2013). Despite these higher rates of violence and increased risk of TBI, the TGNC population remains understudied in the TBI field (Safer et al., 2016). This bias extends to healthcare settings where many TGNC individuals face significant barriers to obtaining high-quality, compassionate medical care at primary care facilities, especially in emergency rooms, where most TBIs are diagnosed (Sanchez et al., 2009; Porter et al., 2016; Reisner et al., 2016; Safer et al., 2016; Dickey and Singh, 2017).

Research regarding transgender health has dramatically increased, yet there is still significant room for improvement as TGNC individuals are at an increased risk for several health issues (Reisner et al., 2016; Ackerley et al., 2019; Neblett and Hipp, 2019; Yeung et al., 2019; Wiepjes et al., 2020). A gender inclusive approach in biomedical research is vital to our understanding and treatment of TBI. The aim of this paper is to call upon lab-based investigators to approach the study of TBI and also biomedical research in a gender inclusive manner.

SEX, GENDER, AND TBI: BEYOND THE BINARY

TBI has a biphasic response. While the primary insult is often short in duration, the secondary phase can linger for hours to weeks after the initial injury. Like the primary injury, if untreated,

this secondary phase can lead to a manifestation of clinical or behavioral symptoms, including death (Lenzlinger et al., 2001; Bramlett and Dietrich, 2007; Maas et al., 2008). The majority of research focuses on both decreasing the chance of injury and decreasing the secondary effects of that injury (Fitch et al., 1999; Day et al., 2013; Kim et al., 2015; Ripley et al., 2020; Bourgeois-Tardif et al., 2021). Severe symptoms include long-term cognitive or behavioral deficits, while moderate to mild symptoms include headaches, dizziness, nausea, and short-term amnesia (Prins et al., 2013). Additionally, if untreated, these long-term effects of TBI can result in increased risk of neurodegenerative diseases such as Alzheimer's disease, chronic traumatic encephalopathy, and Parkinson's disease (Lye and Shores, 2000; McKee et al., 2009; Hutson et al., 2011). Treatment of secondary injuries is complex, as there are a multitude of neurobiochemical and metabolic pathways that are activated across multiple time scales and can differ depending on sex (Prins et al., 2013; Saldanha et al., 2013; Rahimian et al., 2019; Mikolić et al., 2020).

Hormone production or availability likely contribute to TBI outcome which results in females largely being reported to be more resilient than their male counterparts (Mollayeva et al., 2018; Ma et al., 2019; Rubin and Lipton, 2019). Furthermore, when comparing prepubescent, premenopausal, and post-menopausal women, premenopausal, and pubescent females generally have lower rates of mortality and better prognoses than older, premenopausal adults (Du et al., 2004; Ley et al., 2013; Albrecht et al., 2016; Ranganathan et al., 2016; Ma et al., 2019). These conclusions are supported by studies directly examining the role of estrogens, progesterone, androgens, and their metabolites following TBI. The studies have identified these steroids via activation of their receptors (which can vary by sex following injury) as being neuroprotective by preventing the brain from edema, necrosis, apoptosis, and inflammation (Stein and Hoffman, 2003; Bryant et al., 2006; Dubal et al., 2006; Spence and Voskuhl, 2012; Acaz-Fonseca et al., 2016; Brotfain et al., 2016; Duncan and Saldanha, 2020). These effects can occur acutely after the injury, but can have prolonged effects lasting weeks after the initial injury (Suzuki et al., 2007). However, the majority of these studies have focused on endogenous release vs. exogenous therapy and when comparing humans to lab models, we can see the opposite result (Hall et al., 2005; Stein, 2015; Gupte et al., 2019).

How to Study TBI Through a Transgender Lens?

The process of transitioning is complex and can be heavily individualized, which partially explains some of the difficulties in developing a lab-based model. Despite the limited information regarding the development of human gender identity, there has been significant progress in using animal models to demonstrate the neurodevelopment pathways leading to sex differences in brain and behavior (Joel and McCarthy, 2017; Choleris et al., 2018; Theisen et al., 2019). From these studies, three main factors: environment, genes, and hormones, have all been identified as mechanisms key to understanding human gender identity.

Environment (social or physical) plays a major role in the development of human gender including transgender identity by directly or indirectly (epigenetics) altering gene expression and behavior (Szyf et al., 2008; Arnold, 2017). However, the role of the environment is difficult to model in non-human subjects; and therefore, we will focus primarily on the other two factors identified (genes and hormones).

Factors Affecting TBI Outcome in TGNC Populations

Genes

A number of studies have suggested a genetic contribution to the development of transgender identity (Lippa and Hershberger, 1999; Bentz et al., 2007, 2008; Fernández et al., 2015; Smith et al., 2015; Fisher et al., 2018; Polderman et al., 2018; Foreman et al., 2019). Specifically, twin studies have found heritability anywhere between 38–47% in adolescent natal females and 25–43% in adolescent natal males, while these numbers decrease to 11–44% and 27–47% in adults (Fisher et al., 2018; Polderman et al., 2018; Theisen et al., 2019). A number of genes were identified from these studies, many of which were also previously identified in studies of sexual differentiation in animal models. These include COMT, PIK3CA, RYR3, SRD5A2, STS, and SULT2A1, as well as variants of genes coding aromatase, androgen receptor (AR), estrogen receptors (ER) α & β, and 17α-hydroxylase (Fernández et al., 2015, 2018; Smith et al., 2015; Yang et al., 2017; Fisher et al., 2018; Foreman et al., 2019; Theisen et al., 2019). In terms of sex differences in TBI, we have also identified differences in a number of these genes as well, including PIK3C, SULT2A1, aromatase, AR and ERs, and 17 hydroxylase (Garcia-Segura et al., 2003; Duncan and Saldanha, 2011, 2013; Saldanha et al., 2013; Pedersen et al., 2018; Cook et al., 2020; Duncan, 2020). Suggesting that these genes may have variations in their response following TBI and can serve as first candidates for examining differences in gene expression. For example, ERα mediates the estrogenic neuroprotective effects of TBI (Dubal et al., 2006; Duncan and Saldanha, 2020) and TGNC individuals have differences in constitutive receptor expression or different polymorphisms that may affect their ability to activate these neuroprotective pathways (Fernández et al., 2018). Discerning how these receptors and genes may change in TGNC individuals is key to our understanding of their activation following TBI.

Furthermore, the use of the four core genotype mouse model which uncouples chromosomal (X, Y) effects from gonadal influence along with the XY* mice could be a powerful tool in determining the chromosomal/genetic contributions during TBI (Arnold and Chen, 2009; Corre et al., 2016; Arnold, 2020). Comparison of XX and XY mice with the same type of gonads, but different sex chromosomes can help in determining the role of sex linked genes vs. hormone availability. While not fully a model for TGNC populations, this is a powerful tool for determining the relative contribution of a sex difference. Currently, two studies have examined TBI using one of these models and found that in young animals, hormones and not chromosomes shaped response; however, this was reversed in aged populations (Manwani et al., 2015; McCullough et al., 2016).

More work is on-going to determine the differences in gene expression following TBI, and if these differences are the same between cis and transgender individuals. The use of these two powerful models can help to determine if a sex difference in TBI are mediated by chromosomes, hormones, or both, especially when paired with cross hormone therapies.

Hormones

Steroid hormone levels and receptors are markedly different in natal males and females throughout most of their lifespan and clearly play a role following TBIs (Arnold, 2017; Gölz et al., 2019; Giordano et al., 2020). In terms of TGNC individuals, life-long hormonal therapy is often a key component of their transition and can be implemented as early as adolescence (Deutsch et al., 2015; Hembree et al., 2017; Nguyen et al., 2018; T'Sjoen et al., 2019). One major issue with studying individuals that are currently transitioning or have transitioned is identifying the specific medical plan used to transition. A number of various plans are used (**Table 1**), for both medical and personal reasons, and thus modeling can become difficult to mimic exactly (Feldman and Safer, 2009; Hembree et al., 2009; The World Professional Association for Transgender Health, 2012; American Psychological Association, 2015; Unger, 2016; Funabashi et al., 2018; Defreyne and T'Sjoen, 2019; Hamidi and Davidge-Pitts, 2019). It is important to note that many of the hormones used for transitioning differ to what are commonly used in the lab, specifically in terms of long-term use and "stacking" of multiple drugs. What we currently know about exogenous hormones and neural damage and recovery comes from teasing out the various contributions of a transition plan, but more research is needed to combine all of these components into a comprehensive model of a transitioning or transitioned individual.

Trans-masculine

TBI has historically been viewed as a problem that predominantly affects natal males, as they are both more likely to receive TBIs and have less of the circulating neuroprotective steroids: estrogen and progesterone (Späni et al., 2018; Gupte et al., 2019; Rubin and Lipton, 2019; Mikolić et al., 2020). When comparing age-matched natal males and females, younger females appear to be protected against neuronal damage, suggesting that androgens may not be advantageous following injury (Dubal and Wise, 2002; Gupte et al., 2019). However, this is complicated by research that shows that males with lower testosterone have worse clinical outcomes than males within normal ranges, suggesting that while testosterone isn't detrimental in males, that other steroids may be more beneficial. This is supported by studies of natal males given testosterone for myelin repair for relapsing-remitting Multiple Sclerosis that show a significant increase in neuroprotection over controls (Kurth et al., 2014). More research is necessary to further identify the role of testosterone following TBI.

In trans-masculine procedures, hormone therapy is sometimes paired with removal of the uterus and ovaries (hysterectomy and oophorectomy) via gender confirmation surgery (Coleman et al., 2012; American Psychological Association, 2015). Ovary removal has profound effects on both circulating hormone levels and TBI outcome. Cisgender women undergoing oophorectomy show lower levels of estrogen than age-matched women experiencing natural menopause (Korse et al., 2009; Perera et al., 2013; Orozco et al., 2014). Post-menopausal cisgender women, characterized by decreased circulating estrogens and progestins, show worse outcomes than premenopausal females, but better than age-matched natal males (Niemeier et al., 2013). This suggests that removing circulating hormones affects TBI severity and

TABLE 1 | Drugs used for gender affirming hormonal treatments in TGNC individuals.

	Drug	Common drug name(s)	Route of administration	Proposed dosage with frequency	Blood levels (in humans)
Trans-masculine (FtM)	Testosterone Undecanoate (UK) or Testosterone Enanthate (US) or Testosterone cypionate (US)	• Andriol® • Delatestryl® • Depo®-Testosterone • Aveed® • Androgel®, Androderm®	Oral, Subcutaneous, Intramuscular, Transdermal	• Undecanoate: 160–240 mg/day • Enanthate, cypionate: 20–100 mg/week • Transdermal: 2.5–10 mg/day	Testosterone: 300–1,000 ng/dL
	Progesterone (optional)	• Provera®	Oral	• 12.5 mg/daily	
Trans-feminine (MtF)	Estradiol or Estradiol valerate or Estradiol cypionate	• Depo®-Estradiol, Depofemin®, Estradep® • Delestrogen®, Progynon Depot®, Progynova®	Oral, Subcutaneous, Intramuscular, Transdermal	• Estradiol: 2–6 mg daily • Estradiol valerate: 2–20 mg/2weeks • Estradiol transdermal: 0.025–0.2 mg/daily	Estrogen: 100–200 pg/mL
	Anti-Androgens: Progesterone Spironolactone Histrelin implant	• Provera® • CaroSpir®,Aldactone® • Vantas®, Supprelin LA®	Oral, implant	• Progesterone: 25–50 mg PO daily • Spironolactone: 100–300 mg PO daily • Histrelin: 3.75 mg monthly	Testosterone: <50 ng/dL
Puberty blockers	GnRH analogs/agonists: Leuprolide acetate Histrelin	• Lupron Depot® • Vantas®, Supprelin LA®	Subcutaneous, Intramuscular, Implanted pellet	• Lupron Depot: 7.5 mg/monthly • Histrelin: 3.75 mg monthly	Peak LH < 4 mIU/mL after GnRHa stimulation.

could potentially make FTM individuals more susceptible to neurodegeneration. Furthermore, when studying risk of neural damage, epidemiological evidence clearly shows that sex and estrogen levels are important factors in long-term outcome (Rocca, 2017; Bazzigaluppi et al., 2018). Studies of ovariectomy prior to TBI in rats showed larger areas of damage compared to intact females and thus worse outcomes (Bramlett and Dietrich, 2001). Together, these data suggest that although steroid hormones may be protective, their sudden withdrawal either before or after injury may be a key factor contributing to worse outcomes in individuals assigned female at birth (Wunderle et al., 2014). Put together, these data suggest that transgender males that elect for gender affirmation surgeries may be more susceptible to negative outcomes of TBI. However, more research is needed to understand how this can be alleviated.

Trans-feminine

If natal females are indeed better protected from TBI, then individuals undergoing trans-feminine transition may see better outcomes following hormonal transition as they typically take exogenous estrogen and progesterone, as well as anti-androgens. Typically, younger-aged cisgender women appear to be protected against neuronal damage, compared with cisgender men, but lose this advantage in their post-menopausal years (Niemeier et al., 2013; Ranganathan et al., 2016). A typical cycling female shows monthly variation in both estrogen and progesterone signaling. By using this natural variation in estrogen and progesterone response, we have been able to identify the relative contribution of estrogen and progesterone following TBI. Cisgender women in the luteal phase of their menstrual cycle, in which progesterone is highest, had worse outcomes than those in the follicular phase, in which progesterone is initially low and can therefore not decrease significantly (Wunderle et al., 2014). However, exogenous progestin use from oral contraceptives leads to better outcomes than controls in individuals assigned female at birth (Wunderle et al., 2014). The potential use of steroids in natal men following TBI has led to mixed results [see Späni et al. (Späni et al., 2018) for review]. For estrogens, the negative effects (cardiovascular disease and breast cancer) associated with short-term or long-term use overshadow any potential neuroprotective effects (Späni et al., 2018). Progesterone, however, has been included as a treatment option in two large Phase III trials [ProTECT and SYNAPSE (Wright et al., 2007; Stein, 2015)]. Results from these two trials were not conclusive as some saw no difference in cisgender males, while a small subset saw a slight improvement in them (Lu et al., 2016; Späni et al., 2018). Specifically, sex and hormones present at the time of injury were cited as factors that mediated the effectiveness of estrogens and progesterone following injury (Stein, 2015; Stein et al., 2016; Späni et al., 2018).

Individuals undergoing trans-feminine gender affirming surgeries sometimes remove the testes, a significant source of testosterone (Coleman et al., 2012; American Psychological Association, 2015). Paired with antiandrogen hormonal treatments, this significantly removes the amount of androgens in circulation. Therefore, one would assume that these individuals would show better responses to TBI than transgender men. However, inherent differences in gene expression in neuroinflammatory pathways or vasculature could lead to differences in response to TBI.

The bulk of research into hormone use following TBI has been done in adult or possibly aged populations. However, a significant number of individuals begin this sort of transition in early adulthood or adolescence (Smith et al., 2001; Menvielle and Gomez-Lobo, 2011; Olson, 2016). Currently, there is relatively little research on the role of cross sex hormone therapies in younger populations and nothing known about this topic in TBI research. TBI is the leading cause of death and disability in children (Ley et al., 2013; Araki et al., 2017), and children and adolescents diagnosed with a TBI are at higher risk of being diagnosed with a central endocrinopathy (Ortiz et al., 2020). These TBI induced differences in endocrine function are varied, but have been known to increase disturbances in puberty (Auble et al., 2014). To date, no studies have examined how use of CSHT could affect these pediatric outcomes and or recovery from TBI specifically.

DISCUSSION

Although we have placed a great emphasis on the lack of research in TGNC individuals, sex/gender as a biological variable is underrepresented in TBI research and requires further analysis. The increase in the number of individuals undergoing hormonal or surgical treatment to aid in gender transition calls for a substantial increase in TBI research in these underserved and marginalized populations. There is a need for evidence-based guidelines, common hormone plans, and clinically translatable diagnostic and prognostic models. Furthermore, expanding our knowledge of how exogenous hormone use affects TBI could have profound effects not only on TGNC populations, but also cisgender males and females. Such work and studies will not only help to develop better treatment options for those identifying as TGNC, but will also create a conceptual framework which can be used to extrapolate to others undergoing hormone replacement or depletion in the future.

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Selective Estrogen Receptor Modulators: A Potential Option For Non-Binary Gender-Affirming Hormonal Care?

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Gender dysphoria describes the distress associated with having a gender identity that differs from one's birth-assigned sex. To relieve this distress, transgender, and gender diverse (henceforth, trans) individuals commonly undergo medical transition involving hormonal treatments. Current hormonal treatment guidelines cater almost exclusively for those who wish to transition from male to female or vice versa. In contrast, there is a dearth of hormonal options for those trans individuals who identify as non-binary and seek an androgynous appearance that is neither overtly male nor female. Though prolonged puberty suppression with gonadotrophin releasing hormone agonists (GnRHa) could in theory be gender-affirming by preventing the development of unwanted secondary sex characteristics, this treatment option would be limited to pre- or peri-pubertal adolescents and likely have harmful effects. Here, we discuss the theoretical use of Selective Estrogen Receptor Modulators (SERMs) for non-binary people assigned male at birth (AMAB) who are seeking an androgynous appearance through partial feminization without breast growth. Given their unique range of pharmacodynamic effects, SERMs may represent a potential gender-affirming treatment for this population, but there is a lack of knowledge regarding their use and potentially adverse effects in this context.

Keywords: transgender, gender non-binary, gender dysphoria, hormone replacement therapy, selective estrogen receptor modulators

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INTRODUCTION

Trans individuals have a gender identity that is different from the sex they were assigned at birth, as opposed to cisgender people, whose gender identity corresponds with their assigned sex (1). Although some trans individuals identify as either male or female, others have a non-binary gender identity that does not accord with either of these traditional options. Whilst non-binary is a gender

Abbreviations: AFAB, assigned female at birth; AMAB, assigned male at birth; GnRHa, gonadotrophin releasing hormone agonists; SERM, selective estrogen receptor modulator.

identity in itself, it is also an umbrella term that encompasses any gender identity that is not exclusively male or female and may include people who identify as genderfluid, gender non-conforming, transmasculine, transfeminine or agender amongst others. Non-binary individuals in recent studies constitute more than 25% of the trans population (1–3).

Like trans people with binary identities, those with a non-binary gender identity frequently face social difficulties, including social exclusion at school/work and feelings of isolation. As a result, these individuals often experience poor mental health that is worse than those with binary identities (1, 4). In studies using self-report surveys, non-binary people were less likely to have family support, while significantly more likely to feel isolated, report poorer mental health, and consider suicide than trans people with binary identities (1, 2, 4, 5). Moreover, non-binary individuals frequently encounter a lack of understanding within society regarding their gender identity, including from health professionals (6).

A common reason for poor mental health in trans (including non-binary) individuals is gender dysphoria (GD), which is the distress associated with having a gender identity that differs from one's sex assigned at birth (7). Non-binary people seeking assistance to reduce their GD now constitute a significant proportion (10-30%) of referrals to paediatric and adult gender clinics (1, 8). Given the growing population of non-binary individuals seeking gender-affirming clinical care and the highly vulnerable nature of this population (9), it is important to have appropriate medical options to help them achieve a body they regard as consistent with their identity and to reduce their GD.

Medical treatment with gender-affirming hormones, such as testosterone and estradiol may be appropriate for some non-binary individuals who wish to masculinize or feminize respectively (10, 11). However, other non-binary individuals may seek an androgynous body that is neither overtly male nor female, and there is a lack of medical treatment options specifically tested or approved for use in this group to prevent the development of secondary sex characteristics on an ongoing basis (12). In this article, we therefore consider Selective Estrogen Receptor Modulators (SERMs) as a potential treatment option for non-binary people assigned male at birth (AMAB) who wish to partially feminize without undergoing breast development.

CURRENT TREATMENT OPTIONS FOR NON-BINARY TRANS PEOPLE

Currently, medical transition options for trans people vary depending on an individual's pubertal stage. For trans adults, treatment options to induce secondary sex characteristics consistent with a person's gender identity include gender-affirming hormone therapy and surgical interventions (10, 11). Gender-affirming hormone therapy may involve testosterone for trans people assigned female at birth (AFAB) and estradiol and anti-androgens for trans people AMAB (10, 11). For trans adolescents in early/mid puberty, gonadotropin-releasing hormone agonists (GnRHa), commonly known as "puberty blockers", may be given to suppress puberty before or in conjunction with the use of gender-affirming hormone treatment (10, 11, 13, 14). The effects of GnRHa are considered reversible insofar as stopping treatment allows puberty to resume (10, 11, 13, 14). While many effects of testosterone and estradiol are reversible, some including voice deepening and breast development are not (10, 13). The availability of irreversible surgical interventions for adolescents depends on the age and needs of the individual. Individuals in some jurisdictions are able to access chest reconstructive surgery but rarely genital surgery before the age of 18 years (13).

Under current treatment schema, non-binary people who wish for an androgynous body have limited treatment options. For those AFAB, low-dose testosterone is being used empirically with the aim of reducing feminine features by helping to re-distribute adipose tissue and deepen the voice (12). Such effects may be difficult to selectively titrate while avoiding other potentially unwanted masculinizing changes such as facial hair growth. In addition, chest dysphoria is regularly experienced by trans individuals AFAB, and chest reconstructive surgery can enable removal of unwanted breast tissue for those who have progressed through puberty (13). Those AMAB may choose to use low-dose estradiol to partially feminize, though the widespread effects of estradiol could potentially cause unwanted feminising changes, such as breast growth (12). For non-binary adolescents who present in early puberty, long-term GnRHa use may provide a theoretical solution to maintain an androgynous appearance without any secondary sex characteristics (15). However, such a strategy carries substantial risks as described below, which limits its use for this purpose.

MAINTAINING ANDROGYNY *VIA* LONG-TERM PUBERTY SUPPRESSION

Pubertal suppression with GnRHa blocks the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, preventing production of testosterone and estradiol from the testes and ovaries respectively (16). Without testosterone or estradiol, sex-specific secondary sex characteristics, such as breast development in those AFAB or voice deepening in those AMAB, do not progress (16). In this way, for many trans adolescents, puberty blockers can help prevent an exacerbation of GD and the attendant anxiety that often accompanies typical progression through puberty (17).

However, since the pubertal increase in either testosterone or estradiol levels is important for the development and function of other body systems, use of GnRHa in trans adolescents can also cause unwanted outcomes. For example, pubertal sex hormone is essential for healthy bone development, with testosterone and estradiol acting as key promoters of new bone deposition and increased bone mass (18). Consistent with this, use of GnRHa in trans adolescents is associated with reduced bone mineral density (BMD) (19, 20), and the potential functional impact of this on

bone quality and fracture risk in adulthood remains unknown (21). Long-term GnRHa therapy from early puberty would also be expected to inhibit typical pubertal development of reproductive potential (15, 22); this effect is considered likely reversible but has not been directly studied (22). Finally, significant brain and cognitive development occurs during adolescence. Although it is unclear to what extent pubertal hormones positively influence this development, it stands to reason that puberty suppression might impact this process (16, 17, 21).

For trans adolescents who receive puberty blockers and wish to subsequently feminize or masculinize their bodies, use of gender-affirming hormones is likely to ameliorate some of these concerns associated with GnRHa use, though further study is required (14, 23). However, for non-binary adolescents who wish to undertake *long-term* puberty suppression to maintain an androgynous body, these risks will presumably be exacerbated. Although such an approach might be ethically justified in some circumstances (15, 24), most physicians would be unlikely to prescribe puberty blockers as independent, long-term treatment (25). In addition, this treatment would not be effective for non-binary individuals who have completed puberty. Having alternative therapeutic options in such cases would therefore be valuable.

SERMs: A POSSIBLE SOLUTION?

Overview

SERMs are chemically diverse compounds that bind to alpha or beta subunits of the estrogen receptor and produce both agonistic (i.e., estrogenic) and antagonistic (i.e., anti-estrogenic) effects within different cell types and tissues (**Table 1**) (23, 43). With the advent of newer drug designs in recent decades, SERMs are categorized into three generations. In general, each generation has estrogenic and anti-estrogenic effects on different parts of the body. In reality, many effects are drug-specific, still debated, or unknown (23). Across the first, second and third generations of SERMs, the most popular approved treatments are tamoxifen, raloxifene (RLX), and lasofoxifene respectively (29). Tamoxifen is most commonly used to treat pre-menopausal breast cancer (23). RLX is widely approved for post-menopausal osteoporosis (35). Lasofoxifene has been approved in the EU for both osteoporosis and vaginal atrophy (45) and is currently being reviewed by the FDA as a treatment for breast cancer (48).

Theoretical Use of SERMs as a Treatment for Non-Binary Individuals AMAB

Given their tissue-specific antagonistic and agonistic effects, SERMs could potentially be suitable either as an independent or combined treatment modality for the gender-affirming care of non-binary individuals AMAB who desire partial feminization without breast growth. Specific SERMs (**Table 1**) have been shown to inhibit breast and endometrial tissue growth, while exhibiting beneficial estrogenic effects on bones in pre- and post-menopausal cisgender women (35, 43). These effects are not well-studied in people AMAB. Theoretically, a SERM with anti-estrogenic effects on breast but estrogenic effects on bone, fat composition, and skin might offer appeal as a hormone treatment for a non-binary person AMAB who desires an androgynous body.

While SERMs could be used independently for non-binary adults AMAB to induce partial feminization, SERMs alone would not be expected to block unwanted pubertal masculinization in non-binary adolescents AMAB. Androgen blockade would still be required in this younger population. In the following sections, we explore the theoretical use of SERMs in this context, extrapolating from what is known about their use in other situations.

SERMs May Promote Feminine Body Shape and Changes in Skin

Similar to estradiol hormone treatment, SERMs have been found to promote changes in body composition and skin that may be desired by non-binary people AMAB. In post-menopausal cisgender women, studies have found RLX to be an estrogen receptor agonist in adipose tissue, promoting shifts from android to gynoid fat patterns (40) and increasing fat-free mass and total body water (41). In this way, RLX may contribute to a more feminine body shape. In other studies of post-menopausal cisgender women, RLX has been shown to increase skin elasticity (31) and stimulate collagen biosynthesis (32). Thus, RLX is likely to promote softer, more stereotypically feminine

TABLE 1 | Differential effects of GnRHa, SERMs, and Estrogen.

Treatment Type	Breast Tissue	Vertebral Bones	Non-Vertebral Bones	Body Composition/Gynoid Fat Distribution	Skin	DVT Risk
GnRHa	– (13, 21)	– (19–21)	– (19–21)	MR (21, 26)	UN	+ (27)
Estradiol	+++ (28)	+++ (28, 29)	+++ (28, 29)	+ (30)	++ (31–33)	+ (28, 34)
Tamoxifen (SERM Gen 1)	– (29, 35)	+ (29)	+ (35, 36)	UN	– (33)	++ (29, 35, 37)
Raloxifene (SERM Gen 2)	– (29, 35)	++ (29, 35, 38)	0 (29, 35, 38, 39)	+ (40, 41)	+ (31, 32)	++ (35)
Lasofoxifene (SERM Gen 3)	– (35, 42)	+++ (43, 44)	++ (35, 42, 45)	UN	UN	++* (35, 42, 46)

–, antagonistic effect; 0, neutral effect; +, mild agonistic effect; ++, moderate agonistic effect; +++, major agonistic effects; UN, unclear/unknown; MR, mixed results.
**Literature indicates increased DVT risk but lowered risk of heart disease and stroke associated with lasofoxifene (26, 47).*

skin. Though these changes may assist in the partial feminization of a non-binary individual AMAB, the effects of SERMs on skin and body composition are not widely studied outside of the population of post-menopausal cisgender women and further research is needed.

SERMs Can Inhibit Breast Tissue Growth

Most SERMs inhibit the effects of estradiol on breast tissue growth and consequently are used to treat estradiol-responsive breast cancers (23, 49). Historically, tamoxifen has been the most commonly utilized SERM for treatment of metastatic, estrogen receptor-positive (ER+) cancers, where it is effective in lowering recurrence and mortality rates (23). Though not as commonly used for breast cancer treatment, newer SERMs such as RLX are also anti-estrogenic in breast tissue and deter breast cancer growth (35).

Consistent with their anti-estrogenic effect on breast tissue, SERMs such as tamoxifen have been reported as an effective alternative to surgery to treat persistent gynaecomastia in cisgender adolescent and adult males (49, 50). They have also been shown to improve symptoms associated with juvenile breast hypertrophy in adolescent cisgender females (51, 52). Although these studies involved small numbers of patients, they suggest that SERMs could help avoid breast growth in non-binary people AMAB who desire some feminization without breast development.

SERMs and Bone Health

While promoting partial feminization without breast growth, SERMs have pro-estrogenic effects on bones and could also theoretically mitigate negative bone effects associated with GnRHa and/or anti-androgen use in non-binary adolescents and adults AMAB. A variety of SERMs have now been approved for treating osteoporosis in adults (23, 43) based upon their ability to improve BMD and/or fracture risk by up to 55% (39) in post-menopausal cisgender women. However, whether this occurs in people AMAB who may also be androgen deficient is unknown.

Though tamoxifen, RLX and lasofoxifene have all been associated with increases in BMD in post-menopausal cisgender women, there may be important differences between different generation SERMs in terms of bone health. For instance, only RLX and lasofoxifene have shown fracture benefit. Moreover, while lasofoxifene reduces the incidence of both vertebral and non-vertebral fractures (45), RLX was found to reduce the incidence of vertebral but not non-vertebral fractures (38, 39). It should also be noted that the reduction of fracture risk (particularly at the hip) for SERMs is less than that of bisphosphonates. As such, SERMs are frequently reserved for use in osteoporosis under specific scenarios (i.e. for younger post-menopausal cisgender women or those with a family history of invasive breast cancer) (43).

Importantly, the positive bone health effects of SERMs in post-menopausal cisgender women cannot be directly extrapolated to gender non-binary young people AMAB. Post-menopausal cisgender women have already attained peak bone mass (PBM) in early adulthood (53), and it is the physiological estradiol deficiency that causes post-menopausal declines in BMD (54). In contrast, adolescence is associated with a significant increase in BMD directly resulting from effects of pubertal hormones. Adolescents exhibit an average annual accrual of 6-10% and an overall doubling from childhood through the mid 20s (55). Hence, use of SERMs to potentially promote bone health in trans adolescents AMAB would be physiologically distinct from their use in post-menopausal cisgender women. Specifically, while the latter seeks to maintain BMD that has already peaked and has strong supporting evidence, the former aims to promote the attainment of PBM which is a context in which SERMs are untested. Thus, it is unclear to what extent – if at all – SERMs increase BMD during adolescence and how PBM would be affected.

Indeed, there is evidence that questions the extent of SERMs' pro-estrogenic effects on bones. For example, in a three-year prospective study of premenopausal cisgender women treated with tamoxifen for breast cancer, the average annual lumbar spine BMD fell 1.4%, compared to a 0.2% increase in those who received a placebo. These results prompted the authors to conclude that tamoxifen can act as a partial competitive antagonist of endogenous estradiol to cause increased bone remodelling (56). Similarly, another study on post-chemotherapy tamoxifen usage in cisgender women with breast cancer found that premenopausal patients experienced a 4.6% decrease in their baseline BMD compared to a 0.6% gain in BMD in those not on tamoxifen. However, it should be noted that this result may have been confounded by the fact that those who received tamoxifen were from the outset inherently different to those who did not (based on hormone receptor status of their tumors) (57). While both of these studies focused on tamoxifen use in adults with breast cancer, if similar findings held true in healthy adolescents with SERMs, the use of SERMs might in fact be detrimental to the bone health of trans young people.

Hence, caution is required. With no studies having assessed bone outcomes in trans people using SERMs, further research is required, including in those AMAB. Thus far, pre-clinical data has shown that lasofoxifene prevented bone loss by inhibiting bone turnover related to aging and low serum testosterone levels in male rats (58). In addition, a study of cisgender men with prostate cancer found that RLX prevented harmful effects of GnRHa on bone, including bone loss (59). While the use of SERMs in this context might arguably be the most directly analogous to their potential use in trans people AMAB, clearly further assessment of the skeletal effects of SERMs in adolescents and adults AMAB are required.

Effects of SERMs on Other Body Systems

Given that significant brain maturation occurs during adolescence, testosterone and estradiol may potentially facilitate adolescent brain development and functioning. It is unknown whether the absence of sex steroids secondary to GnRHa use in trans adolescents has any cognitive effects (17). Similarly, the effects of SERMs on brain development and function are uncertain. *In vitro* studies have suggested that SERMs promote synaptic transmission, increase axonal growth, and facilitate the regeneration of damaged synapses (60, 61). *In vivo* use of tamoxifen in animal models and cisgender women with breast cancer is associated with improved cognitive function (62, 63).

The relevance of such findings for the use of SERMs in non-binary individuals AMAB is unclear.

Among cisgender males, lower sex hormone concentrations in adulthood have also been associated with an increased risk of cardiovascular disease (64, 65). Interestingly, a recent study of >8000 60-80 year-old post-menopausal cisgender women receiving daily lasofoxifene showed a 32% reduction in major coronary events over five years compared to hypogonadal post-menopausal cisgender women (45). While these findings suggest that SERMs might promote cardiovascular health, such effects again cannot be directly extrapolated to non-binary people AMAB, given likely different mechanisms underlying their cardiovascular risk profile.

Health Risks of SERMs

Common side effects of SERMs include hair loss, nausea, mood disturbance, and hot flashes (23, 43). In addition, long-term use of SERMs have been associated with several other risks, all of which are important to consider when balancing their potential use as a gender affirming treatment.

First, as previously observed with estrogen replacement therapy for postmenopausal cisgender women (34), an increased risk of thromboembolic events has been associated with SERM use. RLX has been shown to increase the risk of deep vein thrombosis by up to 54% and the risk of pulmonary embolism by 91% (66). Similar data is available for tamoxifen (37). Whether the rates of stroke and myocardial infarctions are also increased remains unclear, with previous studies showing inconsistent results (29, 67, 68).

Secondly, long-term SERM use has been associated with hypertriglyceridemia and hepatic steatosis (69), as well as altered glucose metabolism and increased risk of diabetes (70). In a 12-year longitudinal study involving over 22,000 cisgender women with breast cancer, a 31% increased diabetes risk was observed in tamoxifen users versus non-users (71). Notably, the majority of SERM-based metabolic studies have been conducted on cisgender women with and without breast cancer (69). Whether similar metabolic effects would occur in those AMAB is unclear.

Finally, SERM use can increase testosterone production in those AMAB, owing to their antagonistic actions at the estrogen receptor within the hypothalamus and pituitary. Specifically, tamoxifen was found to increase serum FSH, LH and testosterone levels in cisgender men by less than 2-fold, while raloxifene showed only minimal increase (47). The clinical relevance of such changes is unclear but, may cause or further exacerbate unwanted masculinization.

CLINICAL IMPLICATIONS

Whilst SERMs should not be routinely recommended as treatment options, clinicians may encounter scenarios where SERMs may be requested by patients AMAB who desire feminization of skin or fat distribution without breast growth. Indeed, we are aware of SERMs already being used anecdotally by adults in this context. For patients wanting to use SERMs to affirm their gender, the lack of evidence for efficacy or safety must be highlighted, and there should be a clear explanation that treatment is experimental. To provide informed consent, individuals need to understand the selective differential effects of SERMs, their potential lack of benefit, and the risk of adverse effects. If a shared decision between patient and doctor is made to trial the use of a SERM, then clear goals of treatment (e.g., fat redistribution) over a pre-specified timeline (e.g., 3-6 months) should be determined. If treatment goals are not achieved, treatment should be ceased. Given Raloxifene is the only SERM with evidence for agonistic effects on fat and body composition (40, 41), it would appear to be the most suitable choice at present and would ideally be used in the context of a prospective research study. Consistent with this, clinical trials – ideally across multiple centers to aid in patient recruitment – are required to provide greater evidence regarding the potential benefits and harms of using SERMs in non-binary individuals.

CONCLUSION

In the clinical setting, increasing numbers of non-binary individuals are seeking gender-affirming hormone therapy, and some desire androgynous physical characteristics to align with their gender identity. This can pose challenges in tailoring treatment options, and a lack of well-defined therapeutic pathways leaves non-binary people at risk of persistent GD and ongoing vulnerability to adverse mental health outcomes. SERMs may be a theoretically attractive option for non-binary people AMAB who desire partial feminization without breast growth. However, there is a dearth of evidence regarding the safety and efficacy of SERMs in this context. An individualised, shared decision-making approach to care is therefore needed when considering the use of SERMs in non-binary people, with acknowledgement of their experimental nature, significant potential risk and need for further research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JX performed the relevant literature review, screened studies for inclusion, extracted relevant information from the included articles, drafted the initial manuscript, and revised the manuscript. KP conceptualised the manuscript and, together with LN, AC, MO'C, and SZ reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Feminizing Hormone Therapy Prescription Patterns and Cardiovascular Risk Factors in Aging Transgender Individuals in Australia

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Context: The safety and efficacy of feminizing hormone therapy in aging transgender (trans) individuals is unclear. Current recommendations suggest transdermal estradiol beyond the age of 45 years, especially if cardiometabolic risk factors are present.

Objective: To evaluate feminizing hormone therapy regimens and cardiovascular risk factors in aging trans individuals.

Design: Retrospective cross-sectional analysis.

Setting: Primary care and endocrine specialist clinic in Melbourne, Australia.

Participants: Trans individuals on feminizing therapy for ≥6 months.

Main Outcomes Measures: Feminizing hormone regimens and serum estradiol concentrations by age group: (a) ≥45 years, (b) <45 years, and prevalence of cardiometabolic risk factors in individuals ≥45 years.

Results: 296 individuals were stratified by age group: ≥45 years (n=55) and <45 years (n=241). There was no difference in median estradiol concentration between groups (328 nmol/L *vs*. 300 nmol/L, p=0.22). However, there was a higher proportion of individuals ≥45 years treated with transdermal estradiol (31% vs. 8%, p<0.00001). Of those treated with oral estradiol, the median dose was lower in the ≥45 years group (4mg vs. 6mg, p=0.01). The most prevalent cardiometabolic risk factor in the ≥45 years group was hypertension (29%), followed by current smoking (24%), obesity (20%), dyslipidaemia (16%) and diabetes (9%).

Conclusions: A greater proportion of trans individuals ≥45 years of age were treated with transdermal estradiol. Of those who received oral estradiol, the median dose was lower. This is important given the high prevalence of cardiometabolic risk factors in this age group, however cardiovascular risk management guidelines in this demographic are lacking.

Keywords: transgender, aging, estrogen, estradiol, cardiovascular

INTRODUCTION

The intersection of aging and healthcare in transgender (trans) individuals is a novel area of research, and there is a paucity of data regarding the safety and efficacy of feminizing hormone therapy in older trans individuals. Important considerations in this demographic include estradiol dose, formulation, route of administration, target sex steroid concentrations and duration of treatment.

Of particular interest in trans individuals undergoing feminizing hormone therapy, is the reversal of the traditional sex difference in the prevalence of cardiovascular disease. In the cisgender population, the prevalence of cardiovascular disease is higher in men until the age of 75 years (1). However, in the trans population, individuals on feminizing therapy (presumed male at birth) have a greater incidence of cardiovascular mortality than those on masculinizing therapy (123 per 100,000 person years vs. 15 per 100,000 person years respectively) (1). The mechanisms underlying this discrepancy are poorly understood.

Retrospective studies suggest the risk of thrombosis in trans individuals undergoing feminizing hormone therapy is highest with ethinyl estradiol, which is no longer recommended (2). There is limited information regarding the thrombotic risk of different feminizing hormone regimens, and safety data for transdermal estradiol has therefore been extrapolated from the menopausal hormone therapy literature, but notably higher doses of transdermal estradiol are used for gender affirmation (3, 4). In fact, a preliminary study suggested no apparent difference in global coagulation assays in trans people using oral or transdermal estradiol formulations (5). Nonetheless, despite the limited data, current European Network for the Investigation of Gender Incongruence (ENIGI) guidelines recommend transdermal estradiol beyond the age of 45 years (6), with continuation of the lowest dose possible to maintain adequate feminization (or in line with individual treatment goals). This is particularly prudent in aging trans individuals with established cardiovascular disease, cardiovascular risk factors and/or a history of thromboembolism.

In this retrospective analysis of individuals on feminizing hormone therapy for ≥6 months, we aimed to evaluate prescribing patterns in older (age ≥45) versus younger (age <45) trans individuals, in the context of cardiovascular risk factors that emerge with aging. We hypothesized that there would be a reduction in estradiol dose, a shift from oral to transdermal route of administration, and reduced serum estradiol concentration with aging.

METHODS

A retrospective audit of consultations for gender dysphoria was performed across two gender clinics in Melbourne, Victoria, Australia. Firstly, Equinox Gender Diverse Clinic (an Adult Primary Care General Practice), and secondly, an Adult Endocrinology Specialist Clinic. Data were collected from consecutive new consultations between 1st January 2011 and 30th April 2020. The audit was approved by the Austin Health Human Research Ethics Committee (LNR/17/Austin/102) and Thorne Harbour Health (THH/CREP 19/015), and informed consent was not required given the retrospective nature of the study.

This cross-sectional analysis included trans individuals (including those with a binary and/or non-binary gender identity) on feminizing hormone therapy for ≥6 months, with sufficient documentation of estradiol dose, formulation, route and serum estradiol concentration at their most recent consultation.

The primary outcome of interest was feminizing hormone regimen and serum estradiol concentration by age group: (a) ≥45 years, (b) <45 years. We chose an arbitrary cut-off of 45 years given this is the age at which ENIGI recommend changing to transdermal estradiol to minimize venous thromboembolic risk (6). Cardiometabolic risk factors were collected in the ≥45 years group (hypertension, dyslipidaemia, diabetes, obesity and smoking history), as well as history of ischaemic heart disease, cerebrovascular disease, or venous thromboembolic disease.

Estradiol concentration was measured using immunoassays available as standard care for clinical decision-making and only laboratories accredited by the National Association of Testing Authorities (NATA) were used.

Statistical analyses were performed using STATA version 16.1 software (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LP). As data were not normally distributed, median (IQR) are presented. Differences between groups were tested using the Mann-Whitney U test or Kruskall-Wallis test. Fisher's exact test was used for the proportion of individuals treated with oral vs. transdermal estradiol by age group. A p-value of <0.05 was considered statistically significant.

RESULTS

Data were collected from 390 individuals of whom 296 had been treated with estradiol for at least 6 months and had adequate data available for analysis. Of these, 55 individuals were aged ≥45 years. Individuals treated with ethinyl estradiol (n=12) or anti-androgen monotherapy (n=3) were excluded from this analysis.

Median age of individuals was 26.3 (22.1, 38.6) years and duration of feminizing therapy 25.2 (10.7, 32.9) months. In all, 254 (85.8%) individuals were treated with oral estradiol. Median estradiol concentration achieved was 325 (233, 428) pmol/L.

Age ≥45 Years

Clinical and laboratory parameters are outlined in **Table 1**. Oral estradiol valerate was the most frequently prescribed formulation (58%), followed by transdermal estradiol (31%), and combination oral and transdermal estradiol (11%). There was no significant difference between groups in serum estradiol concentration achieved (322 pmol/L for oral estradiol, 293 pmol/L for transdermal estradiol, and 413 pmol/L for combination oral and transdermal estradiol, overall p=0.108). Box plots of serum

Abbreviations: BMI, body mass index; CPA, cyproterone acetate; MPA, medroxyprogesterone acetate; SHBG, sex hormone binding globulin; trans, transgender; WHI, Women's Health Initiative.

TABLE 1 | Clinical Characteristics (≥45 years age group).

	Overall (n = 55)	Oral estradiol (n = 32)	Transdermal estradiol (n = 17)	Combination therapy (oral and transdermal) (n = 6)	P-value[#]
Age (years)	52.8 (49.7, 58.3)	52.5 (47.6, 55.6)	59.1 (52.5, 63.1)	48.1 (46.0, 52.0)	0.014
BMI (kg/m^2)	27.1 (25.0, 30.1)	27.7 (25.5, 29.4)	29.6 (24.8, 34.1)	25 (24.3, 27)	0.358
Duration of feminizing therapy (months)	69.0 (45.9, 134.9)	78.3 (52.2, 168.9)	48.0 (23.8, 87.7)	132.6 (49.7, 240.5)	0.076
Estradiol dose	–	4 (4-6) mg	Patch 62.5 (50-100) mcg Gel 1 (1-1.25) mg	Oral 6 (2.5-8) mg Patch 50 (50-100) mcg Gel 1 (1-1) mg	–
Estradiol concentration (pmol/L)	300 (222, 414)	322 (233, 414)	292.5 (133.3, 361)	412.5 (281.8, 563.5)	0.108
SHBG (nmol/L)	77 (48, 107)	88 (71, 126)	46 (38, 63)	93 (62, 106)	0.005

Median (IQR) are shown. BMI, body mass index; SHBG, sex hormone binding globulin. [#]P-value from Kruskal-Wallis test.

estradiol concentration by estradiol formulation are shown in **Figure 1**.

There was no significant difference in median serum estradiol concentration when comparing individuals with or without previous orchidectomy and/or genitoplasty (264 nmol/L *vs.* 316 nmol/L respectively, p=0.55). Individuals with previous orchidectomy and/or genitoplasty were treated with lower oral estradiol doses (3.4 mg *vs.* 5.7 mg, p=0.003).

Trans individuals prescribed transdermal estradiol were older than those prescribed oral estradiol (59.1 years *vs.* 52.5 years respectively, p=0.03). A strong association was observed between sex hormone binding globulin (SHBG) concentration and route of estradiol administration, with a median SHBG concentration of 46 nmol/L in the transdermal estradiol group, compared to 88 nmol/L in the oral estradiol group (p=0.0012).

Cyproterone acetate (CPA) was the most commonly prescribed anti-androgen (*n*=15), followed by spironolactone (*n*=11) and goserelin (*n*=1), with a median serum testosterone concentration of 0.5 (0-4) pmol/L. Median dose of CPA and spironolactone were 25 (18.75-25) mg and 100 (50-150) mg respectively, and there was no significant difference in median serum testosterone concentration achieved (0.4 vs. 0.5 nmol/L respectively, p=0.09). Eight individuals (15%) were co-prescribed a progestogen; two individuals were treated with micronised progesterone, and six with medroxyprogesterone acetate (MPA).

The median body mass index (BMI) of all individuals was in the overweight range at 27.1 (25.0, 30.1) kg/m^2, whereas individuals treated with transdermal estradiol had median BMI of 29.6 (24.8-34.1) kg/m^2. Cardiometabolic risk factors are shown in **Table 2**. The most prevalent cardiovascular risk

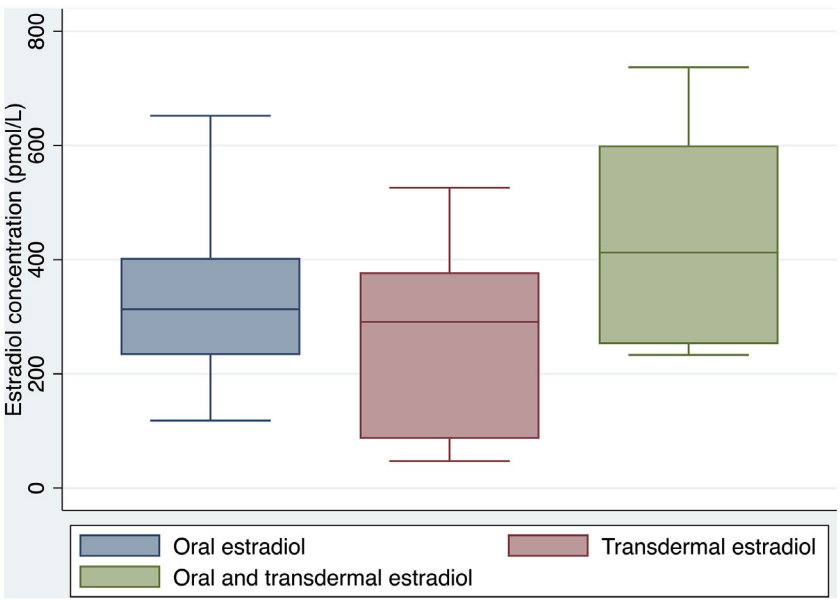

FIGURE 1 | Serum estradiol concentration by estradiol formulation (age ≥45 years).

TABLE 2 | Cardiometabolic risk factors (≥45 years age group).

	Overall (n=55)	Oral estradiol (n=32)	Transdermal estradiol (n=17)	Combination therapy (oral and transdermal) (n=6)	P-value[#]
Current smoker	13 (24%)	7 (22%)	4 (24%)	2 (33%)	0.810
Ex-smoker	12 (22%)	8 (25%)	2 (12%)	2 (33%)	0.455
Dyslipidaemia	9 (16%)	1 (3%)	8 (47%)	0 (0%)	0.0004
Diabetes	5 (9%)	2 (6%)	3 (18%)	0 (0%)	0.368
Hypertension	16 (29%)	8 (25%)	6 (35%)	2 (33%)	0.684
Obesity (BMI > 30kg/m^2)	11 (20%)	6 (19%)	4 (24%)	1 (17%)	0.885
IHD	3 (5%)	1 (3%)	2 (12%)	0 (0%)	0.489
CVA	2 (4%)	1 (2%)	1 (6%)	0 (0%)	0.999
VTE	1 (2%)	0 (0%)	1 (6%)	0 (0%)	0.418

[#]*P-value from Fisher's exact test for categorical variables. BMI, body mass index; IHD, ischaemic heart disease; CVA, cerebrovascular disease; VTE, venous thromboembolic disease*

factor was hypertension (29%), followed by current smoking (24%), obesity (20%), dyslipidaemia (16%) and diabetes (9%). Of note, there was a higher proportion of individuals treated with transdermal estradiol with a history of dyslipidaemia (p=0.0004).

Comparison to Group Aged <45 Years

Clinical and laboratory parameters of the <45 years group are outlined in **Table 3**. There was no difference in serum estradiol concentration achieved between the ≥45 and <45 years age groups (300 nmol/L *vs*. 328 nmol/L respectively, p=0.22) (**Figure 2**). However, there was a significant difference in the proportion of individuals treated with transdermal estradiol between the two age groups (31% in the ≥45 years group *vs*. 8% in the <45 years group, p<0.00001).

The median oral estradiol dose was 4 (4-6) mg in the ≥45 years group, which was lower than the median dose of 6 (4-8) mg in the <45 years group (p=0.01). Individuals in the <45 years group were more commonly prescribed a progestogen (32% *vs*. 15%).

DISCUSSION

In this retrospective cross-sectional analysis of feminizing hormone therapy regimens for trans individuals attending gender clinics in Australia, there was a higher proportion of individuals over the age of 45 years who were treated with transdermal estradiol compared to those aged less than 45 years (31% *vs*. 8%, p<0.00001). This is in keeping with the ENIGI Guidelines, which recommend transdermal estradiol in patients older than 45 years (6). There remain a considerable 58% treated with oral estradiol. Of those treated with oral estradiol, the median dose was lower in the ≥45 years group (4mg vs. 6mg, p=0.01), and the SHBG was higher (88nmol/L *vs*. 46nmol/L, p=0.004).

TABLE 3 | Clinical Characteristics (<45 years age group).

Age (years)	24.6 (21.5, 28.9)
BMI (kg/m^2)	24.2 (21.6, 28.5)
Duration of feminizing therapy (months)	23.8 (15.2, 32.4)
Estradiol dose	Oral 6 (4-8) mg
Estradiol concentration (pmol/L)	328 (237, 434)

Median (IQR) are shown. BMI, body mass index.

Comparison to Previous Studies

To our knowledge, there is no published data specifically evaluating feminizing hormone regimens and estradiol concentrations in older trans individuals. Previous studies that have enrolled older trans women have noted increased mortality from ischaemic heart disease in those aged 40-64 years (2), however they have not reported on estradiol dose, formulation, route and concentration with aging. Furthermore, current recommendations for aging trans women are predominantly expert opinion pieces (7), with many recommendations being extrapolated from the menopausal hormone therapy literature. Our study provides an insight into the prescribing patterns of experienced Australian transgender healthcare providers, in the context of cardiovascular risk factors that emerge with aging.

Current Guidelines for Feminizing Hormone Therapy in Aging Transgender Individuals

There is no evidence to guide continuation or cessation of feminizing hormone therapy in aging trans individuals (8). Recommendations are mainly speculative and reflect experience with hormone replacement in postmenopausal women. ENIGI guidelines recommend changing to transdermal preparations beyond the age of 45 years due to the 'first pass effect' of the liver and associated thrombotic risk of oral estrogens. Other expert groups suggest the lowest effective dose, with preference for transdermal formulation in those beyond 50-55 years, due to potential cardiovascular side effects becoming more apparent (7). Whilst ranges of estradiol are provided by some guidelines, these are based upon expert opinion and their applicability to older individuals is unclear (9, 10).

Complete discontinuation of hormones in aging trans individuals, especially in those who have undergone orchidectomy, may lead to profound bone loss (10). Additionally, in people who have not undergone orchidectomy, withdrawal of feminizing hormone therapy may result in virilization (8). As such, continuation of estradiol in an 'age-appropriate' dose beyond the age of 50 years is recommended (11).

In terms of our study population, 58% of patients over the age of 45 years were prescribed oral estradiol, with an additional 11% being prescribed combination oral and transdermal therapy estradiol. 31% were prescribed transdermal monotherapy. Whilst there was a significant transition from oral to

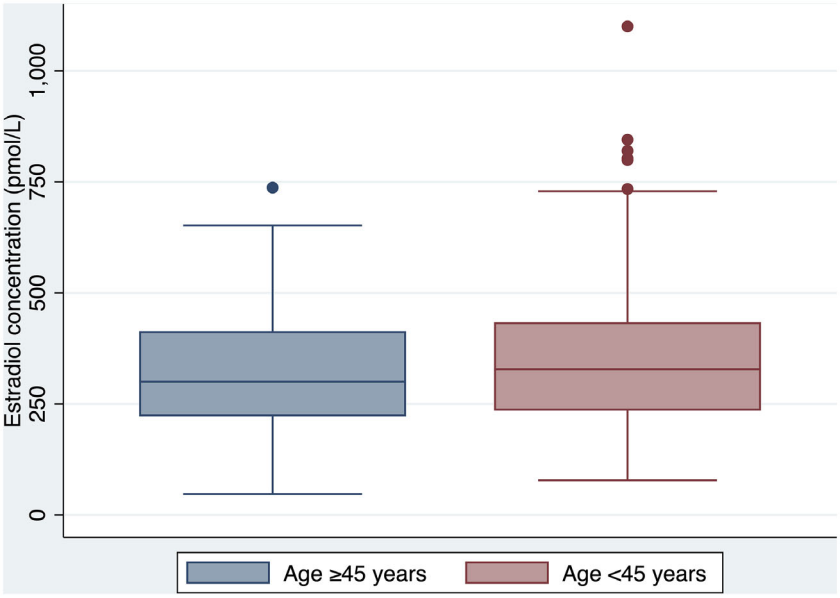

FIGURE 2 | Serum estradiol concentration by age group (≥45 *vs* <45 years).

transdermal therapy beyond the age of 45 years, preference for the oral route as first-line therapy may reflect patient/clinician preference or practical difficulties with transdermal patches, such as adherence issues due to excessive hair or sweat on the skin (12).

Overall, the risks and benefits of continuing versus withdrawing feminizing hormone therapy is an individualised discussion, and shared decision making is recommended (8). Unless there are clear contraindications, feminizing therapy should not be withheld or withdrawn from trans individuals based purely on age (7).

Extrapolations From the Menopausal Hormone Therapy Literature

No prospective, randomized controlled trials have been performed comparing oral versus transdermal estradiol in postmenopausal women, nor the trans population (12). A previous large population-based study of postmenopausal women demonstrated a similar increased risk of stroke when comparing oral estradiol (both high and low dose) to high-dose transdermal estradiol (>50mcg) (13). However, this risk has not been borne out in subsequent meta-analyses, which have suggested transdermal estrogens carry minimal or no thrombotic risk, even in women with a prior history of thrombosis (3, 4). Transdermal estrogens have negligible effects on haemostatic variables, which has led many authors to recommend this route in postmenopausal women with thrombotic risk factors (14).

This concept has been extrapolated to the trans population, however, notably trans people using feminizing hormones typically use far higher doses than cisgender women using menopausal hormone therapy (15). In fact, a study of 26 trans people on estradiol therapy demonstrated hypercoagulable global coagulation assay parameters compared with cisgender men with a shift towards cisgender female parameters (5). There was no difference between transdermal or oral routes of administration with both groups showing hypercoagulable global coagulation assay parameters. As such, the benefit of using transdermal estradiol in older trans women to minimise thrombosis risk factors is unclear. As a harm minimisation approach, as there is a potential dose-response relationship (12), the lowest transdermal dose that maintains feminization should be used.

The effect of estrone, the main metabolite of oral estradiol, on thrombin generation has also been posited as a potential mediator of VTE risk with oral estradiol therapy in postmenopausal women (16). Higher estrone concentrations have been demonstrated in trans women and non-binary adults taking sublingual estradiol compared to transdermal or injectable preparations (17). However, the role of monitoring serum estrone concentrations in individuals taking feminizing hormone therapy is yet to be determined and requires further investigation.

An expected finding is the higher SHBG concentration in those treated with oral estradiol (18). SHBG is elevated in the presence of estrogen, more so with oral estradiol than parenteral routes, due to first pass hepatic metabolism (19). SHBG has been postulated as a marker of estrogenicity and a potential surrogate indicator of VTE risk (19). The low prevalence of VTE in our study precluded analysis of this biochemical marker in relation to VTE risk, however future studies are warranted to further evaluate SHBG concentration in trans patients receiving feminizing hormone therapy (20).

Prevalence of Cardiovascular Disease in Transgender Individuals Receiving Feminizing Hormone Therapy

The relationship between feminizing hormone therapy and cardiovascular risk in trans women is complex and is influenced by many factors including estradiol formulation, route of administration, dose, and baseline cardiovascular status (21). In the general population, cisgender men have a cardiovascular survival disadvantage relative to cisgender women up until the age of 65-75 years (7). However, in the trans population, individuals on feminizing therapy have a greater incidence of cardiovascular mortality than individuals on masculinizing therapy. This survival disadvantage was demonstrated in a recent large cohort study (22), which highlighted an increased incidence of VTE and ischaemic stroke in trans women compared to both cisgender men and women.

Increased mortality from ischaemic heart disease has also been demonstrated in trans individuals aged 40-64 years receiving feminizing therapy for a median duration of 18.5 years (2). It should be noted that most cardiac events occurred in individuals treated with ethinyl estradiol (2), which is no longer recommended in consensus guidelines (10).

The risk of oral estradiol versus transdermal estradiol is less clear, as there have been no prospective studies comparing these two formulations. A previous cross-sectional study demonstrated an increased rate of myocardial infarction in trans women after a median duration of 6 years of feminizing therapy [prescribed estradiol formulations were transdermal estradiol (49.1%), estradiol valerate (42.5%) and ethinyl estradiol (3.2%)] compared to their cisgender counterparts (21). The mean age at time of myocardial infarction was 48 years, and the majority of trans women with cardiovascular events had one or more cardiovascular risk factors, mainly smoking.

A number of changes in surrogate cardiovascular risk markers occur after commencing feminizing hormone therapy, including increased weight, visceral fat, total body fat, reduced insulin sensitivity, and potentially increased systolic and diastolic blood pressure (7, 23). Endocrine Society guidelines suggest cardiovascular risk factors be treated as they emerge, in accordance with established guidelines (10), and other groups advocate managing hypertension, hypercholesterolaemia, diabetes and smoking before initiating feminizing therapy as a risk mitigation strategy (9, 21).

The underpinnings of the reversed sex difference in cardiovascular mortality are yet to be fully elucidated, particularly what degree of risk is genetically determined, and whether this risk is modified by changes in the sex steroid milieu (7). Further research is required to investigate possible mechanisms contributing to the increased prevalence of cardiovascular disease in trans women receiving feminizing hormone therapy.

Cardiovascular Risk Factors in Transgender Individuals Receiving Feminizing Hormone Therapy Compared to the General Australian Population

The most prevalent cardiovascular risk factor in our study was hypertension, followed by current smoking. Nearly 1 in 4 patients in the ≥45 years group were current smokers (24%). This is considerably higher than recent Australian population data highlighting a smoking prevalence of 16.9% in the general population in persons aged 45-54 years (males 19.3%, females 14.7%) (24). Smoking rates in trans individuals aged ≥45 in our study were also higher than a recent cross-sectional survey of Australian trans adults (current smoking 15%), noting that this survey reflected a younger demographic with a median age of 28 years (25).

Obesity was the third most prevalent cardiovascular risk factor in our study population. The median BMI in the ≥45 years group was in the overweight range 27.1 (25.0-30.1) kg/m^2. This is considerably higher than the median BMI of 24.4 kg/m^2 in a previous Belgian cross-sectional study evaluating 214 trans women with a median age of 43.7 years (21). However, the median BMI of the ≥45 years group was not considerably higher than the median BMI of Australians aged 45-54 years based on 2017-2018 Australian Institute of Health and Welfare data (26).

Almost half of the trans women in the transdermal estradiol group (47%) had dyslipidaemia, compared to just 3% of patients in the oral estradiol group (p=0.0004). A recent systematic review highlighted the effect of oral estrogens on increasing triglycerides (27), which may be one factor influencing the preference for prescribing transdermal therapy to older trans women with dyslipidaemia in our study.

Irrespective of the low prevalence of cardiovascular events in our study, the disproportionately high prevalence of cardiovascular risk factors highlights an at-risk population, and we therefore advocate for proactive and aggressive management of modifiable risk factors to mitigate the risk of developing overt cardiovascular complications.

Limitations

There are inherent limitations to this study given the retrospective cross-sectional design. These include missing data (duration of feminizing therapy 13/55, estradiol concentration 5/55, SHBG 7/55), lack of randomisation of estradiol formulation, and reliance on clinical records to determine prevalence of cardiovascular risk factors. Similarly, data regarding cardiovascular risk factors and cardiovascular disease were not collected in the <45 years age group. Potential confounders include prescriber preference for estradiol formulation, individual adherence with prescribed feminizing therapy, as well as adherence with any other prescribed therapy for cardiovascular risk reduction. We were unable to determine prescriber or patient reasons for choice of therapy.

We examined prescribing patterns across two clinics experienced in trans health care, an Adult Primary Care General Practice and an Adult Endocrinology Specialist Clinic, both located in Melbourne, Australia. Prescribing patterns were in accordance with National Guidelines (9), and are likely to reflect overall trends in Australia. It should be noted that oral and transdermal estradiol are available on the Pharmaceutical Benefits Scheme (PBS) in Australia, so prescribing patterns may vary between countries.

Estradiol concentrations reported represent a single point in time, acknowledging that there can be significant intraindividual variability between samples. Estradiol concentrations were also measured *via* different immunoassays as standard care. Furthermore, we did not have data describing feminine physical characteristics achieved by individuals.

There were small patient numbers treated with combination oral and transdermal estradiol therapy, however this is representative of feminizing hormone prescribing in Australia (12). Similarly, there were a limited number of individuals treated with estradiol for greater than 5 years, and the relationship between duration of therapy and prescribing patterns could be a consideration for future research. The prevalence of VTE and cardiovascular events were low, which can lead to imprecision of estimates, however this is one of the largest cohorts to date specifically evaluating feminizing hormone regimens and cardiometabolic risk factors in aging trans women.

CONCLUSIONS

A greater proportion of trans women ≥45 years of age are treated with transdermal estradiol, and of those who were not treated with transdermal therapy, the median oral estradiol dose was lower. This highlights a preference for transdermal therapy in aging trans women, however this is not universal given the lack of evidence comparing the safety of different routes of administration on thrombosis and cardiovascular risk. Well-designed prospective studies with larger subject numbers and longer duration of follow up are required to evaluate the safety and efficacy of modern feminizing hormone therapy regimens in trans individuals across their lifespan.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Austin Health Human Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MB and BN contributed to conception, design, statistical analysis and drafting of the manuscript, which was overseen by AC. All authors contributed to the article and approved the submitted version.

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Short-Term Effects of Gender-Affirming Hormone Therapy on Dysphoria and Quality of Life in Transgender Individuals: A Prospective Controlled Study

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Background: Gender affirming hormone therapy (GAHT), whilst considered the standard of care in clinical guidelines for the treatment of many transgender (trans) people is supported by low quality evidence. In this prospective longitudinal controlled study, we aimed to examine the effect of newly commencing GAHT on gender dysphoria and quality of life (QoL) over a 6 month period.

Methods: Adult trans (including those with binary and/or non-binary identities) people newly commencing standard full-doses of masculinising (n = 42; 35 = trans masculine, 7 = non-binary) or feminising (n = 35; 33 = trans feminine, 2 = non-binary) GAHT and cisgender participants (n=53 male, n=50 female) were recruited to participate in this longitudinal prospective study. This analysis of gender dysphoria measured by the Gender Preoccupation and Stability Questionnaire and QoL measured by the RAND Short-Form 36 Health survey at baseline, 3 and 6 months after commencement of GAHT was a prespecified secondary outcome. Dysphoria and QoL over time in those starting GAHT compared to cisgender comparison group matched for their presumed sex at birth is reported as the mean difference (95% confidence interval) adjusted for age.

Results: In trans people initiating masculinising GAHT, there was a decrease in gender dysphoria with adjusted mean difference -6.80 (-8.68, -4.91), $p < 0.001$, and a clinically significant improvement in emotional well-being [adjusted mean difference 7.48 (1.32, 13.64), $p = 0.018$] and social functioning [adjusted mean difference 12.50 (2.84, 22.15), $p = 0.011$] aspects of QoL over the first 6 months of treatment relative to the cisgender female comparison group. No significant differences were observed in other QoL domains. In trans people initiating feminising GAHT, there was a decrease in gender dysphoria [adjusted mean difference -4.22 (-6.21, -2.24), $p < 0.001$] but no differences in any aspects of QoL were observed.

Conclusions: In the short-term, our findings support the benefit of initiating masculinising or feminising GAHT for gender dysphoria. Masculinising GAHT improves emotional well-being and social functioning within 6 months of treatment. Multidisciplinary input with speech pathology and surgery to support trans people seeking feminisation is likely needed. Further longitudinal studies controlled for other confounders (such as the presence of social supports) contributing to QoL are needed.

Keywords: transgender, gender dysphoria, quality of life, testosterone, oestradiol

INTRODUCTION

Transgender individuals (trans) experience a gender identity (which may be binary or non-binary) different from that which they were presumed at birth, and often, but not always, this gender incongruency is associated with significant psychological distress, known as gender dysphoria (1, 2). Despite considerable progress made by the trans community over the past decade in regard to visibility and acceptance, trans people still face significant obstacles and prejudice (3, 4), social stigma and discrimination, leading to job and housing insecurity, verbal and physical violence, and barriers to accessing appropriate healthcare (3). Similarly, trans people experience alarmingly high rates of mental health disorders compared to the general population (5, 6). A recent study looking at self-reported mental health diagnoses within the trans community has indicated that 73% of trans adults have been diagnosed with depression in their lifetime, 67% have been diagnosed with anxiety, and 43% attempted suicide in their lifetime (3). In contrast, self-reported rates of depression, anxiety and suicide attempts for the general population are indicated at 11.6%, 26.3%, and 3.2% respectively (7). While transgender health research is still in its infancy, research thus far has indicated that gender-affirming interventions (gender counselling, gender confirmation surgery and hormone therapy), as well as sociocultural factors (external validation of one's gender, support from family and friends) are associated with improved well-being among transgender individuals (8, 9). Access to such healthcare can be challenging for some, and the largest contributing factor to this, as reported by trans individuals, is an overall lack of medical professionals who are experienced in the field (6, 9, 10). Other reported factors include: financial and socioeconomic status, and discrimination (10). Gender affirming hormone therapy (GAHT) is the current standard treatment for people who experience gender dysphoria (5). While there is a significant amount of expert-opinion based evidence available regarding the benefits of GAHT for trans people, the majority of existing research into gender dysphoria and quality of life (QoL) focuses on mental health outcomes following surgery, and there is scant clinical evidence available regarding the effects of GAHT alone (5), particularly in regard to gender dysphoria outcomes (11). Of the research that does exist, a beneficial role of GAHT has been consistently suggested (6, 11–22), however evidence quality is low, primarily consisting of uncontrolled cross-sectional and retrospective cohort studies, and only two studies specifically quantify changes in gender dysphoria following GAHT (11, 23, 24). Consequently, existing guidelines for the treatment of trans individuals contain broad recommendations based on expert-opinion which has been valuable in providing clinical care for the trans community but is also the subject of critiques from some clinicians (2, 5). This guideline ambiguity leads to varied interpretation of treatment recommendations amongst clinicians, and a lack of consistent training practices (5).

We aimed to assess the short-term effects (0-6 months) of newly commencing GAHT on the QoL and gender dysphoria experienced by trans individuals and compare these results with age-matched cisgender individuals of the same presumed sex at birth. Based on previous literature (11, 22–24), it was hypothesised that GAHT would be associated with improved QoL and reduced gender dysphoria for trans people relative to cisgender comparison groups of the same presumed sex at birth.

METHODS

Participants

A total of 77 trans (n = 35 initiating feminising GAHT, n = 42 initiating masculinising GAHT) and 103 cisgender (n = 53 male, n = 50 female) participants were recruited from online and local print advertisements, and primary or secondary care clinics specialising in trans health in Melbourne, Australia. Cisgender females and males in the community were individuals who responded to local advertisements for a study on bone health. Recruitment occurred between April 2017 – April 2020 for the primary outcome of bone microarchitecture, and gender dysphoria and QoL were prespecified secondary outcomes. The research study is registered with the ANZ Clinical Trials Registry ACTRN12617000584336. Trans individuals were included for the study if they were aged 18 years or over, newly commencing standard (full) doses of masculinising or feminising GAHT, and were able to provide written informed consent to the study and comply with study protocols. Cisgender individuals without medical conditions or medications that contributed to metabolic bone disease were included. Trans individuals were excluded if they had any contraindications to GAHT use, had previously used masculinising or feminising GAHT, or had a history of gender-affirming surgery. Exclusion criteria for all participants were the presence of metabolic bone disease or receiving therapy that affects bone (glucocorticoids, bisphosphonates, antiepileptic medication, use of HIV pre-exposure prophylaxis). People presumed to be menopausal (age > 50 years) (trans men and cisgender females) were

excluded. Baseline study visits occurred prior to or within 4 weeks of commencing GAHT. The research study is registered with the ANZ Clinical Trials Registry ACTRN12617000584336.

Ethics

Ethics approval was received from the Austin Health Human Research Ethics Committee (Austin/17/HREC/74) and all participants provided written informed consent.

Design

The study incorporated a prospective controlled observational study design. Trans participants newly initiating were divided into the following groups: masculinising GAHT or feminising GAHT. Comparison individuals were presumed sex at birth-matched cisgender males and females who were not undergoing hormone therapy and we had intended to match for age as closely as possible. Final statistical analyses were adjusted for age. A comparison group who were trans but not using GAHT were not recruited as trans community members deemed that it would not be ethical or acceptable to withhold GAHT for research purposes. Participants completed the following outcomes at baseline and 6 months.

Outcomes

Gender Dysphoria

Gender dysphoria was assessed using the Gender Preoccupation and Stability Questionnaire (GPSQ). The GPSQ has been validated in Australia for use as a tool to measure gender dysphoria within the transgender community (25) and was chosen for this study due to its ability to evaluate the effectiveness of gender-affirming interventions in both binary and non-binary transgender individuals. The questionnaire includes 14 multiple choice questions designed to measure the extent of an individuals' preoccupation with gender, and the stability of their own gender identity over the past 2 weeks (25). Each question had 5 possible answers, corresponding to a score of 1-5, with higher scores indicating higher levels of gender dysphoria. Total scores were calculated by taking the summation of all values. Total scores 28 were considered highly suggestive of clinical gender dysphoria, and a change in score of 11 points or more reliably indicated a change in the degree of gender dysphoria (25).

Quality of Life

QoL was measured using the RAND Short Form-36 (SF-36) Health Survey, a 36 item questionnaire which has been validated for use to assess 8 domains of QoL: physical functioning (relating to the extent of physical limitations), social functioning (relating to one's ability to participate in social activities), role limitations due to physical health (relating to the extent individuals were limited in work/activities due to physical health), role limitations due to emotional health (relating to the extent individuals were limited in work/activities due to mental health), pain (relating to physical pain), energy/fatigue (relating to one's energy levels/vitality), general health (relating to one's perceived physical wellbeing), and emotional wellbeing (relating to perceived mental wellbeing) (26). Each item was denoted a value of 0-100, with higher scores indicating a better health state. Scores were calculated by taking the averages from the values pertaining to each QoL domain, thus producing eight different total scores between 0-100 per visit (27). The SF-36 has been reliably used to assess the QoL of individuals in various populations and circumstances (26, 28, 29), including several that have assessed the QoL of transgender individuals on GAHT (20, 21, 30). Furthermore, it is a fast, inexpensive, and accurate tool designed to be self-reported by individuals in order to monitor and assess treatment outcomes (27, 28). The minimum clinically important difference for the SF-36 is typically in the range of 3 to 5 points which translates into a 0.09-0.28 effect size range (31).

Statistical Analysis

Age was not normally distributed and is presented as median (interquartile range). Descriptive statistics were otherwise presented as mean standard deviation (SD for continuous variables). Results were summarised as the mean difference [95% confidence interval (CI)] between groups (transgender versus comparison group) over time. Comparison between two groups at baseline was conducted using a two-sample independent t-test whenever a variable followed normal distribution, otherwise nonparametric Mann-Whitney test was used. Categorical variables at baseline were compared using Chi-squared test, or Fisher's exact test where cell counts are low. Linear mixed effects model was used to test the differences between groups across time, with a time by group interaction term and a random intercept for individual participant, assuming an unstructured variance-covariance structure. The interaction term coefficients were reported, which indicates the change in trend across time between groups (i.e., whether the groups follow the same pattern of change). All models were adjusted for age. All participants recruited into the study at baseline (n=180) were used in the linear mixed effects model, in which missing data were handled *via* maximum likelihood estimation. The significance level was defined as $P < 0.05$ (two-tailed).

RESULTS

Baseline characteristics for the trans participants and cisgender comparison group are summarised in **Table 1** for each group. The median age of the masculinising hormone therapy group was 24.0 years (22.0-29.3) and feminising hormone therapy group was 28.0 years (22.5-39.5) relative to cisgender females [27.5 years (24.0-37.5)] and cisgender males [31.0 years (25.0-41.0)] (**Table 1**).

Gender Dysphoria

Compared to the cisgender comparison groups, a significant reduction in gender dysphoria within the first 6 months of GAHT was observed for both the masculinising hormone [adjusted mean difference -6.80 points (-8.68, -4.91), $p < 0.001$] and feminising hormone [adjusted mean difference -4.22 points (-6.21, -2.24), $p < 0.001$] groups, as shown in **Table 2**.

TABLE 1 | Characteristics of trans participants and cisgender comparison group matched for presumed sex at birth.

		Masculinising GAHT group	Cisgender Females	*p* value	Feminising GAHT group	Cisgender Males	*p* value
General Characteristics	Total Count	42	50		35	53	
	Age Median (IQR)	24.0 (22.0-29.3)	27.5 (24.0-37.5)	0.028	28.0 (22.5-39.5)	31.0 (25.0-41.0)	0.651
Employment Status: N (%)	*Unemployed*	4 (10.5%)	8 (17.0%)	0.010^	8 (24.2%)	2 (4.1%)	0.018^
	Self Employed	2 (5.3%)	1 (2.1%)		1 (3.0%)	1 (2.0%)	
	Casual/Part-time Employment	21 (55.3%)	15 (31.9%)		13 (39.4%)	14 (28.6%)	
	Full Time Employment	8 (21.1%)	21 (44.7%)		9 (27.3%)	28 (57.1%)	
	Retired	0 (0.0%)	1 (2.1%)		2 (6.1%)	2 (4.1%)	
	Other	3 (7.9%)	1 (2.1%)		0 (0.0%)	2 (4.1%)	
Lifetime Mental Health Diagnoses: N (%)	*Depression*	25 (59.5%)	8 (16.0%)	<0.001	16 (45.7%)	4 (7.5%)	<0.001
	Anxiety	24 (57.1%)	8 (16.0%)	<0.001	14 (40.0%)	3 (5.7%)	<0.001

^*Global p-values have been reported for employment status and no post-hoc analyses were performed.*

Quality of Life

As shown in **Table 2**, individuals initiating masculinising GAHT showed a significant improvement in emotional well-being (mental health) [adjusted mean difference of 7.48 (1.32, 13.64), $p = 0.018$] as well as social functioning aspects of QoL [adjusted mean difference 12.50 (2.84, 22.15), $p = 0.011$] relative to cisgender female comparison group over the first 6 months of treatment. Improvements in both aspects were greater than the minimum clinically important difference (32). No significant differences were observed in the remaining 6 domains: physical functioning, role limitations due to physical functioning, role limitations due to emotional problems, energy/fatigue, bodily pain or general health score.

Individuals initiating feminising GAHT showed no significant changes in QoL over the first 6 months relative to the cisgender male group (**Table 2**).

DISCUSSION

We demonstrate in this prospective controlled study that in trans people initiating masculinising GAHT, that there is an improvement in gender dysphoria and a clinically significant improvement in emotional well-being and social functioning aspects of QoL over the first 6 months of treatment relative to cisgender female comparison group. In trans people initiating feminising GAHT, there is an improvement in gender dysphoria, but no differences in QoL as measured by the RAND SF-36 were observed.

Gender Dysphoria

The beneficial effects of GAHT on gender dysphoria have previously been discussed in relation to changes in psychological state, such as changes in body uneasiness (16, 17), social anxiety (6), and self-esteem (19), however specific changes in gender dysphoria have rarely been quantified. To our knowledge, this is the only longitudinal controlled analysis of gender dysphoria, and our findings of improved dysphoria in both the masculinising-hormone and feminising hormone groups relative to the cisgender comparison group over 6 months, are consistent with 2 previous longitudinal but uncontrolled studies which reported lower levels of gender dysphoria after starting GAHT as measured by the Utrecht Gender Dysphoria Scale (23) or the Gender Identity/Gender Dysphoria questionnaire (11). Notably, whilst our findings were statistically significant, the degree of change (4.2 – 6.8 points) is less than the minimum clinically significant difference of 11 points for GPSQ. This is likely related to the limited duration of 6 months follow up which may be insufficient to have a significant effect on an individual's physical characteristics and in turn, their gender dysphoria. Moreover, contributors to gender dysphoria are complex and GAHT alone may not impact on many other confounders such as social gender role recognition, exposure to discrimination and physical features unaffected by GAHT such as genitalia.

Quality of Life

We have demonstrated improved emotional well-being and social functioning aspects of QoL in trans people initiating masculinising GAHT over 6 months compared with cisgender females. Whilst no other controlled prospective studies have been performed, our findings are consistent with an Italian cohort study which followed trans men over the first 12 months of masculinising GAHT which showed improved QoL related to body image (22). Moreover, several cross-sectional studies in trans men which have all shown better QoL as measured by SF-36 in those using GAHT compared to those not on GAHT (20, 21, 33, 34). Such consistently positive changes in multiple studies may be reflective of the effectiveness of testosterone therapy in inducing masculinising physical characteristics, and significantly reducing dysphoria, to a greater magnitude than those initiating feminising hormone therapy.

In contrast, we did not demonstrate a significant improvement in QoL in trans women commencing feminising GAHT relative to the cisgender comparison group. Multiple cross-sectional studies examining QoL measured by SF-36 have been performed comparing trans women on GAHT compared with trans women not using GAHT. These have shown variable results with some studies finding worse QoL on GAHT (35),

TABLE 2 | Overall mean differences in GPSQ and QoL scores between the trans participants and cisgender comparison groups adjusted for age.

Characteristic	Masculinising GAHT Mean (SD)	*n*	Cisgender Females Mean (SD)	*n*	Adjusted Mean Difference (95% CI)	*p* value	Feminising GAHT Mean (SD)	*n*	Cisgender Males Mean (SD)	*n*	Adjusted Mean Difference (95% CI)	*p* value
Gender Preoccupation and Stability Questionnaire												
0 months	41.4 (7.3)	42	19.9 (3.4)	50			42.1 (7.4)	35	18.4 (3.8)	53		
3 months	36.7 (7.8)	38	19.6 (4.4)	49			38.1 (8.0)	34	18.6 (3.6)	52		
6 months	33.8 (8.1)	36	19.1 (3.4)	46	-6.80 (-8.68, -4.91)	**<0.001**	38.5 (8.4)	30	18.3 (3.6)	42	-4.22 (-6.21, -2.24)	**<0.001**
Physical Functioning Score												
0 months	62.6 (18.1)	42	78.8 (13.6)	50			59.2 (19.1)	35	81.8 (10.8)	53		
3 months	64.8 (15.2)	38	75.6 (16.2)	49			61.2 (20.0)	34	80.8 (12.2)	52		
6 months	65.8 (15.3)	36	75.5 (15.1)	46	8.10 (-2.47, 18.67)	0.133	58.1 (20.9)	30	82.8 (11.0)	42	-7.01 (-17.57, 3.55)	0.192
Role limitations due to physical health												
0 months	46.0 (38.9)	42	89.1 (24.4)	50			48.0 (39.5)	35	91.3 (22.1)	53		
3 months	54.8 (38.1)	38	81.8 (31.7)	49			62.2 (41.7)	34	87.4 (26.9)	52		
6 months	54.3 (36.0)	36	88.6 (29.3)	46	-0.67 (-13.03, 11.69)	0.915	50.6 (45.6)	30	94.9 (14.7)	42	-0.21 (-12.72, 12.30)	0.974
Role limitations due to emotional problems												
0 months	44.9 (19.1)	42	61.2 (21.9)	50			45.9 (16.3)	35	65.7 (18.1)	53		
3 months	48.7 (18.1)	38	62.4 (20.0)	49			46.8 (19.4)	34	65.1 (20.9)	52		
6 months	48.1 (18.2)	36	58.8 (22.2)	46	9.21 (-7.53, 25.96)	0.280	47.8 (18.4)	30	66.7 (18.7)	42	-1.83 (-18.93, 15.28)	0.834
Energy/Fatigue												
0 months	61.7 (20.2)	42	80.3 (15.0)	50			66.3 (15.9)	35	77.6 (16.4)	53		
3 months	67.1 (17.2)	38	81.4 (15.1)	49			67.3 (16.9)	34	72.6 (18.3)	52		
6 months	66.5 (21.0)	36	78.9 (16.9)	46	6.27 (-0.81, 13.35)	0.082	65.4 (15.4)	30	72.1 (19.1)	42	1.93 (-5.24, 9.10)	0.597
Emotional Well-Being												
0 months	77.6 (25.5)	42	87.0 (15.7)	50			88.0 (15.1)	35	87.3 (15.0)	53		
3 months	78.7 (23.9)	38	86.4 (15.6)	49			87.8 (12.7)	34	88.4 (15.0)	52		
6 months	80.8 (23.0)	36	86.1 (15.0)	46	7.48 (1.32, 13.64)	**0.018**	83.6 (11.9)	30	86.3 (15.1)	42	-2.54 (-8.76, 3.67)	0.421
Social Functioning												
0 months	84.4 (23.9)	42	97.0 (8.1)	50			89.9 (20.9)	35	89.3 (25.0)	53		
3 months	84.2 (23.4)	38	93.6 (17.6)	49			92.2 (11.3)	34	96.1 (11.4)	52		
6 months	88.7 (18.7)	36	93.2 (20.7)	46	12.50 (2.84, 22.15)	**0.011**	90.0 (17.2)	30	96.8 (7.3)	42	-7.14 (-16.90, 2.62)	0.151
Bodily Pain score												
0 months	81.0 (35.7)	42	95.7 (15.2)	50			95.0 (13.3)	35	95.0 (15.2)	53		
3 months	85.8 (32.6)	38	92.6 (21.3)	49			89.2 (23.4)	34	90.6 (24.6)	52		
6 months	81.5 (35.8)	36	96.6 (13.4)	46	4.83 (-2.37, 12.02)	0.188	90.7 (22.1)	30	90.9 (23.2)	42	-4.01 (-11.20, 3.17)	0.272
General Health score												
0 months	64.6 (22.9)	42	91.6 (13.2)	50			72.5 (23.0)	35	93.5 (11.9)	53		
3 months	72.1 (23.8)	38	87.8 (19.5)	49			71.7 (19.9)	34	90.6 (17.1)	52		
6 months	72.2 (26.0)	36	87.2 (20.1)	46	4.09 (-1.43, 9.62)	0.146	66.2 (29.0)	30	95.1 (9.3)	42	2.40 (-3.14, 7.94)	0.395

Mean differences are adjusted for the age of the participant and all participants at baseline were used in the linear mixed effects model, in which missing data were handled via maximum likelihood estimation. Gender dysphoria was measured by the Gender Preoccupation and Stability Questionnaire, with lower scores indicating less gender dysphoria. The remaining domains are part of the RAND SF36 Health Survey whereby higher scores indicate better QoL.
Bold indicates statistically significant finding.

better (20, 34, 36) or no change (30, 37). Whilst the SF-36 is widely used, it is unclear which measure is most appropriate in trans women. Utilising the WHO QoL questionnaire, *Manieri et al.* did find improved overall QoL in trans women over the first 12 months (22). Notably our relatively shorter follow-up over 6 months may be insufficient for feminising GAHT to have maximal effect, and whilst dysphoria significantly improved, many physical characteristics, such as voice pitch and bony structure, which may contribute to social functioning or gender role recognition are unchanged with GAHT alone and multidisciplinary support of gender transition (i.e., with speech pathology, surgery) for trans people seeking feminisation may be needed.

Limitations

There are multiple limitations to our study. The short follow-up time of 6 months is likely insufficient to gain a complete and thorough understanding of GAHT's psychological effects, however we were interested in short-term effects of GAHT. Furthermore, we did not recruit a comparison group who

identified as trans. A controlled trial in trans people randomised to GAHT or no GAHT would allow the best understanding of GAHT's effect on gender dysphoria and QoL, however the conduction of such a trial is considered by many trans community members to be unethical, given the existing difficulties in accessing healthcare experienced by many who desire GAHT. As such, a cisgender comparison group were used. We used the locally developed GPSQ to measure gender dysphoria, although this has not been validated as a tool to measure changes in dysphoria over time.

However, this is the only prospective controlled study examining gender dysphoria and QoL in trans individuals newly commencing GAHT, thus validation from future studies and longer term follow up is required.

CONCLUSIONS

In the short-term, our findings support the benefit of initiating feminising or masculinising GAHT on gender dysphoria. Masculinising GAHT improves emotional well-being and social functioning within 6 months of treatment however there are many other confounders which can contribute to gender dysphoria or QoL other than the changes in physical characteristics induced by GAHT. These confounders include the presence of dysphoria towards one's voice, chest or genitalia which may not be relieved with GAHT, the presence of a supportive home, school or work environment, relationship status or sexual function. Multidisciplinary input to holistically support trans people, particularly those seeking feminisation will likely be of benefit. Further longitudinal studies controlled for confounders contributing to QoL will provide greater insights on the benefit of GAHT specifically on gender dysphoria.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the Austin Health Human Research and Ethics Committee (HREC/17/Austin/74). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, IB, JDZ, and AC. Methodology, LF, IB, JZ, and AC. Investigation, LF, IB, and SL. Formal analysis, LF, IB, SL, and AC. Writing – original draft, LF and AC. Writing – review and editing, LF, IB, SL, JZ, and AC. Funding acquisition, AC. Supervision, AC. All authors contributed to the article and approved the submitted version.

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Epigenetics Is Implicated in the Basis of Gender Incongruence: An Epigenome-Wide Association Analysis

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Introduction: The main objective was to carry out a global DNA methylation analysis in a population with gender incongruence before gender-affirming hormone treatment (GAHT), in comparison to a cisgender population.

Methods: A global CpG (cytosine-phosphate-guanine) methylation analysis was performed on blood from 16 transgender people before GAHT vs. 16 cisgender people using the Illumina© Infinium Human Methylation 850k BeadChip, after bisulfite conversion. Changes in the DNA methylome in cisgender vs. transgender populations were analyzed with the Partek® Genomics Suite program by a 2-way ANOVA test comparing populations by group and their sex assigned at birth.

Results: The principal components analysis (PCA) showed that both populations (cis and trans) differ in the degree of global CpG methylation prior to GAHT. The 2-way ANOVA test showed 71,515 CpGs that passed the criterion FDR $p < 0.05$. Subsequently, in male assigned at birth population we found 87 CpGs that passed both criteria (FDR $p < 0.05$; fold change $\geq \pm 2$) of which 22 were located in islands. The most significant CpGs were related to genes: *WDR45B, SLC6A20, NHLH1, PLEKHA5, UBALD1, SLC37A1, ARL6IP1, GRASP,* and *NCOA6*. Regarding the female assigned at birth populations, we found 2 CpGs that passed both criteria (FDR $p < 0.05$; fold change $\geq \pm 2$), but none were located in islands. One of these CpGs, related to the MPPED2 gene, is shared by both, trans men and trans women. The enrichment analysis showed that these genes are involved in functions such as negative regulation of gene expression (GO:0010629), central nervous system development (GO:0007417), brain development (GO:0007420), ribonucleotide binding (GO:0032553), and RNA binding (GO:0003723), among others.

Strengths and Limitations: It is the first time that a global CpG methylation analysis has been carried out in a population with gender incongruence before GAHT.

A prospective study before/during GAHT would provide a better understanding of the influence of epigenetics in this process.

Conclusion: The main finding of this study is that the cis and trans populations have different global CpG methylation profiles prior to GAHT. Therefore, our results suggest that epigenetics may be involved in the etiology of gender incongruence.

Keywords: DNA methylation, epigenetics, gender dysphoria, gender identity, gender incongruence

INTRODUCTION

Sexual development in mammals begins at conception, when the sex chromosome pair is determined as XX or XY. Later, the biological sex will imply differences in gonadal development, hormonal environment, sexual behavior, as well as other physical and behavioral differences. The current hypothesis about brain sexual development points to the existence of a complex "mosaic" model in the mammalian brain with a diversity of mechanisms involved, that allows a variable degree of masculinization/feminization within the brain (Joel et al., 2020).

But in humans, it is possible to differentiate between sex and gender. Whereas, gender identity could be defined as one's innermost concept of self as male, female, a blend of both or neither (American Psychological Association, 2012; Gómez-Gil, 2019) that could be coincident or not, with the sex assigned at birth. According to this concordance between sex and gender, we can differentiate into "cisgender" or "transgender" people, respectively (Polderman et al., 2018). Gender incongruence (GI) as per International Classification of Diseases ICD-11 (World Health Organization [WHO], 2018) is characterized by a pronounced and persistent incongruence between the individual's experience of gender and their sex assigned at birth.

According to the latest research, the origin of GI is complex and multifactorial. It might be associated with neurodevelopmental processes of the brain as well as genetic and epigenetic factors. With regards to the neuroanatomy, whereas post-mortem histological studies showed feminization of the central region of the bed nucleus of the stria terminalis in trans women (Zhou et al., 1995), more recent structural MRI studies indicated different brain phenotypes in trans women, trans men, cis women, and cis men (Guillamon et al., 2016; Kreukels and Guillamon, 2016; Nota et al., 2017; Baldinger-Melich et al., 2020; Mueller et al., 2021).

Paralleling the brain structural research, studies have been searching for a genetic component of GI. Some authors found variations in the DNA sequence of the androgen (AR) and estrogen (ERs, α and β) (Henningsson et al., 2005; Hare et al., 2009; Fernández et al., 2014a,b, 2016, 2018, 2020a; Cortés-Cortés et al., 2017; Foreman et al., 2019) that could hypothetically modulate the sensitivity of the nuclear steroid receptors. Furthermore, since AR and ER (α and β) are, at the same time, hormonal receptors and transcription factors, the modulation of gene expression via activation of AR and ERs by their ligands and coactivators, could be another presumptive mechanism underlying GI (Fernández et al., 2021; Ramírez et al., 2021).

Epigenetics offers an interesting complement to genetic studies because it reflects the interconnection between genes and the environment and could be a mechanism underlying GI given its sensitivity to environmental stimuli. Moreover, it could be possible to detect the capacity of the GAHT to modify gene expression and their stability over time.

DNA methylation (DNAm), which is the most stable and widely studied epigenetic modification to date, involves the covalent addition of a methyl group to cytosine residues adjacent to guanine in DNA (CpG sites) (Bird, 1986) and is associated with changes in gene transcription when they are located in gene promoter regions (Suzuki and Bird, 2008). Using DNA methylation analysis, epigenetic regulation has been shown to be critical in the control of sexual differentiation of the brain (McCarthy and Nugent, 2015; Nugent et al., 2015; Joel and McCarthy, 2017; Li et al., 2017; Joel et al., 2020; McCarthy, 2020). Thus, inhibition of DNA methylation in developing mice brains induces aberrant neurobehavioral profiles and disrupts sexually dimorphic neurobehavioral phenotypes in adulthood (Li et al., 2017; McCarthy, 2020). Furthermore, the sex difference in maternal anogenital licking of male compared to female pups produces a different methylation of the estrogen receptor α promoter in the preoptic area (Kurian et al., 2010).

Previous studies carried out in our laboratory in people with GI have found that certain environmental factors such as GAHT modify the methylation profile of the promoters of the ERα (Aranda et al., 2017; Fernández et al., 2020b), the AR and the ERβ (Aranda et al., 2017). Aranda et al. (2017) found no differences in the DNA methylation of the ERα in trans women, while DNA methylation was increased in trans men at 6 and 12 months of GAHT. The AR showed a significant increase of methylation in trans women after 12 months of estrogen supplementation. With respect to the ER α promoter, before the hormone treatment, trans men showed a lower methylation level with respect to both cis men and women, whereas trans women reached an intermediate methylation level with respect to the cis groups. However, after 6 months of GAHT, trans men showed a methylation increase, and both transgender groups reached a midway methylation level between cis men and cis women (Fernández et al., 2020b). Thus, both studies suggest that epigenetic changes in the sex steroid receptor promoters might be associated to GAHT. In fact, 6 months of GAHT was sufficient to modulate epigenetic changes at the estrogen and androgen receptor promoter regions. Yet, these prior studies focused exclusively on the AR and ER receptors and, to our knowledge, a global CpG analysis has not been performed to date in people with GI.

Therefore, taking into account our previous analyses and to achieve a broader perspective of the influence of epigenetics in GI, our main objective of this study was to carry out a

global CpG methylation analysis in a transgender population before GAHT and cisgender comparisons, assessed by ethnicity, geographical origin and sex.

SUBJECTS AND METHODS

Study Participants and Experimental Design

We analyzed sixteen Flemish Belgian transgender people (9 trans men and 7 trans women) before GAHT, and sixteen Flemish Belgian cisgender people (8 cis men and 8 cis women). The population was recruited at the Center for Sexology and Gender, Dept. of Endocrinology at the Ghent University Hospital (Belgium).

To obtain a homogeneous population avoiding stratification (Michels et al., 2013), the following inclusion and exclusion criteria were applied: for transgender people were the presence of GI according to ICD-11 (World Health Organization [WHO], 2018), identification with the other sex (male or female); and no prior history of hormonal treatment.

The exclusion criteria were the presence of psychiatric, neurological and hormonal diseases, and major medical condition. To the cisgender population the same exclusion criteria were applied. The mean age of the cisgender group at the beginning of the investigation study was 27.75 (SD $\pm$ 7.6)years and 34.1 (SD $\pm$ 14.0) years for the transgender group. Written informed consent was obtained from all participants after a full explanation of the procedures. The study was approved by the Ethical Committees of Gent University Hospital and UNED.

Genomic DNA Methylation Analysis

Genomic DNAs were extracted from peripheral blood samples using the DNeasy Blood and Tissue Kit from Qiagen following the manufacturer's protocol, and an aliquot of 1 μg DNA per subject was processed for bisulfite conversion (Zymo Research EZ Methylation Kit), according to the manufacturer's instructions.

DNA methylome was analyzed using the Illumina© Infinium Human Methylation 850k BeadChip array (Illumina, San Diego, CA, United States) that assesses 862,927 cytosine–phosphate–guanine (CpG) sites throughout the genome, covering 99% of RefSeq genes, 95% of CpG islands and high coverage of enhancer regions. In this study we selected the CpGs located in islands because they often coincide with promoters (Illingworth and Bird, 2009), and methylation modification of the promoter regions has the capacity to modify gene expression (Maurano et al., 2015) because methylation of the promoters prevents the binding of RNA polymerases and/or other diverse transcriptional factors to the promoter region, thereby inhibiting DNA transcription (Kang et al., 2019).

Beadchips were scanned with the Illumina iScan SQ system, and image intensities were extracted with the Genome Studio (2011.1). DNA quality checks, data normalization, and statistical filters were performed with the Partek® Genomics Suite® v7.19.1018 Methylation Module. Probes from the X and Y chromosomes were excluded from the study, and probes based on detection $P > 0.05$ were also filtered to exclude low-quality probes. Analysis of differentially methylated loci in humans and mice often excludes probes on the X and Y chromosomes because of the difficulties caused by the inactivation of one X chromosome in female samples.

Functional normalization, NOOB background correction and dye correction were applied. Principal component analysis (PCA) was performed to visualize clusters in the methylation data, and as a quality control procedure (**Figure 1**).

The raw methylation score for each probe was represented as methylation beta (β), in which β = intensity of the methylated allele (M)/intensity of the unmethylated allele (U) + intensity of the methylated allele (M) + 100. β-values range from 0 (unmethylated) to 1 (fully methylated) and can be broadly interpreted as the percentage of CpG methylation (Bibikova et al., 2011; Moran et al., 2016). Subsequently β-values were converted to *M*-values using the following equation: $M\text{-value} = \log2(\beta/(1 - \beta))$. An *M*-value close to 0 for a CpG site indicates a similar intensity of the methylated and unmethylated probes, which means the CpG site is about half-methylated. Positive *M*-values mean that more molecules are methylated than unmethylated, while negative *M*-values mean that more molecules are unmethylated than methylated. As discussed by Du et al. (2010), the β-value has a more intuitive biological interpretation, but the *M*-value is more statistically valid for the differential analysis of methylation levels. Because we were performing differential methylation analysis, Partek Genomics Suite automatically created the *M*-values to use for statistical analysis. Distribution of *M*-values across the samples was inspected by a box-and-whiskers plot and the distribution of beta-values by a histogram.

Differential methylation analyses (mean M variation, ΔM) aimed to evaluate methylation differences between the studied groups. Individual probes were then filtered based on Illumina detection $P < 0.05$ value, and a false discovery rate correction (FDR) $p < 0.05$ and a fold change $\geq \pm 2$ were applied.

All analyses were done by the Partek® Genomics Suite® software, version 7.0. The human reference genome (GRCh37/hg19 assembly) was used to determine the location and features of the gene region using the UCSC Genome Browser (Kent et al., 2002).

Statistical Analysis

To detect the differential methylation in global CpGs that varies across all samples we performed a 2-way ANOVA test comparing cisgender vs. transgender individuals by their sex assigned at birth. Then, we added two contrast interaction terms to find those genes that specifically change in each group: we contrasted cis men vs. trans people with male sex assigned at birth (trans women), and cis women vs. trans people with female sex assigned at birth (trans men). For each contrast, a *P*-value, Beta difference (Δβ), and M difference (ΔM) were generated. Hierarchical cluster analysis of the significant CpGs was carried out with the Heatmap function in the Partek® Genomics Suite® 7.0 (**Figure 2**). *P*-values were calculated using false discovery rate correction for multiple comparisons, FDR $p < 0.05$; corrected, two-tailed, and fold change $\geq \pm 2$).

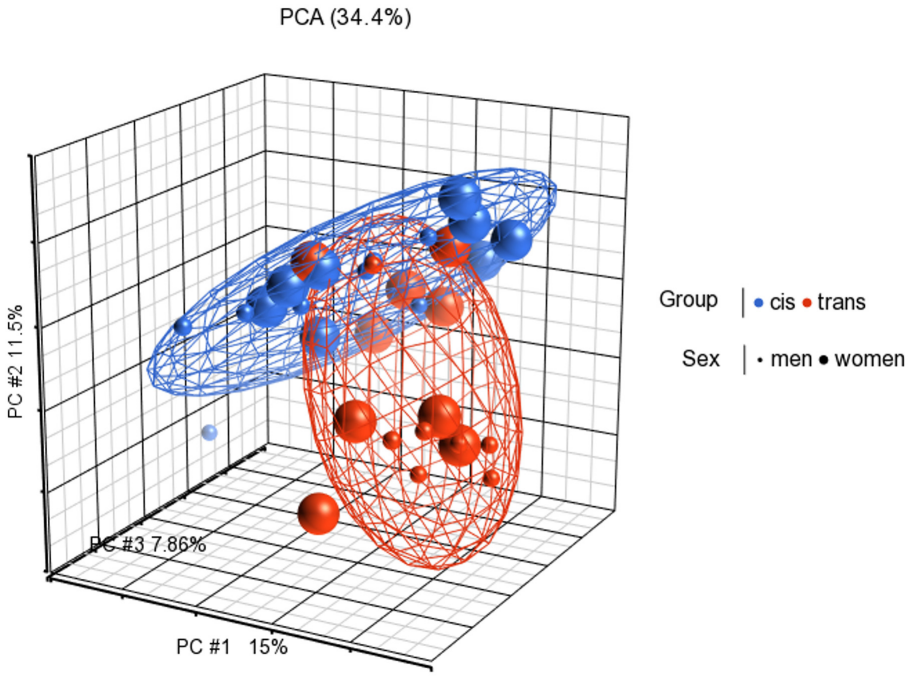

FIGURE 1 | Principal components analysis (PCA) in 3D showing methylation profiles of the study samples. Each sample is represented by a dot, the axes are the first three PCs, the percentages indicate the fraction of variance explained by each PC. The number at the top is the variance explained by the first three PCs. The samples are colored according to the levels of the variable "group" (blue for cisgender individuals and red for transgender individuals), and sized according to the levels of the variable "sex assigned at birth" (small for men and big for women).

Controls Applied to Exclude Genes Associated With Smoking and Age

The most robustly validated findings to date with DNA methylation studies have been the association between DNA methylation in blood and smoking. The genes that have shown the strongest associations to smoking status are: *AHRR*, 2q37.1, 6p21.33, *F2RL3, GPR15, GFI1, CYP1A1, MYO1G*, and *CNTNAP2* (Flanagan, 2015). We have used this knowledge to create a list of genes related to smoking status that was checked from our list of genes related to our variable of interest. This has been done because methylation alterations are detectable in blood DNA even in ex-smokers who stopped smoking up to 10–20 years before (Flanagan, 2015).

Furthermore, several genes appear consistently associated with age, including *ELOVL2, CCDC102B, OTUD7A*, and *FHL2*. Therefore, we have used this list of genes to exclude them from our study, prior to the enrichment analysis (Flanagan, 2015).

Functional and Regulatory Enrichment Analysis

The distribution of significant CpGs differentially methylated in females and males was examined across functional and regulatory annotations. CpG findings were mapped to known genes for enrichment of Gene Ontology (GO) classifications. The GO analysis and pathway enrichment analysis were carried with the Partek® Pathway program and the WebGestalt (WEB-based Gene SeT AnaLysis Toolkit)[1] (Liao et al., 2019) using the Genomes (KEGG) and the Panther databases. The GO ontology includes three independent divisions: biological process (BP), molecular function (MF) and cellular component (CC). The biological process can be defined as a biological objective to which the gene or gene product contributes. The molecular function is defined as the biochemical activity of a gene product, while the cellular component refers to the place in the cell where the gene product is active (Draghici, 2012).

RESULTS

Analysis of 2-Way ANOVA Test

When we compared the DNAm of transgender and cisgender populations by the variable sex assigned at birth, we found a baseline of 71,515 CpGs that passed the criterion FDR $p < 0.05$. Furthermore, 28.5% were in islands. About a third of these positions (32.3%) were hypomethylated while 67.66% were hypermethylated in cis men. In cis women, 27.05% of the CpGs were hypomethylated, while 72.95% were hypermethylated. These statistically significant CpGs were distributed among all autosomes.

[1]http://www.webgestalt.org

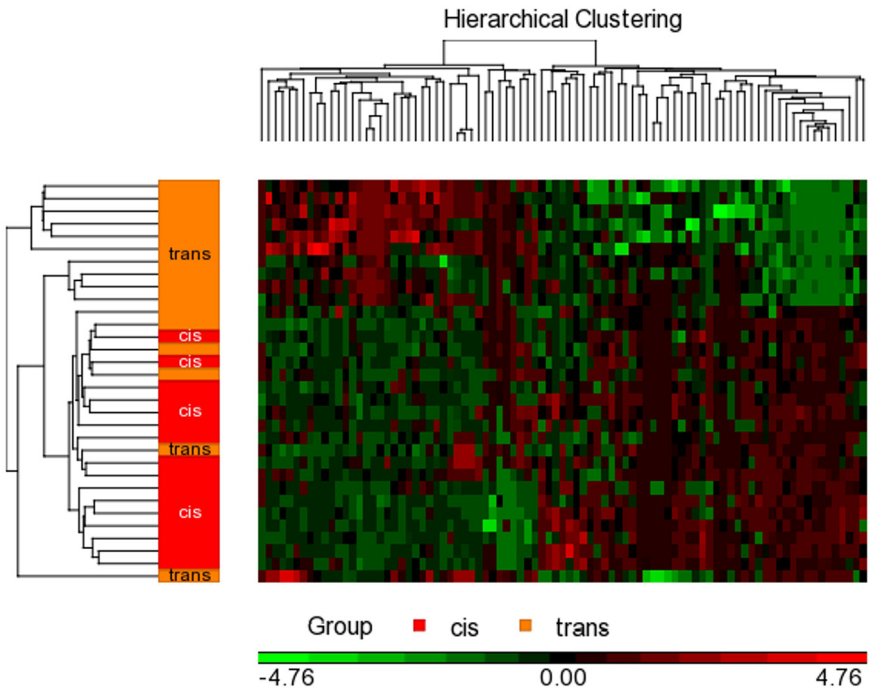

FIGURE 2 | Hierarchical clustering with heat map invoked on the list of significant CpGs FDR $p < 0.05$ and a fold change ≥ 2.0 in male assigned at birth populations. The experimental groups are rows, while the CpGs from the cisgender vs. transgender spreadsheet are columns. CpGs with higher methylation are colored red, CpGs with lower methylation are colored green. Samples of transgender people are colored orange and samples of cisgender people are colored red in the dendrogram on the left-hand side of the heat map.

Analysis of Cis Men vs. Trans Women

Subsequently, when we specifically contrasted the methylome in people who were male assigned at birth, we found 87 CpGs that passed both criteria (FDR $p < 0.05$; fold change $\geq \pm 2$), of which 22 CpGs were located in islands: 14 were hypomethylated while 8 were hypermethylated in the cis population. **Table 1** lists the 22 CpG islands in populations assigned male at birth that passed both criteria. The most significant CpG islands were related to genes: *WDR45B, SLC6A20, UBALD1, GRASP, NHLH1, PLEKHA5, SLC37A1, NCOA6*, and *ARL6IP1* (**Figure 3**).

Analysis of Cis Women vs. Trans Men

With respect to the population with female sex assigned at birth, we found 70 CpGs that passed the criterion FDR $p < 0.05$, of which 2 CpGs also passed the criterion fold change $\geq \pm 2$ (**Table 2**), but none were in islands. **Table 2** lists the 2 CpGs that passed both criteria. The two significant CpGs were cg23944405 related to gene *MPPED2*, and cg16149820 that may be in intragenic areas or information about it remains unknown.

Functional and Regulatory Enrichment Analysis

Once the significant CpGs had been selected, and prior to making the enrichment analysis, we excluded the list of genes involved in age and smoking. Subsequently, the enrichment analysis was done with the Partek® Gateway program and the WebGestalt. The results of the enrichment tests yielded significant over-representation for the categories of biological process, cellular component, and molecular function ontologies. Among the main molecular functions, we can highlight: negative regulation of gene expression (GO:0010629), central nervous system development (GO:0007417), brain development (GO:0007420), purine nucleotide binding (GO:0017076), ribonucleotide binding (GO:0032553), RNA binding (GO:0003723), and ATP binding (GO:0005524), among others (**Table 3**).

DISCUSSION

The main finding of this study is that the cis and trans populations have different global CpG methylation profiles, prior to GAHT. The PCA analysis showed that the spatial representation of the global methylation of these populations clearly differs between them. When comparing male sex assigned at birth individuals (cis men vs. trans women), 22 CpGs with significant methylation were located in islands. However, with respect to female assigned at birth individuals, significant changes of methylation in only 2 CpGs were found, and none were in islands. Furthermore, one of these CpGs, related to the *MPPED2* gene, is shared by both, trans men and trans women. Among the most statistically significant CpGs, we found that at least four of these genes were clearly involved in brain development and neurogenesis. These genes are *SLC6A20, PLEKHA5, NHLH1,* and *MPPED2*. Overall, our results suggest that these genes

TABLE 1 | The 22 CpG islands that passed statistical correction (FDR $p < 0.05$; fold change ≥ 2.0), in the population assigned male at birth.

Probeset ID	Gene symbol	*P*-value(cis vs. trans)	Difference(cis vs. trans)	Difference (description)(cis vs. trans)
cg10401531	*WDR45B*	2.97E-07	−2.20167	Cis men down vs. trans women
cg09700085	*SLC6A20*	3.49E-05	−2.00023	Cis men down vs. trans women
cg21538190	*NHLH1*	4.81E-05	−2.47591	Cis men down vs. trans women
cg24441383	*PLEKHA5*	4.87E-05	2.03159	Cis men up vs. trans women
cg16240751	—	5.29E-05	−2.05596	Cis men down vs. trans women
cg25764197	*UBALD1*	6.39E-05	2.6023	Cis men up vs. trans women
cg12993026	*SLC37A1*	0.000286382	2.19509	Cis men up vs. trans women
cg26358144	*ARL6IP1*	0.000298896	−2.10562	Cis men down vs. trans women
cg09016212	*GRASP*	0.000450551	2.40079	Cis men up vs. trans women
cg04208499	*NCOA6*	0.00138631	−5.96574	Cis men down vs. trans women
cg11502198	*ABT1*	0.00144373	−2.00222	Cis men down vs. trans women
cg02090742	*C17orf79*	0.00158261	−5.81018	Cis men down vs. trans women
cg11738485	*HOOK2*	0.00166823	−4.62858	Cis men down vs. trans women
cg04657146	*HOOK2*	0.00199853	−3.6818	Cis men down vs. trans women
cg09698465	—	0.00229726	6.38536	Cis men up vs. trans women
cg14623093	*GORASP1*	0.00285936	2.37051	Cis men up vs. trans women
cg20544675	*LETM2*	0.00336149	2.15344	Cis men up vs. trans women
cg12688781	*AACS*	0.00360693	−2.22118	Cis men down vs. trans women
cg01655658	*HLA-L*	0.00371637	−2.56482	Cis men down vs. trans women
cg11424828	*MYOM2*	0.00407032	−4.38544	Cis men down vs. trans women
cg05528899	—	0.00437277	−3.36645	Cis men down vs. trans women
cg24418853	*PTPLA*	0.00463796	3.29113	Cis men up vs. trans women

(—) Some CpGs are not located in genes, which means these signature probes may be located in intragenic areas or information about them remains unknown.

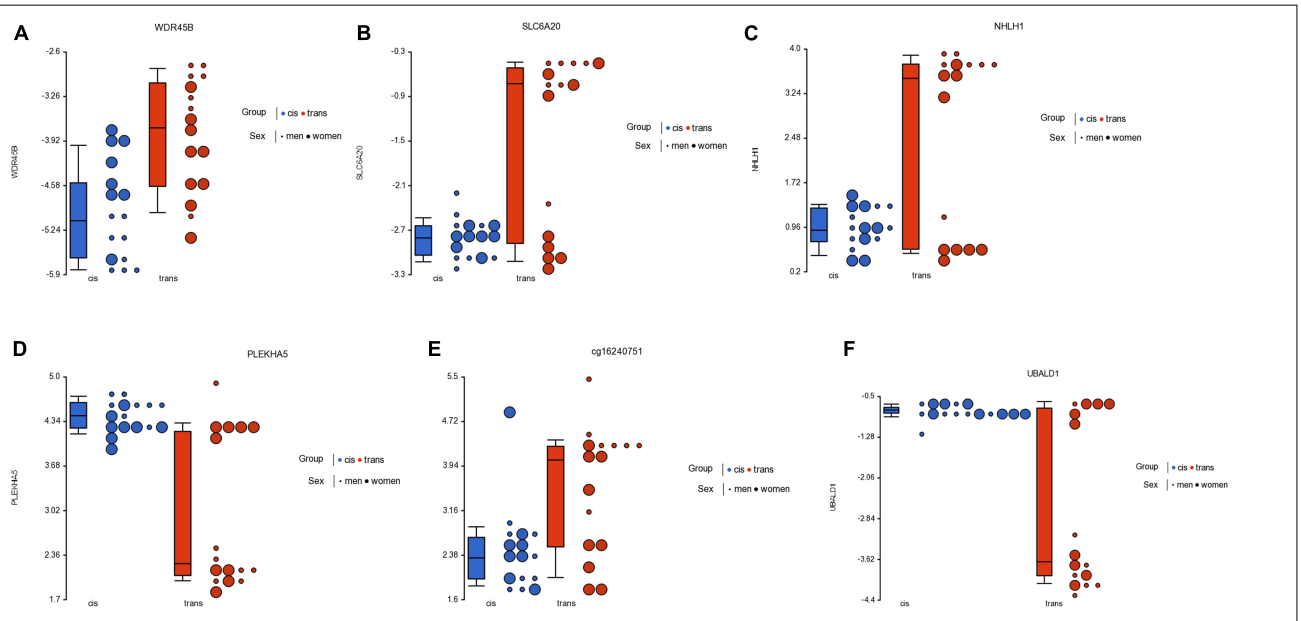

FIGURE 3 | Dot plot showing *M*-value data for genes *WDR45B* **(A)**, *SLC6A20* **(B)**, *NHLH1* **(C)**, *PLEKHA5* **(D)**, one intergenic locus (cg16240751) **(E)**, and *UBALD1* **(F)**, for cisgender vs. transgender populations by their sex assigned at birth. Each sample is represented by a dot, which corresponds to the overall degree of methylation (*M*-value data). The samples are colored according to the levels of the variable "group" (blue for cisgender population and red for transgender population), and sized according to the levels of the variable "sex assigned at birth" (big for women and small for men). The middle line is the median, the box represents the upper and the lower quartile, while the whiskers correspond to the 90th and 10th percentiles of the data.

could be involved in brain development, and that epigenetic factors play a role in a differential development that might be related to GI.

When comparing cis men vs. trans women 87 CpGs passed statistical correction (FDR $p < 0.05$; fold change $\geq \pm 2$), of which 22 CpGs were located in islands: 14 were hypomethylated

TABLE 2 | The 2 CpGs that passed statistical correction (FDR $p < 0.05$; fold change ≥ 2.0) in the population assigned female at birth.

Probeset ID	Gene symbol	Relation_to_UCSC_ CpG_Island	*P*-value (cis vs. trans)	Difference (cis vs. trans)	Difference (description) (cis vs. trans)
cg16149820	—	N_Shelf	3.14E-07	5.38516	Cis women up vs. trans men
cg23944405	MPPED2	N_Shelf	2.85E-06	−5.52554	Cis women down vs. trans men

The cg16149820 CpG is not located in a gene (—), which means this signature probe may be located in intragenic areas or information about it remains unknown. N_Shelf: Location relative to the CpG island, between 2 and 4 kb up- and downstream.

TABLE 3 | The results of the enrichment analysis for the categories of biological process, cellular component, and molecular function ontologies.

Gene set	Description	Size	Expect	Ratio	*P*-value	FDR
Enrichment categories: geneontology_biological_process						
GO:1901566	Organonitrogen compound biosynthetic process	1,776	752.54	1.2544	0	0
GO:0010629	Negative regulation of gene expression	1,733	734.32	1.3318	0	0
GO:0009719	Response to endogenous stimulus	1,595	675.85	1.2384	0	0
GO:0007417	Central nervous system development	949	402.12	1.3727	0	0
GO:0009894	Regulation of catabolic process	875	370.76	1.3944	0	0
GO:0043604	Amide biosynthetic process	766	324.58	1.3957	0	0
GO:0031329	Regulation of cellular catabolic process	764	323.73	1.3870	0	0
GO:0007420	Brain development	714	302.54	1.3816	0	0
GO:0043043	Peptide biosynthetic process	636	269.49	1.4138	0	0
GO:0006412	Translation	613	259.75	1.4206	0	0
Enrichment categories: geneontology_cellular_component						
GO:0005783	Endoplasmic reticulum	1,861	620.62	1.397	0	0
GO:0031984	Organelle subcompartment	1,661	553.92	1.4768	0	0
GO:0044433	Cytoplasmic vesicle part	1,462	487.55	1.5014	0	0
GO:0042175	Nuclear outer membrane-endoplasmic reticulum membrane network	1,072	357.5	1.3958	0	0
GO:0005789	Endoplasmic reticulum membrane	1,049	349.83	1.3978	0	0
GO:0099503	Secretory vesicle	976	325.48	1.4133	0	0
GO:0005773	Vacuole	760	253.45	1.5348	0	0
GO:0000323	Lytic vacuole	670	223.43	1.5217	0	0
GO:0005764	Lysosome	669	223.1	1.524	0	0
GO:0044437	Vacuolar part	552	184.08	1.5373	0	0
Enrichment categories: geneontology_molecular_function						
GO:0017076	Purine nucleotide binding	1,865	750.79	1.4039	0	0
GO:0032553	Ribonucleotide binding	1,865	750.79	1.3959	0	0
GO:0032555	Purine ribonucleotide binding	1,850	744.75	1.4005	0	0
GO:0035639	Purine ribonucleoside triphosphate binding	1,786	718.99	1.4048	0	0
GO:0008144	Drug binding	1,707	687.18	1.3417	0	0
GO:0042802	Identical protein binding	1,696	682.75	1.3050	0	0
GO:0003723	RNA binding	1,603	645.32	1.3962	0	0
GO:0030554	Adenyl nucleotide binding	1,522	612.71	1.4183	0	0
GO:0032559	Adenyl ribonucleotide binding	1,509	607.47	1.4141	0	0
GO:0005524	ATP binding	1,453	584.93	1.4156	0	0

and 8 were hypermethylated in the cis population. In this study we have considered CpGs islands because they often coincide with promoter areas, and they have the capacity to modify gene expression (Maurano et al., 2015).

The most significant CpGs in trans women were related to genes *WDR45B, SLC6A20, NHLH1, PLEKHA5, UBALD1, SLC37A1, ARL6IP1, GRASP, NCOA6, ABT1,* and *C17orf79* (**Table 1** and **Figure 3**). Among the most statistically significant CpGs, at least four of these genes were involved in brain development and neurogenesis (*WDR45B, SLC6A20, NHLH1*, and *PLEKHA5*) and three were related to transcriptional functions (*NHLH1, NCOA6,* and *ABT1*). Furthermore, the gene *C17orf79* is related to chromatin organization and its activation stimulates the transcription of the AR. Finally, another two genes were related to glutamate synapses (*ARL6IP1* and *GRASP*).

When we analyzed specifically the functions of each gene, we found that *WDR45B* is a component of the autophagy machinery that controls the major intracellular degradation process by which cytoplasmic materials are packaged into autophagosomes and delivered to lysosomes for degradation. Experiments with knockout (KO) mice exhibit many swollen axons and show cerebellar atrophy (Ji et al., 2020). On the other hand, the gene *SLC6A20* synthetizes an amino acid transporter as proline and is a regulator of brain glycine levels. Recent studies have reported that this gene is highly expressed in various brain regions and is also highly expressed in astrocytes and microglia, but only modestly expressed in glutamate or minimally in GABAergic neurons (Bae et al., 2021). This could suggest that *SLC6A20* proteins act as the regulator of both proline and glycine homeostasis in the brain.

The gene *NHLH1* is involved in neurogenesis that encodes a helix-loop-helix (HLH) protein that belongs to a family of transcription factors, some of which have been shown to play an important role in the growth and development of a wide variety of tissues. This protein is mainly expressed in the brain, specifically in the cerebellum. Ware et al. (2016) proposed that *NHLH1* is a neuronal marker. Its function might be regulating the expression of specific neuronal genes at the level of the first neurons, establishing the early axon scaffold tracts.

On the other hand, *PLEKHA5* is related to cell migration and cell to cell interactions and might also be a mediator of the brain homing phenotype (Eisele et al., 2015). Yamada et al. (2012) demonstrated that this gene may play an important role in mouse brain development. We also found differences in the methylation profile of the *UBALD1* gene, but its function is still unknown, however, it was associated with IL-8 secretion and NF-kappa-B signaling (Frenkel et al., 2019).

With respect to gene *NCOA6*, the protein encoded by this gene is a transcriptional coactivator that can interact with nuclear hormone receptors to enhance their transcriptional activator functions. It is a nuclear receptor coactivator that directly binds nuclear receptors such as for steroids (glucocorticoid receptors GR and ERs) and stimulates transcriptional activities in a hormone-dependent fashion (Eyster, 2016). Gene ontology annotations related to this gene include chromatin binding and transcription coactivator activity. Besides that, previous DNA analysis of polymorphisms related to SRC-1 and SRC-2 coactivators have pointed out their possible implication in the process of brain dimorphism (Fernández et al., 2021).

A further point in relation to this subject is that studies in mice suggest that the protein encoded by the gene *ABT1* is likely to activate basal transcription from class II promoters by interaction with the class II promoter DNA. GO annotations related to this gene include transcription coactivator activity, DNA binding, RNA binding, transcription coactivator activity, or regulation of transcription by RNA polymerase II among others.

On the other hand, when we compared cis women vs. trans men, we found significant methylation in only 2 CpGs, and none were in islands. The Venn analysis showed that one of the significant CpGs was shared by both trans groups. Thus, the cg23944405, located in the *MPPED2* gene (Metallophosphoesterase Domain Containing 2) showed statistically significant changes in methylation in trans men and trans women. This gene is expressed in most human tissues, also in the brain, both in cis men and cis women, and is expressed predominantly in fetal brains. Furthermore, Liguori et al. (2012) characterized *MPPED2* expression in human tissues of neuronal origin, and demonstrated that *MPPED2* expression is modulated during development, attributing to this gene an important role in the processes of neuronal differentiation that occur at the embryonic stage during CNS development. This gene has also been associated with altered inflammation and adverse clinical outcomes in severe blunt trauma (Schimunek et al., 2019). Furthermore, the functional importance of *MPPED2* regulation is related to cell cycle inhibition as it induces apoptosis and differentiation of neuronal precursors (Liguori et al., 2012).

Cg23944405 related to the *MPPED2* gene is hypermethylated in both trans populations (**Figure 4**). But this CpG is not located in an island (**Table 2**), so we cannot conclude that the hypermethylation in the transgender population was related to a low gene expression. Nevertheless, overall, we must point out that low metallophosphoesterase activity (*in vitro*) may play a role in the development of the CNS.

Our previous studies on the genetic basis of GI pointed to the existence of DNA sequences that modulate the sensitivity of the estrogen and androgen receptors in the trans population. Furthermore, we must remember that these nuclear receptors (ER and AR) are at the same time transcription factors, that modulate gene expression. Furthermore, the direct induction of gene expression through the activation of estrogen receptors and the androgen receptor is the presumed route for masculinization of the brain (Sato et al., 2004; Kudwa et al., 2006).

Now, with the present investigation, another small step is taken to increase our knowledge about GI. The results obtained here tell us that also epigenetics also plays an important role in the etiology of GI. Specifically, the differential methylation of essential genes in brain neurodevelopment such as *SLC6A20, PLEKHA5, NHLH1,* and *MPPED2*, are also involved in the etiology of GI. These are genes that play an important role in brain neurodevelopment, gene expression, and neuronal migration, which makes it possible to consider the existence of characteristic methylation profiles in the trans population.

In summary, our data reaffirm the hypothesis of a complex origin of GI, as the result of a combination of multiple factors such as hormones, hormone receptors, genetics and now also epigenetics.

LIMITATIONS

A potential limitation is that the methylation data was generated for only 32 participants. To make our study more robust, it would be advantageous to repeat this in a larger sample size or validate the conclusions with a new analysis from another trans population with similar characteristics. Also to make it even stronger it would be advantageous to do a longitudinal study that we are also collecting.

Another limitation of our study is that other factors with a known influence on the DNA methylome exist that must be taken into account. For example: sleep profile (Lahtinen et al., 2019),

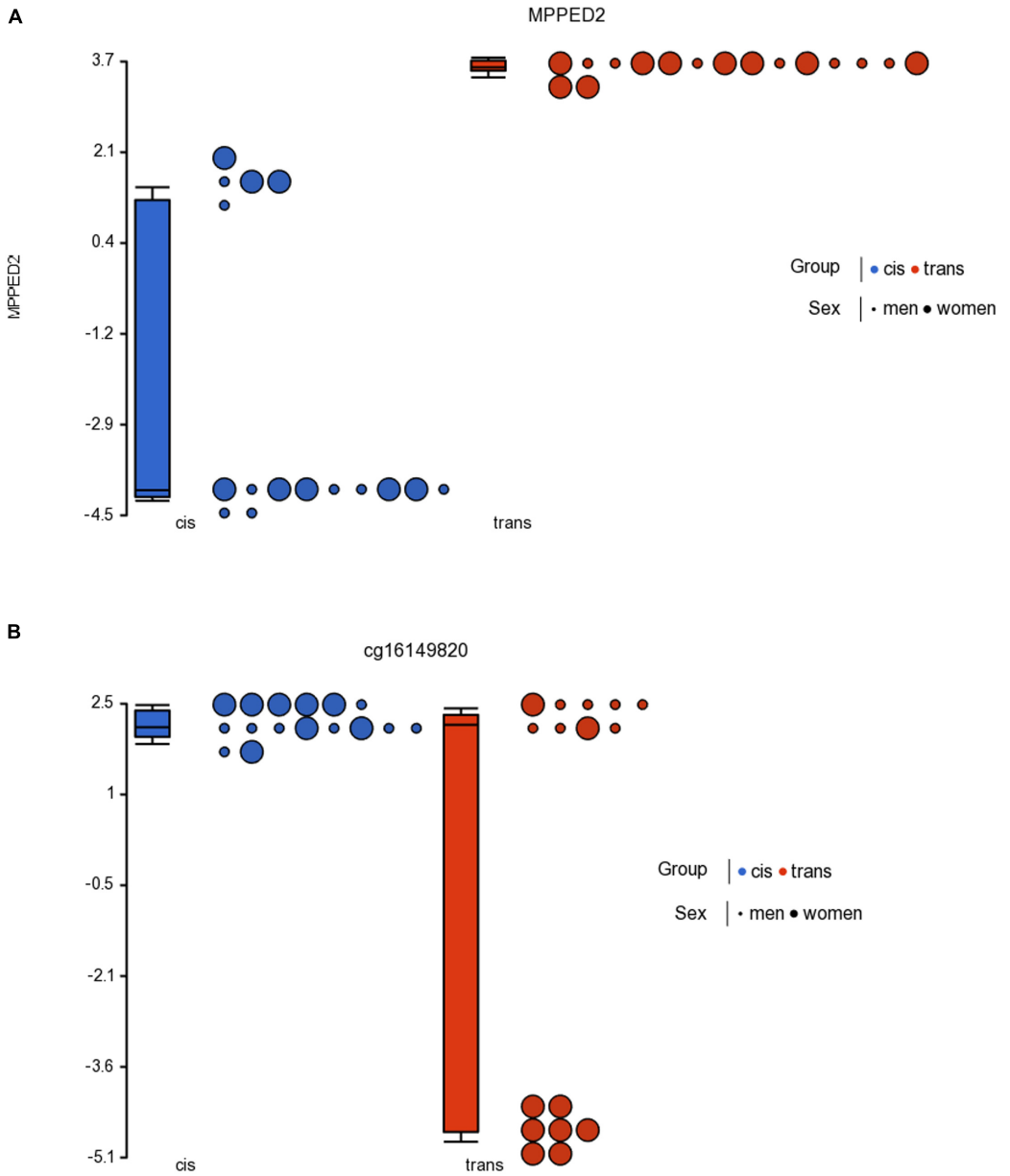

FIGURE 4 | Dot plot showing *M*-value data for cg23944405 **(A)** in the *MPPED2* gene and cg16149820 **(B)** for cisgender vs. transgender populations by sex assigned at birth. Each sample is represented by a dot. The samples are colored according to the levels of the variable "group" (blue for cisgender population and red for transgender population), and sized according to the levels of the variable "sex assigned at birth" (big for women and small for men). The middle line is the median, the box represents the upper and the lower quartile, while the whiskers correspond to the 90th and 10th percentiles of the data.

active/sedentary lifestyle (Voisin et al., 2015), nutritional habits (Kadayifci et al., 2018), or life adversity (Cecil et al., 2020).

The effect of sleep deprivation on transcriptome and methylome has previously been studied both in experimental animal models (Lahtinen et al., 2019). Sleep deprivation induces notable changes in the brain transcriptome of rats, affecting protein synthesis, synaptic plasticity, and metabolism (Cirelli and Tononi, 2000; Cirelli et al., 2004).

Moreover, nutrition is another important factor which plays a direct role in DNA methylation (Kadayifci et al., 2018). It is believed that nutrition affects the epigenetic regulation of DNA methylation by altering the substrates and cofactors that are necessary for DNA methylation, and also by changing the activity of enzymes that regulate the one-carbon cycle, and has a role in DNA demethylation activity too.

On the other hand, multiple studies in animals and also in humans have supported a link between early adversity and DNA methylation. The first piece of evidence for the impact of early adversity on the epigenome stemmed from research in animals. In a series of seminal studies based on rodents,

Weaver et al. (2004, 2005) found that variations in maternal care during the first week of life led to long-term changes in the pup's epigenetic regulation of the glucocorticoid receptor gene (Nr3c1), a gene crucially implicated in HPA axis function. These epigenetic changes stably altered Nr3c1 expression, resulting in variations in the density of glucocorticoid receptors in the brain as well as inter-individual differences in the pup's physiological and behavioral responses to future stressors (Turecki and Meaney, 2016; Cecil et al., 2020).

Since these and other factors have not been taken into account in our study, together with the small sample size, we believe that our study constitutes a preliminary analysis of the influence of epigenetics on gender incongruence.

CONCLUSION

In conclusion, we have identified two global CpG methylation profiles in cis and trans populations, prior to gender affirming hormonal therapy. These epigenetic changes in DNAm were associated with several genes related to crucial processes during development. Moreover, these methylation data, along with our previous genetic data, support the hypothesis that GI is a complex multifactorial trait, involving intricate interactions between sex steroids, sex steroid receptors, genetics and epigenetics. This supports the view that combining genetic and epigenetic approaches in parallel may be a successful approach to understanding the mechanisms underlying brain dimorphism. Furthermore, this hypothesis is consistent with the current complex "mosaic" model of the masculinization/feminization of the brain (Joel, 2021).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, GSE173382.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committees of Gent University Hospital and UNED. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AG, EP, RF, and SM contributed to the conception and design of the study. SC and MK recruited the population, involved in the study, and collected data from participants. SC and TV performed DNA extractions. RF and KR performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Healthcare for Trans*gender People in Germany: Gaps, Challenges, and Perspectives

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People whose gender does not correspond to the binary gender system, i.e., trans*gender people, face two main problems when it comes to healthcare in Germany: (1) They often suffer from general psychiatric comorbidities as well as specific and significant mental distress due to gender dysphoria, and (2) the German healthcare system lacks sufficiently educated and clinically experienced medical personnel who are able to provide specialized healthcare. Aside from transition, it often is extremely difficult for trans*gender people to get access to and be integrated into the medical system. Stigmatization and pathologization in treatment are widespread, as are long waiting times for specialized healthcare providers who are often only accessible to those trans*gender people willing to travel long distances. Frequently, trans*gender people face further difficulties and barriers *after* transition, as some healthcare professionals fail to provide suitable care (e.g., gynecological consultation for transmen). The ICD-11 German Modification (ICD-11-GM), which should be routinely used by 2022, implements a depathologization of trans*gender people in the medical system. This paper compares the issues related to health and healthcare of trans*gender people in Germany with those in other European countries. We review the care offered by specialized centers with regard to treatment of and support for trans*gender people. We conclude with specific proposals that may contribute to establish an improved, up-to-date, gender-sensitive healthcare system.

Keywords: transgender, transidentity, transsexualism, healthcare, internalized homonegativity, gender-affirmative healthcare

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INTRODUCTION – GAPS AND CHALLENGES

Modern societies are widely dominated by a hegemonic binary view of people's gender identity as well as a heteronormative understanding of relationships. Even in liberal democracies, where a pluralist understanding of different sexual, religious and lifestyle orientations are commonly accepted, trans*gender people are confronted with this "heterosexual matrix" (Butler, 1991) on

a daily basis. Correspondingly, the healthcare systems in these societies have institutionalized an exclusive binarity: medicine largely operates with the classification "male" and "female" as the only expected expression of gender, with most of the current models for mental disorders still relying on male data only (Shansky, 2019). This is especially problematic when it comes to healthcare for non-binary people. They face insufficient medical care, which is aggravated by treatment providers' lack of awareness of their concerns and insufficient knowledge of gender-sensitive medicine. People whose gender identity does not correspond to the perceived norm are negatively affected by this lack of knowledge with some of them facing severe stress and discomfort. Unsurprisingly, trans*gender individuals are at higher risk to report mental health problems than cisgender individuals. For example, a recent comparative study of mental health issues among cisgender and trans*gender people indicated that 77% of the included trans*gender participants were diagnosed with a mental disorder vs. 37,8% in cisgender participants (Hanna et al., 2019). Several studies show an elevated risk for affective disorders, anxiety disorders, and addictive disorders in trans*gender people compared to cisgender individuals (Reisner et al., 2016; Bouman et al., 2017; De Freitas et al., 2020). In addition, increased suicidality for trans*gender people compared to the cisgender population has been reported (Goldblum et al., 2012; Bailey et al., 2014; Reisner et al., 2016; Adams et al., 2017; Yüksel et al., 2017). This increased risk of comorbidities could be replicated in several countries worldwide, including data from the Lebanon (Ibrahim et al., 2016), the United States (Hanna et al., 2019), and the Republic of Côte d'Ivoire (Scheim et al., 2019). Consequently, mental health issues do not result from gender incongruence and stress/rejection/discomfort experienced by the individuals alone but are possibly further promoted by the binary-gendered thinking and treatment routines of the healthcare systems as they exist in most societies around the globe.

Interestingly, the question why trans*gender people have increased comorbidity rates can still be considered unanswered (Reisner et al., 2016). Some authors refer to the model of internalized homonegativity in order to explain increased risk and high prevalence of mental comorbidities in trans*gender people (Bockting et al., 2013, Bockting, 2015; Breslow et al., 2015). Internalized homonegativity describes how non-heterosexual people internalize socio-culturally predetermined negative attitudes and images (Göth and Kohn, 2014). This model is in line with societies' heteronormativity as it explains how predominant socio-culturally norms can lead to self-pathologizing (Rauchfleisch et al., 2002; Günther et al., 2019) which in turn can cause psychological distress and may finally result in mental health conditions (Bockting et al., 2013; Breslow et al., 2015; Perez-Brumer et al., 2015; Scandurra et al., 2018). This internalization process can be applied correspondingly to trans*gender persons inasmuch as gender identities are conceived of as stable, binary and invariant personality traits. Accordingly, this can be conceptualized as internalized transphobia (Bockting et al., 2013; Bockting, 2015; Breslow et al., 2015). The notion that mental comorbidities solely arise due to gender incongruence and dysphoria therefore seems decidedly too one-dimensional, ignoring the underlying complexity.

The Evolution and Current Healthcare for Trans*gender in Germany

Trans*gender healthcare in Germany has a centennial history already. In 1922, the German sexologist Magnus Hirschfeld, founder of the first Institute for Sexology, carried out the worldwide first sex reassignment surgery in Berlin (Bhinder and Upadhyaya, 2021). In the post-war German society, the situation of trans*gender persons was recognized only very haltingly. The so-called "transsexual law" (TSG) from 1980 implemented changes of personal and civil status. The law since required trans*gender persons to undergo surgical alteration of their genitals in order to have key identity documents changed. This was declared unconstitutional only in 2011.

Besides the legal framework there were no regulations for medical and psychotherapeutic healthcare for trans*gender people whatsoever until the publication of the German Standards for the Treatment and Diagnostic Assessment of Transsexuals (1997) (Nieder and Strauß, 2015). These standards provided temporal and diagnostic frameworks and concrete guidelines according to which gender-affirming procedures may take place. Stemming from the desire to enable trans*gender people to follow a self-determined and individualized transition, the new S3 guidelines from 2018 ["Gender incongruence, gender dysphoria, and trans health: S3 guideline on diagnosis, counseling, and treatment" (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften [AWMF], 2019)] have been developed in collaboration with experts and interest groups. In contrast to the precursor from 1997, the new guidelines take a less directive and more participatory approach (Nieder and Strauß, 2019). Hence, treatment seekers and treatment providers are now able to find individual solutions together on equal terms. Access restrictions should no longer exist. Thus, gender-affirming hormone treatment can already be used after diagnosis, at the beginning of the transition. Psychotherapy should no longer be a prerequisite for gender-affirming therapy but should accompany the transition and promote self-acceptance and stability (Nieder and Strauß, 2019). However, the report guidelines of the medical service of the health insurance funds (MDS) contradict the S3 guidelines by continuing to set strict framework conditions for the treatment costs to be covered by the public health insurance funds. Also, the guidelines for the diagnosis of trans*gender criteria from the ICD-10 catalog are less flexible and more stigmatizing than the S3 guidelines. Trans*gender is coded as "transsexualism" (Graubner, 2013). There, the main criterion is the desire of a person to belong to the binary opposite gender. This may include the desire to change sex characteristics (primary or secondary) and to be recognized as belonging to this gender. The desire must be constant for 2 years and must not result from mental disorder. The ICD-10 defines transsexualism as a disorder, subclassified in the section of disorders of adult personality and behavior (Graubner, 2013).

Therefore, practitioners in Germany find themselves in a field of tension between the prevailing strict conditions imposed

by health insurance and the ICD-10 catalog and attempts to loosen the regulations in accordance with the individual needs of trans*gender people. This also explains ambivalent reactions and uncertainties on the part of the practitioners to the S3 guidelines (Nieder and Strauß, 2019). In this constellation, it is expected that the new ICD-11 catalog 2022 will bring further change, as transsexualism will be coded in the section "Conditions affecting sexual health," thus separating trans*gender from somatic or mental illness (Jakob, 2018). This was already successfully implemented in the Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V), according to which it is only possible to speak of a disorder when there is relevant suffering due to the gender incongruence (dysphoria) (American Psychiatric Association, 2013). According to MDS the assessment instructions will have to be revised after ICD-11 has been established.

Trans*gender healthcare in Germany is provided in different institutions. Usually, medical services are provided in private practices. In addition, interdisciplinary healthcare supplies are available via outpatient care, such as the regional "Qualitätszirkel." These are regional associations of multidisciplinary trans*gender healthcare specialists. There are hardly any centers that offer multiprofessional treatment. The interdisciplinary care center at the University Hospital of Hamburg plays a pioneering role in this area. Some university hospitals offer specialized consultation hours, such as the specialized outpatient clinic for transsexuality and trans*gender in Tübingen, which was established in October 2020. This service is primarily aimed at trans*gender people before and during transition. To the best of our knowledge, there are no central registers for medical services for trans*gender people. Online, there are lists of addresses maintained by interest groups. **Figure 1** depicts the institutions providing treatment in Germany and the "Qualitätszirkel" (individual practices or clinics that only cover somatic needs are not listed). They offer various services: psychotherapeutic support, indication letters, medical reports to the TSG and partly interdisciplinary services. Healthcare services offered to trans*gender persons are covered by the health insurance and thus are covered publically, as was decided in 1987 by the Federal Social Court, the Bundessozialgericht (BSG 3 RK 15/86). However, letters of indication from experts are necessary in order that services (e.g., hormonal treatment, surgery) are covered by the health insurance.

How Does the Healthcare System Understand Trans*gender Nowadays?

Trans*gender people experience incongruence between the sex assigned at birth and their gender identity. Sex assignment is based on the external genital, which are usually defined in medical literature as indicators of the so-called biological sex. To avoid classifying non-binary gender identities as a deviation from the biological sex, the terminology "assigned gender" or "assignment gender" seems more suitable than the term "biological sex" (Günther et al., 2019). Gender identity describes a person's certainty and conviction to belong to a certain gender (Eckloff, 2012). This develops during the course of a person's life and is shaped by biological and social conditions equally (Göth and Kohn, 2014). In trans*gender people, gender identity does not develop in accordance with the assigned sex; the result can be a binary or a non-binary form of gender identity: Binary trans*gender indicates that individuals experience themselves as belonging to the binary opposite gender (i.e., transman or transwoman). However, there are also people who feel they belong to neither the female nor the male gender and/or experience their gender on a continuum between the sexes (Günther et al., 2019).

In terms of prevalence rates in European countries, similar rates have been reported, always indicating a slightly higher prevalence rate for trans*women. The prevalence of the ICD-10 diagnosis of transsexualism is estimated at 1:12000 for trans*women and 1:30000 for trans*men in Germany (Schneider et al., 2007). In Belgium, 1:12900 trans*women have undergone gender-affirming surgery, while in men this ratio is approximately 1:33800 in trans*men (De Cuypere et al., 2007). Netherlands show similar prevalence rates (1:11900 for trans*women and 1:30400 for trans*men) (Bakker et al., 1993). However, an increase in prevalence has been reported in several countries: in Germany, for example, a 2.6-fold increase in the number of inpatients who were diagnosed with a gender identity disorder between 2000 and 2014 has been reported (data of the German Federal Statistical Office) (Brunner et al., 2017). Brunner et al. (2017) discuss the increased amount of informational martials and the facilitated access to gender-affirming therapy as a cause of the reported increase in prevalence. Whether and how destigmatization of trans*gender individuals further contributes to the increased prevalence rates needs to be investigated. Unfortunately, standardized prevalence rates of trans*gender individuals are rarely to be found (Collin et al., 2016), as different definitions of trans*gender samples lead to different results in prevalence, obscuring the systematic investigation. Furthermore, the prevalence might be underestimated, as not all trans*gender persons seek gender affirming therapy (De Freitas et al., 2020). After the introduction of the new ICD-11, it should be possible to record comparable prevalence rates of the diagnosis gender dysphoria instead of transsexualism.

The Healthcare System's Influence on the Emergence and Maintenance of Suffering of Trans*gender People Focused on the Situation in Germany

The German medical system has institutionalized stigmatization of non-binary people, which has to be especially considered a substantial factor of trans*gender persons' healthcare situation. This mainly applies to non-trans*gender specific medical care, but also partly to trans*gender healthcare. The variety of experiences of discrimination within the healthcare system have already been pointed out (Franzen and Sauer, 2010; Grant et al., 2011; LesMigras, 2012; Bradford et al., 2013; Roberts and Fantz, 2014; Günther et al., 2019). However, since discrimination refers to distinctions that lead to, produce, or give rise to disadvantage (Scherr et al., 2017), it often seems more appropriate to speak of stigmatization in the context of trans-specific healthcare in

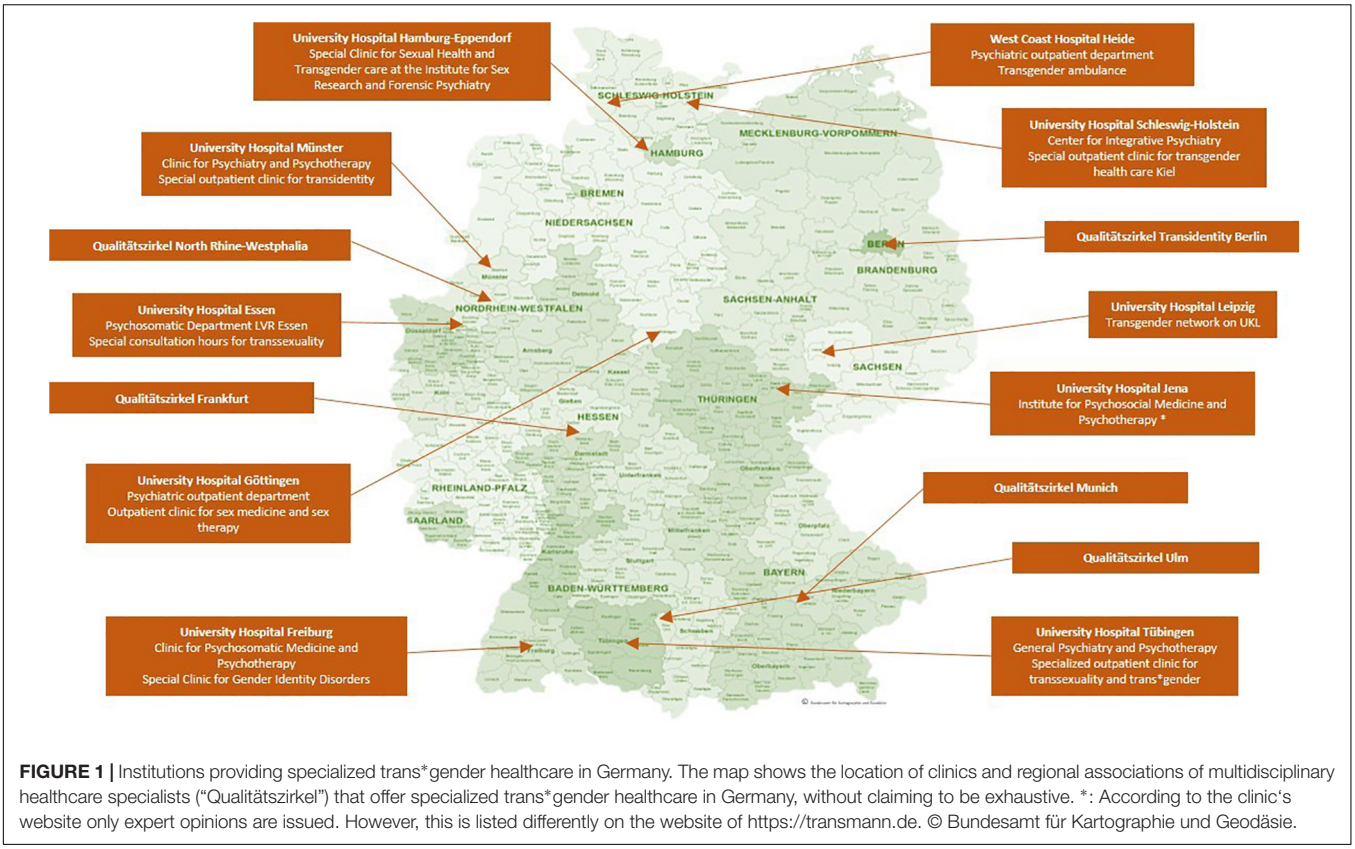

FIGURE 1 | Institutions providing specialized trans*gender healthcare in Germany. The map shows the location of clinics and regional associations of multidisciplinary healthcare specialists ("Qualitätszirkel") that offer specialized trans*gender healthcare in Germany, without claiming to be exhaustive. *: According to the clinic's website only expert opinions are issued. However, this is listed differently on the website of https://transmann.de. © Bundesamt für Kartographie und Geodäsie.

Germany. Stigmatization means the designation and marking of a deviation from a norm which is given or desired within a society (Goffman, 1963). Stigmatized persons are denied the status of a normal member of society because of an attribution of characteristics marked as a deviation. Institutional stigmatization occurs within social systems or organizations, where routines in communication and actions perpetuate "normality," which force the presentation and treatment of deviations from this norm as explicit deviations. Trans*gender people experience this institutional stigmatization in modern medicine in Germany and worldwide (Franzen and Sauer, 2010; Fuchs et al., 2012; LesMigras, 2012).

In itself, the structure of the healthcare system in Germany can be experienced as exclusionary by trans*gender individuals: Identification documents, such as health insurance cards, may not match the gender, cause confusion in providers and can lead to misgendering which in turn is experienced as stigmatizing (Roberts and Fantz, 2014). In the context of medical treatment in Germany, they presumably experience not so much discriminatory disadvantage as invalidation of their gender identity. Günther et al., suggest that exposition to the healthcare system may trigger internalized transphobia among trans*gender people, due to the fact that it occasionally puts the trans*gender individual under pressure to legitimize their own gender identity (Günther et al., 2019).

Because of experienced and/or feared stigmatization, some people are not willing to utilize the medical system. Studies from different countries show that the use of the healthcare system in trans*gender people is reduced due to fear of discrimination (Bauer et al., 2014). In the US-American "national transgender survey" stigmatization experiences of trans*gender persons were documented. One of the key findings reports a high likelihood of discrimination if the medical provider knows about their patients trans*gender identity. They also identify a lack of knowledge by the medical providers, so most of trans*gender people themselves have to inform their doctors about trans*gender healthcare (Grant et al., 2011). In Germany trans*gender persons report that their experiences with the healthcare system depend on whether their trans*identity remains hidden or becomes visible (LesMigras, 2012). Stigmatizing experiences in the healthcare system are among the most common negative experiences of trans*gender persons in Germany, after discrimination at the workplace (LesMigras, 2012). As a result, the health of this group of people is under-supplied, as they typically leave the health system after negative experiences and seek help elsewhere (Mizock and Lewis, 2008).

Due to the deeply embedded heteronormativity in Germany's society, it is unsurprising that medical areas that are not primarily oriented toward trans*gender healthcare show an unprofessional handling when they face gender identities that do not correspond to this supposed norm. Correspondingly, a study in North-Rhine-Westphalia (Germany) shows that trans*gender persons were hardly satisfied with their psychotherapeutic support (Fuchs et al., 2012). The same study reveals the administrative

and treatment burdens caused by the MDS review procedure. It has also been shown, that the institutional pathologizing of trans*identity is experienced as a tremendous burden by trans*gender people (LesMigras, 2012). As outlined in section "The Evolution and Current Healthcare for Trans*gender in Germany," the German healthcare system has been developing new ways of dealing with trans*gender healthcare. It is in a transition period between strict regulation and self-determination of the trans*gender community. Studies on the fears and wishes of the trans*gender community for multiprofessional treatment centers (as the one in Hamburg) show that also on the part of the treatment providers this development is being worked on and the offers are being adapted to the needs of the trans*gender community (Eysell et al., 2017).

Trans*gender people depend on the healthcare system as they require medical professionals before, during and after gender affirming therapy. Even after a successful transition, psychotherapeutic and somatic care must be ensured. Due to hormone therapy, trans*gender persons have a different lifetime risk profile for cardiovascular diseases (Aranda et al., 2019; Dutra et al., 2019; Pyra et al., 2020). The risk for sex-hormone dependent cancers is not higher during gender-affirming hormone therapy, but the cancer screening recommendations have to be considered in trans*gender people as well, i.e., prostate cancer screening in transwomen or breast and cervical cancer screening in transmen (McFarlane et al., 2018). Because of this need, it is alarming that the stigmatization in the healthcare system increases the chance for trans*gender people to avoid medical care and balk preventive measures, such as cancer screenings (Günther et al., 2019; Weyers et al., 2021).

In addition, studies show that after gender-affirming therapy, psychological stress can also occur, which may lead to increased suicidality (Rolle et al., 2015; Wiepjes et al., 2020). The lifetime prevalence of suicidality is also affected – amongst other variables – by negative experience with medical providers (Haas et al., 2014).

Psychotherapeutic services should also strive to offer gender-sensitive counseling in order to adequately address internalized transphobia, specific role conflicts, and so forth. The need for specialized counseling usually is not met after transition, as trans*gender persons are constantly confronted with their minority status in a binary, heterosexual environment (Verbeek et al., 2020). Unfortunately, specialized training programs for psychotherapists are hardly established. Since medical professionals are usually not trained in gender-sensitive medicine and may be out of their depth with regard to the healthcare of trans*gender persons, this ongoing stigmatization comes as no surprise. Therefore, gender sensitive medicine must become a part of the medical curriculum. There seems to be an interest on the part of medical students (Turner et al., 2014). Finally, gender sensitive medicine has to be implemented in the standard medical care in Germany (Chase et al., 2014).

Medical Care Services and Barriers for Trans*gender Individuals in Europe

As we propose, the institutionally co-generated psychological strain on trans*gender persons, promotes comorbidities and further increases economic costs. It seems imperative that stigma-free and need-oriented trans-specific treatment is provided by trained personnel. Only then can we reasonably expect that the psychological distress due to gender dysphoria can be minimized and fused conflicts can be addressed e.g., via psychotherapy. There is evidence for a reduction of distress through access to gender-affirming therapy (Bränström and Pachankis, 2020; Almazan and Keuroghlian, 2021).

The mission statement of the European Professional Association for Transgender Health (EPATH), a sub-organization of the World Professional Association for Transgender Health (WPATH), envisions the establishment of uniform European healthcare for trans*gender persons. By drafting a "standard of care" position paper, EPATH tries to formulate a uniform guideline for trans*gender sensitive health care beyond transition. The guideline furthermore establishes basic principles, addressing medical professionals. There is a consensus that healthcare providers worldwide should adhere to these basic principles, regardless of socio-cultural norms and legal requirements of their respective country. *Inter alia*, EPATH urges medical personnel to treat trans*gender persons respectfully and in a non-pathologizing manner. Access to treatment options should be ensured and medical personnel should be further trained in gender-sensitive medicine (Coleman et al., 2012).

However, uniform and comprehensive care for trans*gender persons is far from guaranteed in Europe, as the legal and medical situation is highly diverse: While some countries have been trying to ensure appropriate treatment of trans*gender persons in the legal and medical domain, trans*gender people in other countries are faced with persecution and discrimination (ILGA Europe Annual Review, 2021d). Apart from that, a legal and medical situation that considers the needs of trans*gender people does not necessarily imply that sufficient medical care is provided or that medical staff are sufficiently informed. While there are specialized treatment centers in many European countries nowadays, trans*gender individuals generally face the problem of long waiting times due to the structural lack of healthcare providers in the area of gender-affirming treatment services. In Netherlands treatment options (i.e., diagnostic classification, subsequent gender-affirming therapy such as hormone therapy, gender-affirming surgery) are offered in health centers (Amsterdam UMC, Groningen UMC and Radboud UMC Nijmegen). Like the center in Hamburg, they provide an interdisciplinary treatment – the so-called "gender team." However, these centers are far from meeting the demand and a lack of healthcare providers in Netherlands has been pointed out recently (Verbeek et al., 2020). In Belgium, care is also provided in healthcare centers (Belgien Universitär ziekenhuis Ghent, Université libre de Bruxelles and Le centre hospitalier universitaire de Liège), with the center in Ghent offering interdisciplinary care (Elaut, 2014). In contrast to Germany, hormonal treatment in Belgium is already possible during full-time real-life experience (Steinmetzer and Groß, 2008). Here, too, the long waiting times have been pointed out as problematic and as an obstacle to the access of appropriate services (Motmans et al., 2010). Specialized care centers in England, Scotland, and Northern Ireland are listed by Vincent (2018).

He points out that trans*gender persons have the longest waiting times of all patients in need of specialized treatment services. In Spain, the healthcare is installed in multidisciplinary gender units in different communities all over the country and the Canary Islands (Gómez-Gil et al., 2019). New healthcare models deviate from the central multidisciplinary gender units, for example by offering gender-affirming healthcare without psychological assessment. These new healthcare models are the subject of controversy, because the decentralization can be considered a missed opportunity: (a) for research and (b) to collected data to evaluate the quality of healthcare (Gómez-Gil et al., 2020a,b).

The procedure for gender-affirming surgery in Denmark is prescribed by the Danish Health Authority and is centralized in three clinics (e.g., The Sexological Clinic, Ringshospitalet Copenhagen) (ILGA Europe Annual Review, 2021c). Italy is a positive example of a publicly accessible database of medical care professionals. On the website https://www.infotrans.it/, published in 2020, trans*gender people can find out about treatment services (ILGA Europe Annual Review, 2021b).

The situation in Poland stands out as a negative example in a discrepancy to the, as not sufficiently marked, but existing care situation in most European countries. There, no medical care for trans*gender persons is guaranteed. In addition, there is talk of a hate campain against the LGBTIQ community (ILGA Europe Annual Review, 2021a).

Overall, healthcare in Europe is taking important steps toward depathologization, and many countries are attempting to establish the requirements of WPATH/EPATH. Worldwide, however, conditions remain poor and self-determination rights are denied to the trans*gender community. In some countries trans*gender persons are still criminalized (e.g., Indonesia, Niger, Malaysia, United Arab Emirates).

Key Findings

The article reviews the medical care situation for trans*gender people nowadays and it provides a more detailed description of the situation in Germany. Three main deficiencies were identified, that are linked to medical care for trans*gender persons in the German healthcare system: (1) A lack of specialized medical care to support transition. Mental comorbidities could be reduced by individualized support during transition. However, this is usually hindered by significant organizational and institutional barriers. Deficits in the structure of specialized healthcare services in Germany and Europe have been pointed out. There is a lack of specialized care offers that ensure a safe place for good care and that alleviate individual suffering through a professionally accompanied transition. (2) A lack of gender-sensitive psychotherapeutic support before and after transition, which could address the trans*gender specific dysfunctional internalization processes in a patient-oriented, professional manner. (3) A lack of sensitivity to special treatment needs in post-transition healthcare. We elaborated that even after transition, a non-discriminatory integration into the healthcare system remains necessary. Due to exclusively binary gender thinking, medicine is prone to institutional stigmatization. Accordingly, trans*gender people are frequently confronted with deficits and hurdles with the safeguard of their health. The multifactorial condition of suffering is modeled in **Figure 2**.

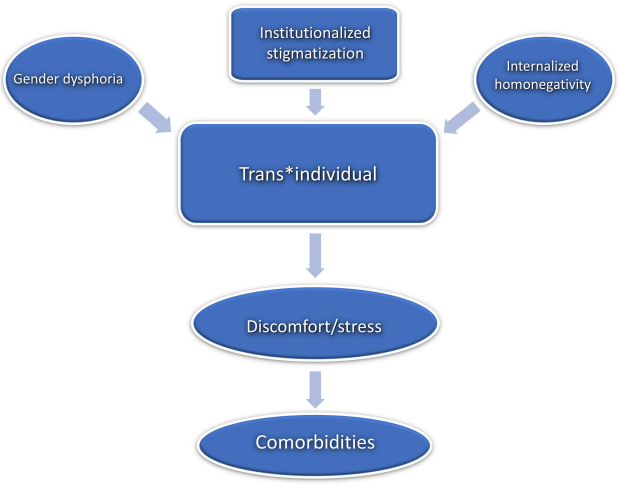

FIGURE 2 | Multifactorial condition of suffering. © Bundesamt für Kartographie und Geodäsie.

PERSPECTIVE

We see an urgent need for the establishment of comprehensive gender-affirmative healthcare. We propose three starting points: (1) A nationwide structure of specialized treatment centers for trans*gender healthcare is needed. In particular, the problem of unacceptably long waiting times must be addressed. (2) Specific sexual medicine training at an early stage (i.e., at university level during education and later on in specialist training) should lay the groundwork to minimize the institutional stigmatization of trans*gender individuals. (3) Finally, we call for the establishment of psychotherapeutic specialization as well as further education programs to support appropriate treatment of the diverse and multifactorial psychological issues of trans*gender people.

It should be pointed out, that through increased cooperation between medical providers and advocacy groups (e.g., Transgender Europe, TGEU), the European healthcare system can be transformed into a system based on self-determination and informed consent. It is time to face and address the many faceted barriers trans*gender people are facing when confronted with the healthcare system in different European countries (and probably world-wide).

In Germany we see a significant progression within the medical system toward the recognition of the trans*gender community and its needs in the recent years. The implementation of the new S3 guidelines is becoming more and more important and the trans*gender community is becoming more and more involved. Unfortunately, this development has not yet reached all areas. The new ICD catalog in 2022 will be an important step to further improving healthcare of trans*gender individuals.

We hope to contribute to establishing improved, gender-sensitive medical care in line with the variable demands of trans*gender people.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

NG prepared the first draft of the manuscript. All authors contributed to critically revising and editing the content of the manuscript and approved the final version of the manuscript for submission.

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Centralized and Decentralized Delivery of Transgender Health Care Services: A Systematic Review and a Global Expert Survey in 39 Countries

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Introduction: Transgender health care is delivered in both centralized (by one interdisciplinary institution) and decentralized settings (by different medical institutions spread over several locations). However, the health care delivery setting has not gained attention in research so far. Based on a systematic review and a global expert survey, we aim to investigate its role in transgender health care quality.

Methods: We performed two studies. In 2019, we systematically reviewed the literature published in databases (Cochrane, MEDLINE, EMBASE, Web of Science) from January 2000 to April 2019. Secondly, we conducted a cross-sectional global expert survey. To complete the evidence on the question of (de-)centralized delivery of transgender health care, we performed a grey literature search for additional information than the systematic review and the expert survey revealed. These analyses were conducted in 2020.

Results: Eleven articles met the inclusion criteria of the systematic review. 125 participants from 39 countries took part in the expert survey. With insights from the grey literature search, we found transgender health care in Europe was primarily delivered centralized. In most other countries, both centralized and decentralized delivery structures were present. Comprehensive care with medical standards and individual access to care were central topics associated with the different health care delivery settings.

Discussion: The setting in which transgender health care is delivered differs between countries and health systems and could influence different aspects of transgender health care quality. Consequently, it should gain significant attention in clinical practice and future health care research.

Keywords: transgender health care, transgender health, access to health care, quality of health care, delivery of health care

INTRODUCTION

Transgender people's gender identity does not match their sex assigned at birth. Their gender identities may be located binary within the sexes (female, male) or beyond (e.g., agender). The latter might also be referred to as non-binary or genderqueer genders (1). Health care for transgender people is primarily focusing on gender-affirming medical interventions (e.g., hormone therapy) and associated needs (e.g., mental health care). The current 7th version of the standards of care, published by the World Professional Association for Transgender Health (WPATH), and country-specific guidelines outline the relevance of gender-affirming medical treatment and high-quality care for transgender health (2, 3). The majority of previous research found a positive effect of gender-affirming medical interventions on the health of transgender people (4, 5). A high number of follow-up studies indicate benefits from hormone therapy and gender-affirming surgery (6–8) as well as from other treatment options, e.g., phonosurgery (9). In general, it has been shown that evidence-based gender-affirming medical approaches, in which the transgender person's gender is respected and safe space to explore their gender is created, improves mental health, quality of life, and several additional factors, e. g., substance abuse (10).

To date, transgender health care is delivered in both centralized and decentralized settings (11, 12). A centralized setting of delivery can be described as an interdisciplinary institution that can provide all relevant transition-related treatment options at one location. Decentralized delivery of transgender health care, in contrast, is characterized as the provision of gender-affirming medical treatments by different medical institutions (**Figure 1**). The gender clinic or "gender unit", a specialized institution especially common at University Medical Centers in Europe (13),

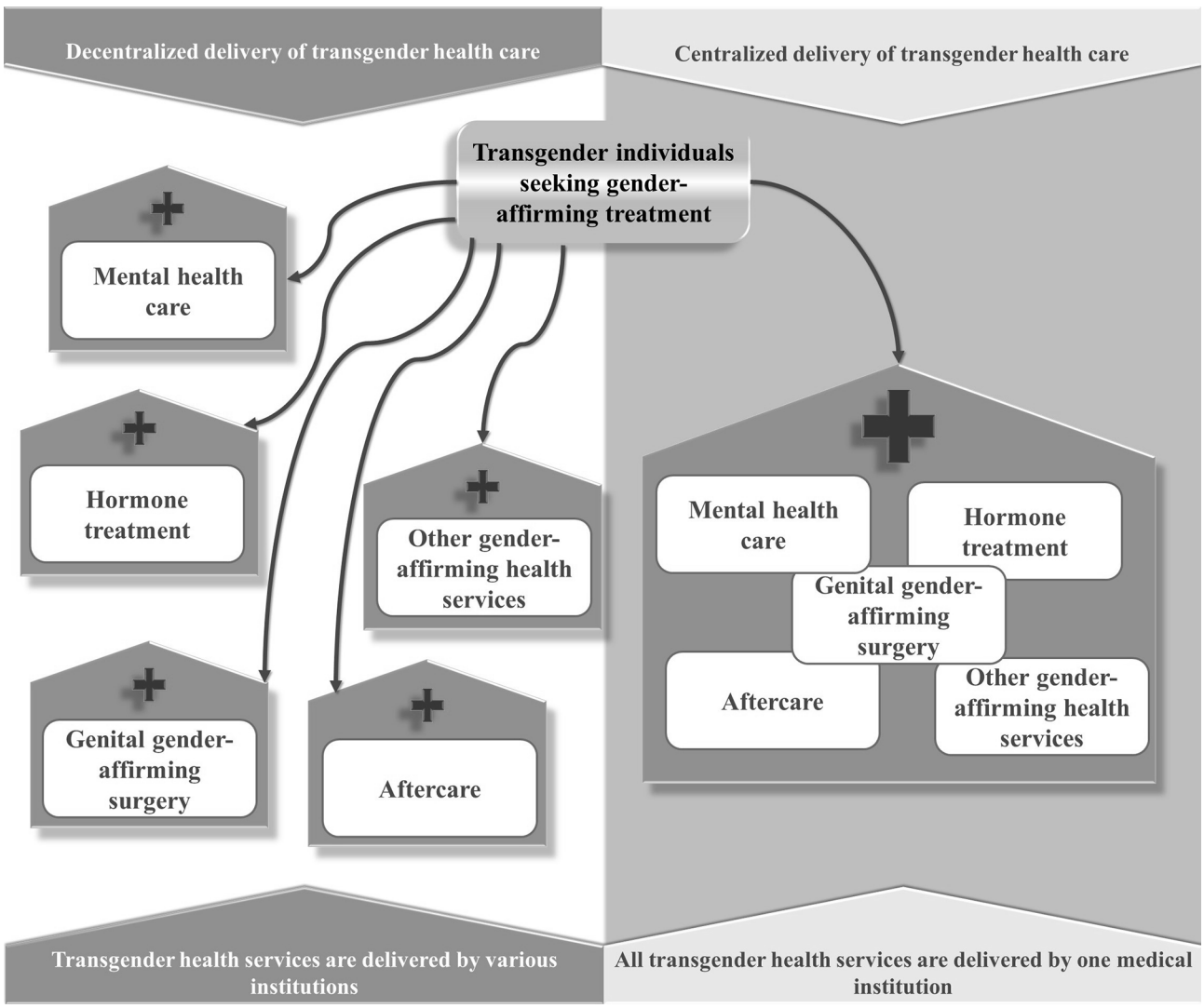

FIGURE 1 | Centralized and decentralized delivery of transgender health care.

often represents a centralized transgender health care service (14, 15). Historically, transgender health service delivery was organized in a centralized setting in the U.S., too. In the 1960s and 1970s, an increasing number of university-based centers offered health care for transgender people (e. g., Johns Hopkins, Stanford University) (16). However, the influence of Paul McHugh, who became the director of the Department of Psychiatry at Johns Hopkins in 1975 (16–18), and a methodologically questionable study (e.g., underpowered sample, inadequate outcome criteria (19) by Meyer and Rether (20), led to the closing of university-based clinics offering gender-affirming medical treatment (16). The study by Meyer and Rether suggested that gender-affirming health care has no health benefit for transgender people [for more details on the history of transgender health care in the U.S see (21)]. This led to the current situation of transgender health service delivery in the U.S., with private practices and community health centers mainly offering primary care for transgender people (e. g., hormone therapy) and university-based departments offering single gender-affirming medical interventions, e. g., gender-affirming surgery (12, 22). However, in both Europe and the U.S., other types of health care delivery exist (i.e., decentralized structures in Europe and centralized structures in the U.S.). In other parts of the world, health care delivery structures are not even developed sufficiently to ensure access to health care for transgender people, e.g., in certain African countries (23).

So far, research on the influence of centralized and decentralized health care delivery was primarily conducted on a superordinate political level and was focusing on health policy aspects, e.g., socioeconomic profits of increasing inter-jurisdictional competition between different medical institutions because of decentralization of health care (24). Decentralized health care delivery in practice has been considered as superior for some medical issues, e.g., HIV (25, 26), as it could ensure easy access to medical care in economically underdeveloped regions. For gender-affirming treatments, the factor of (de-)centralization has not gained sufficient attention so far, even though the setting of health service delivery differs between and within countries and, therefore, could be considered a potential influence on the quality and outcome of transgender health care (13, 27, 28).

There is a need to investigate the gaps in transgender health research as well as to identify where health services might produce inequalities and exclusions (29). Therefore, it appears to be an essential research question if and how the setting of health service delivery affects access to health care, influences the quality of services, and alters the outcome of gender-affirming medical treatment in research and clinical practice. We conducted a systematic literature review, an expert survey, and a grey literature search to shed light on how the setting of health care delivery was addressed in transgender health care research so far.

METHODS

Systematic Review (1st Study)

The present systematic review is registered on PROSPERO (30). Following the PRISMA guidelines (31), we systematically reviewed studies with a follow-up design evaluating at least one gender-affirming medical treatment (e.g., hormone therapy). At least one psychosocial or somatic outcome (e.g., quality of life) had to be assessed. We completed the final literature search on April 1, 2019. Due to an increase in transgender health-related publications since the early 2000s (32), we restricted the search to publications from the year 2000 up to 2019 and used *Cochrane*, *Medline, EMBASE*, and *Web of Science* databases. The search string was as follows:

[(Gender-nonconform*) OR (Gender-diverse) OR (Gender-Identity-Disorder*) OR (GID) OR (Transsexualism) OR (transsex*) OR (gender-dysphor*) OR (Gender-Incongru*) OR (Transgender*) OR (gender-identity)] AND [(follow-up) OR (longitudinal) OR (prospective)].

The inclusion criteria were kept as general as possible to ensure high sensitivity to detect potentially relevant studies, as the topic has not gained sufficient attention in research so far. The studies were reviewed in a three-step procedure with primary, secondary, and tertiary inclusion criteria. The primary inclusion criteria included published articles of studies with a follow-up design, evaluating at least one relevant transgender health care service (mental health care, hormone therapy, gender-affirming surgery, speech therapy, hair removal). The secondary inclusion criteria included articles published in English or German language, except for case studies. The tertiary inclusion criteria included published articles with information on the (de-)centralized delivery of transgender health care services (**Figure 2**).

If we were unable to access full texts, the corresponding authors of the article were contacted and asked to provide a copy. The exclusion criteria excluded studies with no follow-up; no evaluation of at least one transgender health care intervention; studies that were limited to qualitative outcomes; and studies that were found in non-scientific publications. We imported eligible articles into Endnote software (Thomson Reuters, Endnote X9, 2018). The first author AK conducted the literature analysis after joint planning with the last author TN, sorting materials based on the primary inclusion criteria after reviewing both title and abstract ("Yes", "no", "maybe"). Afterward, the included publications were sorted based on the secondary inclusion criteria after reviewing both title and abstract ("Yes", "no", "maybe"). In a third step, the included publications were sorted based on the tertiary inclusion criteria after reviewing the available full texts ("Yes", "no", "maybe"). See **Figure 2** for a flowchart of the different steps of the study selection process and results.

The databases from the different selection steps were sorted based on the evaluated transgender health care service (mental health counseling, hormone therapy, gender-affirming surgery, speech therapy, hair removal) and analyzed further (Tables of the databases after applying the first and second selection criteria are available from the corresponding author upon request). After applying the tertiary selection criteria, the final sample included articles of follow-up studies that were further analyzed, focusing on information concerning (de-) centralized delivery of health care services. Extracted data

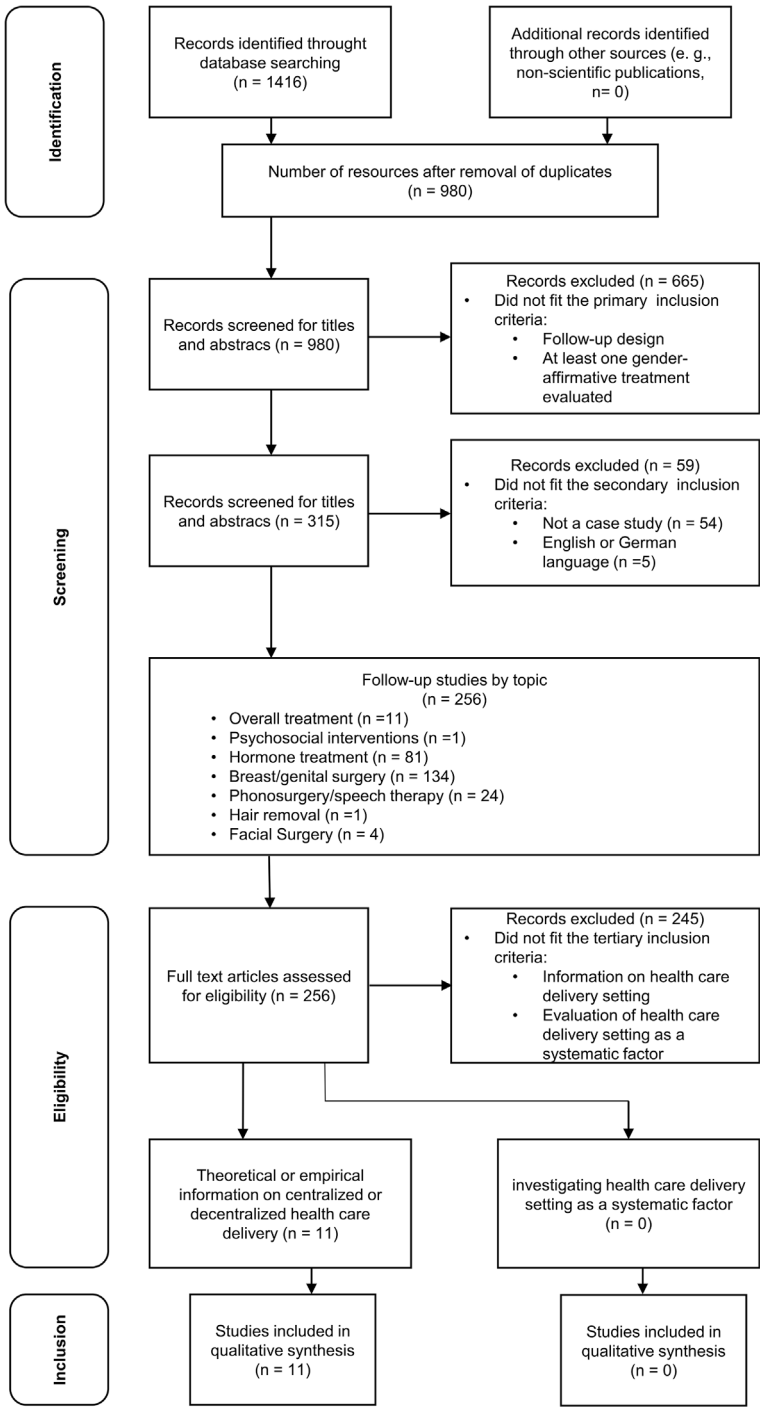

FIGURE 2 | PRISMA Flowchart.

from the articles included authors, study design, country, participants, evaluated gender-affirming medical treatment, outcomes, and information regarding (de-)centralized delivery of health care services.

We used the NIH Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group (33) to assess study quality for non-randomized studies. The tool primarily focuses on the internal validity of a study. It, therefore, helps to determine to what extent an outcome can be attributed to an intervention and not to biases or other confounding factors. Amongst others, aspects like representativeness of study participants for the clinical population, description of the

intervention(s), or outcome measures were assessed. AK and TN conducted the quality rating of the studies included.

Expert Survey (2nd Study)

The TransCareExpert survey was a web-based survey designed to investigate the experiences of transgender health care providers and researchers with the centralized and decentralized delivery of transgender health care. Data collection took part between November 2019 and April 2020. This cross-sectional survey was developed in the English language, followed strict ethical guidelines, and received ethical approval from the Local Psychological Ethics Committee (LPEK) at the Center for Psychosocial Medicine, University Medical Center Hamburg-Eppendorf (No.: LPEK-0071, date: 09/15/2019). Informed consent was obtained from all individual participants included in the study.

The survey was open to anyone who worked as a researcher or provider of transgender health care and was at least 16 years of age. Participants were recruited *via* mailing lists of specialized medical associations, the professional network of the authors, and through snowball sampling. We were not able to provide a paper-pencil version of the survey due to the global range of the study and a lack of financial and human resources. Therefore, access to a web-enabled device and technical affinity need to be considered potential biases for participation in the study. By encouraging participants to promote the survey within their professional network, we tried to address these issues.

All questions of the survey were developed by the authors of this article. A pilot study was conducted with ten researchers from different countries who had experience in the field of transgender health care. The survey was reviewed according to the suggestions from the pilot study. The TransCareExpert survey collected demographical data regarding age, gender, and country of practice. Questions regarding professional experiences with providing or researching transgender health care were focusing on the participant's occupation within transgender health care (e.g., clinician), their (medical) specialty, transgender-specific education (e.g., fellowship training), type of institution the participant works at (e.g., university hospital), and transgender health care services this institution offers. Experiences with working in centralized or decentralized transgender health care delivery settings and a description of the transgender health service system of the country of practice were assessed. Participants were asked to evaluate potential effects of the setting of health care delivery on the involvement of the transgender community into health care provision, the professional exchange between providers, the collaboration with non-medical community-linked institutions, and the collaboration with health insurances. Finally, pros and cons of centralized and decentralized transgender health service delivery were assessed. All aspects were investigated using closed-ended questions with predefined response options, and open-ended, free-response questions (see **Table S9** for the questionnaire).

Grey Literature Search

We conducted a grey literature search to find further information about the health care delivery setting of the countries included in our study that were not published in peer-reviewed journals or derived from the answers of the expert survey. Therefore, we searched for websites of government agencies, professional organizations, and other organizations. Furthermore, we carried out a grey literature database search using the following databases: OpenGrey, New York Academy of Medicine´s Grey Literature Report. Finally, a general search engine search was conducted using Google web search. AK and DS independently reviewed the identified grey literature compared to the evidence from the systematic review and the expert survey.

Statistical Analyses

To assess changes in study quality of studies included in the systematic review over the time of publication, linear regression was conducted.

Continuous data of the expert survey are presented as mean (SD). Categorical data are presented as n (%). Chi-square tests were calculated to assess frequency differences between the groups regarding questions on involvement of the community in care, exchange between providers, and collaboration with non-medical institutions and insurances. Free text responses were analyzed following qualitative content analysis (34), where subcategories and superordinated categories are built based on the qualitative material.

The health care delivery setting of a certain country was classified depending on the presence of at least one specialized center offering the most common treatments of transgender health care (e.g., mental health counselling, hormone therapy, genital gender-affirming surgery). Countries without a center were classified as having a decentralized structure of health care delivery. Countries with at least one center, but other non-interdisciplinary health care institutions, were classified as having both a centralized and decentralized structure of transgender health care delivery. Countries where transgender health services are mainly delivered by specialized centers were classified as having a centralized structure of health care delivery. We are aware that this classification does not adequately represent the whole health service delivery system of a certain country, as it appears that in almost every country, transgender health care is also provided by providers in private practice. However, our classification intents to point out, what institutions supply a major part of transgender health service in that certain country.

RESULTS

Figures 3 and **4** summarizes the results from the systematic review, the expert survey, and the grey literature search concerning the structure and (de-)centralization of transgender health service delivery in 40 countries all over the world. Information on 39 countries derived from the systematic review, the expert survey, and the grey literature search. Information on 1 country (Denmark) derived only from the systematic review and the grey literature search. **Table S1** gives a detailed overview over the structures on the national level.

FIGURE 3 | Transgender health care delivery worldwide.

Systematic Review (1st Study)

We identified a total of 11 articles that fit the primary, secondary, and tertiary inclusion criteria (**Figure 2** and **Table 1**). None of the articles investigated the delivery of health care as a standardized factor (35, 40, 41, 47–54). Hence, the studies included in the qualitative synthesis are providing unsystematic information on the health care delivery structure concerning the extent of centralization.

None of the studies were randomized controlled trials; seven were single-center clinical follow-up studies (35–39, 41, 45); one was a multi-center clinical follow-up study (42); one was a population-based matched cohort study (40); two were nation-wide cohort studies (43, 44). According to the quality assessment using the NIH Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group (33), studies were mostly ranked poor or fair (see supplementary material for the detailed assessment). Linear regression did not show a change in study quality over the time of publication (B=.03, P=.45, f^2=0.01). The majority of studies were conducted in Europe (n=10) (35–38, 40–45), one was located in the United States (39). Sample sizes ranged from n=42 (42) to n=2307 (41). The average sample size was n=424. Most studies evaluated gender-affirming surgery (n=6) (36, 38, 40, 42–44). N=2 studies investigated general hormone therapy (39, 45), n=2 studies investigated general hormone therapy and gender-affirming surgery (35, 41), and n=1 study evaluated psychological support and specific hormone therapy (GnRHA treatment) (37). N=6 studies reported psychosocial outcomes or sample descriptions only (e.g., mental health, age) (35–38, 40, 42, 43). N=4 studies reported somatic outcomes only (e.g., body mass index) (39, 41, 44, 45).

None of the selected articles investigated the setting of health care service delivery as a systematic factor. N=7 studies reported information about the setting of health care delivery in the introduction (35, 36, 39, 42–45). N=4 studies reported information concerning the setting of health care delivery in the methods section (37, 38, 40, 41). Information regarding the (de-)centralized delivery of health care was given on a systemic and an individual level. Information on a systemic level refers to the superordinated structure of the health care system regarding the delivery of health care in a particular country overall. On an individual level, the study authors gave information regarding the specific delivery structure in which the study was conducted.

N=9 studies reported on a systemic level about centralized health care in the health care system of the country in which the study was conducted (35, 36, 39–45). Six of these studies reported of only one specialized center for the whole country (35, 36, 41, 43–45). The studies were conducted in Denmark (36, 43, 44), the Netherlands (41, 45), and Belgium (35). Two studies gave information on the centralized delivery of transgender health care in Sweden by six hospitals (40, 42). One study (39) addressed differences between the U.S. and Europe regarding the health care delivery setting: transgender health care in Europe is

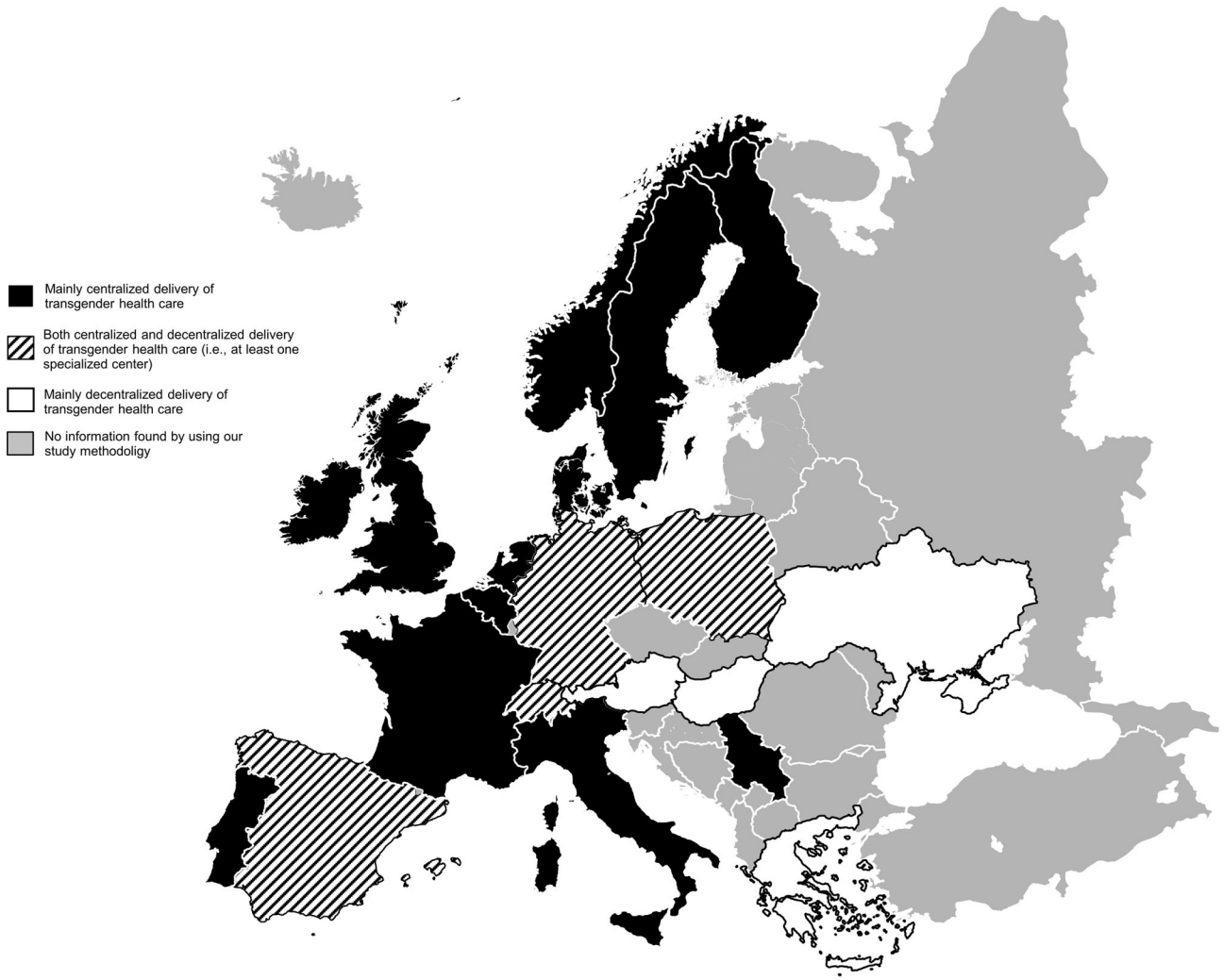

FIGURE 4 | Transgender health care delivery in Europe.

mostly delivered in centralized settings by specialized centers, whereas in the U.S., treatment is provided by a patchwork of community clinics. N=2 studies gave information regarding a centralized setting of transgender health care delivery on an individual level (37, 38). Both studies were conducted in a specialized center. One study was located in England (37), the other in Belgium (38). For Belgium, another study (45) gave information that the clinic the study was conducted in is the only in the country.

Expert Survey (2nd Study)

125 participants from 39 countries took part in the survey (**Figures 3** and **4**, all marked countries except for Denmark). 57 (45.6%) were involved in medical care for transgender people, 55 (44.0%) were involved in mental health care for transgender people, and 65 (52.0) were researchers. Most of the participants (57, 45.6%) were physicians. On average, participants were 46.4 (SD 11.0) years of age and had 12.5 (SD 8.0) years of experience in working with transgender people. 13 (10.4%) were transgender. 47 (37.6%) worked in a centralized setting of transgender health care delivery, 78 (62.4%) worked in a decentralized setting. Detailed participants' characteristics are presented in in **Table 2**. Their evaluation of the effects of the health care delivery setting on involvement of the community in care, exchange between providers, and collaboration with non-medical institutions and insurances are presented in **Table S2**. Categories of the pro and contra free text responses regarding centralized and decentralized delivery of transgender health care are summarized in **Table 3** (see **Table S4–S7** for a detailed summary of the answers).

Grey Literature Search

Results from the grey literature search are presented in **Tables S1**, **S8**. Grey literature sources confirmed the information derived

TABLE 1 | Studies included in the systematic review.

Authors	Study design	Country	Participants	Treatment(s) evaluated	Outcome	Information regarding (de-) centralized delivery of health care	Investigating health care delivery setting as a systematic factor
(35)	Single-center, clinical follow-up	Belgium	57 transgender adults	Hormone therapy, gender-affirming surgery	Mental health	- Section of the paper: introduction - One specialized clinic for the whole country - for insurance coverage, transgender people have to receive treatment at this clinic - the only possibility to bypass the public health care system is undergoing gender-affirming surgery in private sector or abroad - Participants of the study received all procedures from the one specialized clinic	No
(36)	single-center, clinical follow-up	Denmark	158 transgender adults	Gender-affirming surgery	Gender distribution, age trends, surgeries performed	- Section of the paper: methods - All participants of the study received mental health counseling and hormone therapy from the same specialized gender identity service	No
(37)	single-center, clinical follow-up	England	201 transgender adolescents	Psychological support, GnRHa treatment	Gender Dysphoria, psychosocial functioning	- Section of the paper: methods - Participants of the study received all treatments in an interdisciplinary gender clinic	No
(38)	Single-center, clinical follow-up	Belgium	55 transgender adults	Gender-affirming surgery	General health, sexual health	- Section of the paper: introduction - Transgender health care in Europe mostly centralized in nationally sanctioned gender centers - Transgender health care in the U.S. often in a patchwork of community clinics (decentralized)	No
(39)	Single-center (community health center), clinical follow-up	United States	57 transgender adults	Hormone therapy	Body mass index, blood pressure, lipids, sex hormone levels, persistence if menses	- Section of the paper: methods - Six specialized clinics for the whole country offering all treatments necessary for gender affirmation - Participants of the study received treatment in one of the six specialized clinics	No
(40)	Population-based matched cohort study	Sweden	324 transgender adults	Gender-affirming surgery	Mortality, psychiatric morbidity	- Section of the paper: methods - One specialized clinic for the whole country offering all treatments necessary for gender affirmation - Participants of the study received hormone therapy and gender-affirming surgery from the same clinic	No
(41)	Single-center, clinical follow-up	The Netherlands	2307 transgender adults	Hormone therapy, gender-affirming surgery	Prostate cancer	- Section of the paper: introduction - One specialized clinic for the whole country offering all treatments necessary for gender affirmation - Participants of the study received hormone therapy and gender-affirming surgery from the same clinic	No
(42)	Multi-center, clinical follow-up	Sweden	42 transgender adults	Gender-affirming surgery	Gender dysphoria, satisfaction with surgery, social functioning, work, relationships, sexuality	- Section of the paper: introduction - Six specialized clinics for the whole country, two offering gender-affirming surgery - Participants of the study received transgender health care from two of the six specialized clinics	No

(Continued)

TABLE 1 | Continued

Authors	Study design	Country	Participants	Treatment(s) evaluated	Outcome	Information regarding (de-) centralized delivery of health care	Investigating health care delivery setting as a systematic factor
(43)	Nation-wide cohort study, 30-year period follow-up	Denmark	104 transgender adults	Gender-affirming surgery	Psychiatric morbidity & mortality	- Section of the paper: introduction - One specialized clinic for the whole country - for insurance coverage, transgender people have to receive treatment at this clinic - Participants of the study received all procedures from the one specialized clinic	No
(44)	Nation-wide cohort study, 30-year period follow-up	Denmark	104 transgender adults	Gender-affirming surgery	Somatic morbidity, cause of death	- Section of the paper: introduction - One specialized clinic for the whole country - for insurance coverage, transgender people have to receive treatment at this clinic - Participants of the study received all treatments from the one specialized clinic	No
(45)	Single-center, clinical follow-up	The Netherlands	1254 transgender adults	Hormone therapy	Bone mineral density	- Section of the paper: introduction - Refers to (46): 95% of the transgender population receives treatment in one specialized clinic offering all treatments necessary for gender affirmation	No

from the systematic review and the expert survey and added valuable details to get a comprehensive picture of the transgender health care delivery in the different countries.

DISCUSSION

As the first of its kind, the present study investigated centralized and decentralized health care delivery structures for transgender health services. With a systematic review of the existing literature, a global expert survey, and a grey literature search, we were able to shed light on this important, but so far unnoticed, aspect of transgender health care and gained valuable knowledge to further improve transgender health care quality.

We found that previous literature was mainly focusing on the centralized delivery of health care in specialized centers, even though none of the included studies investigated the setting of health care service delivery systematically. In various European countries, transgender health care services are delivered by one specialized center [e.g., Belgium (35, 38)] or by a small network of specialized centers [e.g., Sweden (40, 42)]. With further empirical evidence from our expert survey as well as the grey literature search, we conclude that the (university-based) gender-unit is the standard model for transgender health care delivery in many European countries (**Figures 3** and **4**). Interdisciplinary cooperation from various medical departments offers a broad range of gender-affirming medical treatments, often centrally coordinated by a single superordinated department (13, 55). Outside Europe, transgender health care was delivered centralized only in Japan. One study gave information about decentralized delivery of transgender health care in the U.S (39). In the U.S., transgender health care is typically delivered by private providers or in community health centers, where general health and hormone therapy for transgender people is provided (39). Surgical procedures are usually conducted by specialized departments focusing on gender-affirming surgery. However, specialized centers, offering transgender health services in a centralized setting, are also present in the U.S (56, 57). In fact, the presence of both centralized and decentralized institutions in a certain country was the most common structure of transgender health care delivery in our study (**Figures 3** and **4**). In most countries, our study revealed the presence of at least one center specialized in transgender health care offering the most common interventions. However, often only single or a few specialized centers existed, and transgender health care was delivered by a variety of institutions. In a small number of countries (e.g., Russia, Ukraine, Indonesia), transgender health care was delivered exclusively decentralized, without the presence of specialized, interdisciplinary centers.

If and how the health care delivery setting affects the quality of transgender health care was not investigated in prior research so far. By asking on the involvement and active contribution of the transgender community in care, the professional exchange between caregivers, and the collaboration with non-medical community organizations and health insurance, our expert survey did not find a clear pattern which setting of health care

TABLE 2 | Participants characteristics from the expert survey.

Total number of participants	N° (%)/Mean (SD) 125
Involvement in transgender health care	
Medical care	57 (45.6)
Mental health care	55 (44.0)
Research	65 (52.0)
Type of clinician	
Physician	57 (45.6)
(Advanced Practice) Nurse	5 (4.0)
Physician Assistant	4 (3.2)
Psychologist	23 (18.4)
Licensed Clinical Social Worker	5 (4.0)
Other	13 (10.4)
Medical speciality	
Surgery	25 (20.0)
Endocrinology	12 (9.6)
Mental health	51 (40.8)
Other	18 (14.4)
sub-speciality, fellowship training, or licensure comments	62 (49.6)
Years of experience in working with transgender people	12.5 (8.0)
Type of health care institution	
University hospital	47 (37.6)
Non-university hospital	12 (9.6)
Community health center	8 (6.4)
Private practice	42 (33.6)
Other	11 (8.8)
Field of research	
Mental health	36 (28.8)
Children & Adolescents	12 (9.6)
Endocrinology	10 (8.0)
Social Sciences	13 (10.4)
Voice and Communication	1 (0.8)
Surgery	9 (7.2)
Law	5 (4.0)
Other	20 (16.0)
Years of experience in researching transgender health care	10.1 (8.0)
Type of research institution	
University/University hospital	55 (44.0)
Public research institute (not related to a university)	1 (0.8)
Private research institute (not related to a university)	4 (3.2)
Other	5 (4.0)
Age	46.4 (11.0)
Gender	
Man/Male (men of transgender experience and cisgender men)	59 (47.2)
Woman/Female (women of transgender experience and cisgender women)	57 (45.6)
Non-binary/Genderqueer	6 (4.8)
Other	3 (2.4)
Identification as transgender	13 (10.4)
Health care delivery setting participant works in	
centralized	47 (37.6)
decentralized	78 (62.4)
Health care delivery setting of the country participant works in	
(mostly) centralized	31 (24.8)
(mostly) decentralized	41 (32.8)
both	53 (42.4)

delivery could be in favor. Up to 34.0% of the participants of our expert survey reported, that they cannot judge or do not know about these topics, and we found no significant frequency differences favoring on health care setting regarding one of these issues (**Table S2**). As the health care delivery setting has not gained any attention in research so far, providers and researchers might not have a clear position on its effects on their daily practice and were therefore unable to assess positive or negative effects. Therefore, research on a more individual level, e.g., interview studies or focus groups, might be an option to approach this topic in future research with health care providers. Regarding aspects in favor of centralized transgender health care delivery in general, participants of the expert survey mainly focused on the comprehensiveness of care, professional

TABLE 3 | Pros and Cons of (de-)centralized transgender health care delivery.

Centralized health care delivery		Decentralized health care delivery	
Pro	**Con**	**Pro**	**Con**
Comprehensive, interdisciplinary care & professional exchange	Access barriers to health care (travel & waiting list)	Easy access to health care	Lack of expertise
Professional expertise & standardized care	Monopolization of care	Opportunity to choose health care provider & treatment	Fragmentation of care & coordination challenges
Patient-centered care	Detachment from the community	Community-involvement	
Research opportunities	Structural challenges		

expertise, patient-centeredness, and research. In line with that, several interdisciplinary gender clinics have implemented well-established procedures of transgender health care service delivery that promoted positive outcomes following gender-affirming interventions (13, 57–59). On the other hand, centralized care was negatively brought in connection with access barriers to care, especially due to long travel and waiting lists, the monopolization of care, a detachment from the transgender community, and structural challenges. Pros of decentralized transgender health care delivery were arguments to the contrary, focusing on easy access to care, free choice of providers and treatments, and a better involvement of caregivers into the transgender community. Prior research found the lack of providers with sufficient knowledge is the biggest barrier to health care for transgender people (60). A decentralized delivery structure could face this problem by ensuring better access to transgender health services. However, these health care providers still would need specialized training in gender-affirming care to deliver sufficient transgender health care. Newly developed online training, e.g., by the Global Education Institute of WPATH, could be a first step to make it possible to get high-quality training in gender-affirming care remotely. This could be especially important for providers from rural areas with decentralized structures of health care deliveries and health care systems with lacking financial resources for travel to get in-person training. Moreover, community involvement and partnerships across various stakeholders could maximize the quality of transgender health care and improve health-related outcomes, as it takes issues into account, that are not directly related to the delivery of medical services for transgender people (e.g., employment discrimination) (61). However, cons regarding decentralized care focused on the lack of expertise of health care providers and a fragmentation of care. In sum, comprehensive care with certain medical standards on the one hand, and individual care on the other, were the two central topics of our participants regarding pros and cons of centralized and decentralized delivery of transgender health care. This is in line with prior research, theorizing models of high-quality transgender health care (62). For high-quality, patient-centered transgender health care, however, these two main aspects, and their subcategories (**Table 3**), should not be understood as mutually exclusive for one setting of health service delivery, but rather be integrated and receive equal attention by providers and researchers (63).

The central limitation of the present systematic review is that the (de-)centralized delivery of health care was not systematically assessed as an outcome criterion in the included studies. The review, therefore, confirms that investigating the influence of the health care delivery setting is a so far under-studied perspective in transgender health and documents the need for further research. Moreover, countries from certain regions of the world, e.g., Africa, are underrepresented in the present study. Participants of the expert survey needed access to a web-enabled device and technical affinity, which could have excluded certain providers or researchers, e.g., from developing countries. Finally, a grey literature search is prone to overlook certain evidence.

It has been shown that the setting in which transgender health care is delivered differs between countries and health care systems. Moreover, both delivery settings were assessed as having certain advantages and disadvantages against the other. Taking these issues into account in future research and provision of health services might be a new important component to ensure high-quality transgender health care. E.g., if and how the health care delivery setting could affect the clinical outcome of transition-related health care (e.g., quality of life) is already part of ongoing studies (64). It should get more attention in future research studies and be considered as a potentially important variable that influences the health outcome and the quality of health care.

DATA AVAILABILITY STATEMENT

We will consider sharing de-identified, individual participant-level data that underlie the results reported in this Article on receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding author. The corresponding author and lead investigators of this study will discuss all requests and make decisions about whether data sharing is appropriate based on the scientific rigour of the proposal. All applicants will be asked to sign a data access agreement.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Psychological Ethics Committee (LPEK) at the Center for Psychosocial Medicine, University Medical Center Hamburg-Eppendorf (No.: LPEK-0071, date: 09/15/2019). The

participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AK contributed to the design of the study, undertook the statistical analysis, managed the literature searches, contributed to the qualitative analysis, wrote the first draft of the manuscript, and contributed to the correction of the manuscript. BS contributed to the design of the study and the correction of the manuscript. PB contributed to the design of the study and the correction of the manuscript. DS managed the literature searches, contributed to the qualitative analysis, and contributed to the correction of the manuscript. TN contributed to the design of the study, managed the literature searches, and contributed to the correction of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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CASE REPORT
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Case Report: Successful Use of Minoxidil to Promote Facial Hair Growth in an Adolescent Transgender Male

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Increasing numbers of trans and gender diverse young people are presenting to health services seeking gender-affirming medical care. While testosterone therapy in transgender males is generally effective in inducing masculinization, some adolescents encounter barriers to accessing such treatment or may not wish to experience all the changes that usually accompany testosterone. Here, we describe the case of a 17 year old trans male who presented with gender dysphoria but was initially unable to start testosterone therapy. Due to a desire for facial hair, he was therefore treated with topical minoxidil, an easily accessible, over-the-counter medication that has been used to treat androgenic alopecia for several decades. In this case, minoxidil was applied regularly to the lower face and, after three months of treatment, he developed obvious pigmented facial hair that was sufficient to help him avoid being misgendered. The only reported side effect was excessive skin dryness. Unexpectedly, despite no direct application to other areas, there was also an increase in pigmented body hair, suggestive of systemic absorption and effect. Given its long-standing use and safety record in the management of alopecia, minoxidil might thus represent a useful treatment option for trans males who desire an increase in facial hair.

Keywords: transgender, gender dysphoria, adolescent, minoxidil, hair growth

INTRODUCTION

Trans and gender diverse (TGD) individuals have a gender identity that differs from their sex assigned at birth. Gender dysphoria (GD) refers to the psychological distress that may arise from this incongruence. Recent estimates suggest 1.2-2.7% of young people within the general population identify as TGD (1, 2). With a high rate of associated mental health problems observed among the pediatric TGD population (e.g. depression, anxiety, self-harm, suicidality), there is a strong impetus for health professionals to provide early and effective gender-affirming care (3–7).

Abbreviations: TGD, trans and gender diverse; GD, gender dysphoria; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: DSM-5.

TGD individuals assigned female at birth have come to represent the majority of adolescents presenting to specialist gender clinics (8, 9). The majority of these individuals have a transmasculine identity, and testosterone is frequently used to induce reversible changes such as android fat redistribution and increased muscle mass, as well as irreversible changes such as a deeper voice and increased facial/body hair growth (10, 11). The choice of medical interventions varies and is often influenced by the desire to acquire particular masculine characteristics and/or avoid certain side effects. For instance, some individuals might want to delay or avoid testosterone, but desire access to treatments that help remove unwanted breast tissue (e.g. masculinizing chest surgery) or promote facial hair growth to avoid misgendering.

Currently, however, there are no recommended treatments used to specifically stimulate facial hair development in transmasculine individuals. Indeed, a recent literature review of potential therapies for specifically encouraging facial hair growth – including in cis-gender individuals – identified only two previous clinical trials (12). One trial examined use of topical 2.5% testosterone gel applied for six months to the beard area in men with thalassemia major – since male hypogonadism is a known complication of this condition – and reported a significant increase in facial hair density compared to those who received placebo gel (13). The other was a randomized, double-blinded, placebo-controlled trial of 3% minoxidil solution involving 48 Thai men, and which demonstrated a significant increase in facial hair counts after 16 weeks of treatment (14). Consistent with this, some transmasculine adults in online forums have described use of over-the-counter topical minoxidil to augment facial hair growth in the context of pre-existing systemic testosterone therapy. The concomitant use of testosterone in these informal reports, however, makes it difficult to know whether it was minoxidil, testosterone or their combination that was responsible for apparent improvement.

Minoxidil was first approved as an oral vasodilatory anti-hypertensive in 1979 (15). Early users noted increased hair growth (16) and, by the late 1980s, topical 5% formulations were developed to treat alopecia areata. Subsequent trials in cisgender individuals demonstrated beneficial effects in androgenetic alopecia (17–20). Since 1997, topical minoxidil has been available over-the-counter and, whilst oral minoxidil has adverse cardiovascular effects, side effects of topical administration have so far been limited to reversible hypertrichosis, pruritis and local skin irritation (16, 18–20).

Given its well-established role in promoting hair growth and relatively strong safety profile, topical minoxidil may represent an attractive treatment option for some transmasculine individuals, especially those wishing to increase facial hair growth either selectively or as part of a more generalized masculinization. However, we are unaware of any reports in the medical literature describing the use and effectiveness of topical minoxidil to stimulate facial hair growth in trans males. In this case report, we describe the safe and successful application of topical minoxidil in a trans male adolescent in the absence of any testosterone treatment.

CASE DESCRIPTION

Patient Information and Diagnostic Assessment

A 16 year old, previously well individual who was assigned female at birth presented to a pediatric gender clinic at the Royal Children's Hospital in Melbourne, Australia, with a transmasculine gender identity and, upon further mental health assessment, was diagnosed with GD as per DSM-5 criteria by an experienced child and adolescent psychiatrist. Previously, he had begun questioning his gender identity with the onset of puberty around age 10 years, started binding his chest at age 11 years, and subsequently disclosed his male gender identity to his friends and parents at age 14 years. After perceiving that his parents were overwhelmed by this news, he did not raise the issue again with them until a year later, after which he requested access to gender-affirming health care. At the Royal Children's Hospital, he was seen by a specialist multidisciplinary gender team, including a clinical nurse consultant, psychologist, psychiatrist, and pediatrician, who together helped manage his GD, which included facilitating a social transition, ensuring safe use of a chest binder, and prescribing norethisterone to suppress his menses, which had started at age 12 years and had not been previously associated with any problems (apart from gender dysphoria). However, due to legal requirements in Australia at the time, he was required to obtain Family Court approval to initiate therapy with testosterone.

Therapeutic Intervention, Follow-Up and Outcomes

While awaiting this approval and now aged 17 years, the patient asked whether he could try minoxidil to develop facial hair based on online accounts he had read. Clinical characterization at this time was unremarkable (**Table 1**) with no evidence of polycystic ovarian syndrome (e.g., no history of irregular menstrual cycles to suggest ovulatory dysfunction) nor hyperandrogenism [e.g. no signs of male pattern baldness, acne or hirsutism (see **Table 2** for modified Ferriman Gallwey score evaluation at baseline)]. With his pediatrician's agreement, he subsequently purchased minoxidil (5% lotion) and applied 1 mL twice daily to the

TABLE 1 | Patient characteristics.

Assigned sex	Female
Gender identity	Male
Age at referral	16 years
Age at commencement of minoxidil	17 years
Past medical history	Nil relevant
Baseline body mass index	24.5 kg/m^2
Baseline testosterone*	0.7 nmol/L
Baseline estradiol*	55 pmol/L
Baseline LH*	4.5 IU/L
Baseline FSH*	5.3 IU/L
Existing medication	Norethisterone 10 mg twice daily

Ideally these hormone levels – in conjunction with adrenal androgens – would have been measured in the absence of norethisterone treatment to fully gauge the underlying baseline androgenic profile, but the patient did not wish to cease norethisterone at this time.

beard area. Shortly after commencement, he noted skin dryness and irritation, and decreased application to five days/week and began regular moisturizing. Within one month, early fine hair growth was observed and he started shaving. By three months, there was significantly more facial hair, both in terms of number, thickness and length (up to 1 cm) (**Figure 1**). Interestingly, he also noted an increase in hair in areas with no direct topical application, including the eyebrows, inner forearms, fingers, posterior calves, dorsum of feet and toes, chest and lower abdomen (**Table 2**). After three months on minoxidil, he was able to commence testosterone therapy. His subsequent medical transition, which included bilateral mastectomy at age 18, has continued to proceed well and he currently remains on testosterone therapy under the care of adult gender services. At the time of writing, he continues to intermittently apply minoxidil to aid further facial hair growth, targeting areas where he feels additional growth is needed.

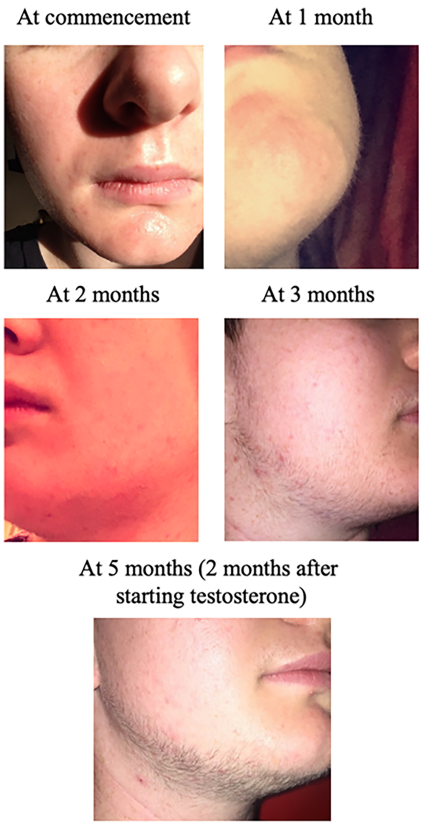

FIGURE 1 | Facial hair growth following topical minoxidil treatment. Minoxidil (5% lotion, 1 mL) was applied twice daily to the beard area. Photos were taken by the patient to document changes in facial hair at the indicated time points.

DISCUSSION

Gender affirming care for TGD youth includes social and psychological support and, in some cases, medical interventions. In trans males, there may be a desire for increased facial hair growth, since this is an archetypal masculine feature and one which often helps reduce misgendering. In this case report, we describe the use of minoxidil 5% lotion with good effect in a trans male adolescent prior to and continuing after testosterone therapy. We believe that this is the first reported use of minoxidil for this purpose in the medical literature, and the facial hair development that occurred within just three months was at least as good as what we have previously observed using testosterone over a similar time frame.

While testosterone monotherapy can encourage facial hair growth, this case report suggests that minoxidil alone may also be effective in doing so and provides a new pharmacological option for TGD individuals assigned female at birth. In particular, we can envisage minoxidil being used in several different contexts. Firstly, for transmasculine individuals on testosterone, facial hair growth may occur slowly and/or to an insufficient extent despite achieving target serum testosterone levels. In such cases, minoxidil may be a means to accelerate and/or augment facial hair growth. Secondly, for individuals desiring testosterone but yet to access it, minoxidil may enable partial masculinization and thus help with GD and reduce misgendering. Although our patient's delay in accessing testosterone was due to legal requirements in Australia at the time, TGD adolescents worldwide commonly incur barriers to accessing gender-affirming care (due to e.g. lack of suitable services, long wait times, insufficient family support) and minoxidil might therefore provide an attractive option for such young people. Thirdly, some TGD individuals may wish to masculinize without experiencing all the effects expected with testosterone. For example, some might wish to avoid impairing their reproductive function or irreversibly deepening their voice but may wish to develop facial hair. This desire for some but not all masculinizing effects is often seen in individuals assigned female at birth who have a non-binary gender identity. At present, it is difficult to accommodate such specific needs, and minoxidil may offer a potential solution.

The mechanism of minoxidil action on hair growth is unclear but has been postulated to act *via* potassium channels (21) or by

TABLE 2 | Modified Ferriman-Gallwey score evaluation before and after 3 months of minoxidil monotherapy.

Area*	Before Minoxidil	After 3 months of Minoxidil
upper lip	0	2
chin	0	3
chest	0	1
upper abdomen	0	1
lower abdomen	1	1
thighs	1	1
back	0	1
arm	1	1
buttocks	0	0
Total score	*3*	*11*

**The modified Ferriman-Gallwey score grades the nine listed body areas from 0 (no hair) to 4 (virile). A total score of ≥8 is considered elevated for a Caucasian individual assigned female at birth.*

causing an influx of intracellular calcium (22). Despite its unclear mechanism, the efficacy of topical minoxidil on scalp hair growth is well established in cisgender men and women. Our patient used the 5% lotion preparation of minoxidil. Typically, the 2% solution is marketed for women and the 5% solution is marketed for men. The 5% preparation has been shown to be more effective in women (18) and men (19), although it was also accompanied by increased adverse effects such as skin irritation. Our patient found the use of 'rest days' and moisturizer helpful in alleviating skin dryness and irritation. Alternative minoxidil preparations less likely to cause skin irritation would have been a foam (20), which is free of propylene glycol, or a 2% solution which is typically water-based.

The observation in our case that areas of skin not contacted by minoxidil demonstrated increased hair growth was unexpected and implies systemic absorption, as has been described by some (23) but not others (17). Given the potential for systemically absorbed minoxidil to have adverse cardiovascular and fetal developmental effects (16, 24), clinicians and individuals should be aware of this possibility.

Looking ahead, several important knowledge gaps remain. Firstly, one limitation of this study is that minoxidil was used as a stand-alone treatment for only 3 months before testosterone was started. A longer duration would have provided better opportunity to assess the full potential of minoxidil monotherapy to promote facial hair growth. Consistent with this, the growth observed was relatively limited, but this is to be expected given the short time frame involved. After all, facial hair takes 4-5 years to fully develop as a result of systemic testosterone treatment in transmasculine individuals (11). Therefore, it is possible that further hair growth would have been observed should our patient have continued on minoxidil alone for a longer period. but we do not know to what extent this would have occurred nor when it would have plateaued. It is thus difficult to predict if there is a limit to the amount of facial hair growth minoxidil can stimulate and how this compares to the effects of testosterone alone. Secondly, the reversibility of minoxidil therapy was not able to be assessed in this case, since the patient subsequently started on testosterone. Previous reports suggest that the effect of minoxidil in promoting hair growth in cisgender individuals is reversible, and that its cessation should lead to regression of hair growth within 3 to 4 months (24). This likely reversibility might increase the attractiveness of minoxidil for use in TGD adolescents, especially given concerns about potential long-term regret following irreversible masculinizing changes, but it would be important to specifically assess reversibility when used in this context. Future research in trans males should look towards establishing the efficacy and safety of topical minoxidil (in its two main strengths and different preparations) using clinically meaningful endpoints for facial hair growth.

In conclusion, we present the case of a 17 year old trans male who was treated with topical minoxidil prior to commencement of testosterone therapy. His outcomes demonstrate the feasibility of using topical minoxidil to augment facial hair growth in transmasculine patients and are likely to be of interest to both other patients as well as clinicians working in this area.

PATIENT PERSPECTIVE

Despite some skin dryness and irritation, our patient was satisfied with the effect of topical minoxidil in promoting facial hair growth. Given the legal requirements in Australia at the time, his use of minoxidil helped him to achieve a more masculine appearance while awaiting formal approval to start testosterone therapy, and he was very pleased by this. In particular, he felt that his facial hair growth while on minoxidil for the first three months helped not only to improve his gender dysphoria by allowing him to feel more comfortable with his facial appearance but also to reduce his social anxiety by decreasing the likelihood of being misgendered by others. At the time of writing, he continues to use minoxidil intermittently, which is a testament to his ongoing satisfaction with its effects.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RU and TN collated resources, drafted and revised the manuscript. KP was the treating clinician, conceptualized the case report, and drafted and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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Cardiovascular Risk Associated With Gender Affirming Hormone Therapy in Transgender Population

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Transgender men and women represent about 0.6 -1.1%% of the general population. Gender affirming hormone therapy (GAHT) helps ameliorate gender dysphoria and promote well-being. However, these treatments' cardiovascular (CV) effects are difficult to evaluate due to the limited number of extensive longitudinal studies focused on CV outcomes in this population. Furthermore, these studies are mainly observational and difficult to interpret due to a variety of hormone regimens and observation periods, together with possible bias by confounding factors (comorbidities, estrogen types, smoking, alcohol abuse, HIV infection). In addition, the introduction of GAHT at increasingly earlier ages, even before the full development of the secondary sexual characteristics, could lead to long-term changes in CV risk compared to current data.

This review examines the impact of GAHT in the transgender population on CV outcomes and surrogate markers of CV health. Furthermore, we review available data on changes in DNA methylation or RNA transcription induced by GAHT that may translate into changes in metabolic parameters that could increase CV risk.

Keywords: transgender women, transgender men, transgender population, gender affirming hormone therapy, cardiovascular risk

INTRODUCTION

Transgender people represent about 0.6 – 1.1% of the general population, 0.7 and 1.1% of people assigned male at birth, and 0.6 and 0.8% of people assigned female at birth, as described in an European study of T'Sjoen et al. (1). Gender affirming hormone therapy (GAHT) helps ameliorate gender dysphoria by changing the physical appearance in accordance with gender identity and expression and promote well-being (2). However, this therapy's cardiovascular (CV) effects are difficult to evaluate because most of the studies are observational and can have a possible bias due to confounding factors such as comorbidities, variety of hormone regimens, smoking, alcohol abuse or HIV infection. Cardiovascular disease (CVD) is the main cause of death for transgender people undergoing GAHT, although suicide is still the leader of all-cause mortality (3). However, for

transgender women, the risk of death from CVD is 3-fold higher than for ciswomen and cismen and has been associated with the use of ethinyl estradiol (EE) (2, 4). Moreover, the introduction of GAHT at increasing earlier ages may lead to changes in CV risk compared to current data.

This review examines the impact of GAHT in transgender people on CV outcomes and surrogate markers of CV health. Furthermore, we review available data on changes in DNA methylation or RNA transcription induced by GAHT that may translate into changes in metabolic parameters that could increase CV risk.

METHODS

We performed a review to evaluate CV health in transgender population. We searched in PubMed/MEDLINE databases for articles with this topic, we included articles published until April 2021, and we limited the search to English language articles. The Keywords were transgender, transgender men, transgender women, hormone therapy, GAHT, estrogen, antiandrogen, progesterone, testosterone, cardiovascular disease, and cardiovascular risk factors.

We included retrospective, observational, cohort, cross-sectional studies, population survey of transgender individuals, with a minimum population size of 100 individuals and a follow-up of 1 year, with GAHT regardless of doses or gender affirming surgery in which CV outcomes (thromboembolism, myocardial infarction, stroke) and surrogate markers of CV risk have been assessed.

FEMINIZING HORMONE THERAPY IN TRANSGENDER WOMEN

Current evidence from Europe and America suggests that GAHT initiated and monitored under medical supervision is associated with very low rates of adverse events (5, 6). However, factors associated with a higher risk of thromboembolic conditions, such as smoking, obesity, and sedentary lifestyle, should be evaluated in transgender women prior to initiating GAHT and modified if possible. In certain cases, using the transdermal route and anticoagulant treatment should be considered to prevent thromboembolisms. In addition, other diseases such as coronary artery and cerebrovascular disease, hormone-sensitive cancers, hyperprolactinemia, hypertriglyceridemia, and cholelithiasis should be assessed, as these conditions can be exacerbated by estrogen and may be considered relative contraindications for GAHT (7, 8). Moreover, information related to fertility preservation should be provided, and options for preservation should be discussed and offered before starting the medication.

Types of Hormonal Therapy in Transgender Women

There are two main classes of medications used in transgender women: estrogenic therapies and androgen-lowering hormone therapies. **Table 1** shows the most frequent regimens used nowadays.

In relation to estrogenic therapies, EE is a synthetic estrogen widely used in Europe prior to 2003 (9). However, given recent safety concerns about its prothrombotic potential and its possible role in CV disease, most clinicians have now switched to oral, cutaneous, or IM estradiol valerate (7). Studies that compare the long-term safety and effectiveness among the different formulations of estradiol are lacking. The Endocrine Society guidelines recommend titrating the doses to serum estradiol levels at 100-200pg/ml (367.1-734.3 pmol/l) (7).

In general, androgen-lowering therapies are required to reduce testosterone levels into the female range. One of the most prescribed androgen-lowering medications is oral cyproterone acetate (CPA) (5, 10). CPA is an androgen receptor blocker but also has some progesterone-like activity (11). Due to reports of increased incidence of meningiomas (12–14), association with depression, and increased

TABLE 1 | Gender affirming hormone therapy.

Hormone	Route	Doses	Considerations
Transgender women			
Estradiol valerate	Oral	2 – 6mg/d	<45 years
Estradiol	Transdermal patch. New patch	0.025 – 0.2mg/d	>45 years
Estradiol valerate or cypionate	placed every 3 – 5 d	5 – 30mg IM every 2wk	
	Parenteral	2 – 10mg IM every wk	
Anti-androgens			
Spironolactone	Oral	100 – 300mg/d	Preferred in USA
Cyproterone acetate	Oral	25 mg/d	Preferred in Europe
Triptorelin (GnRH agonist)	SC	3.75mg/monthly	Preferred in UK instead
		11.25mg/3 monthly	of antiandrogens
Transgender males			
Testosterone			
Testosterone enanthate or cypionate	Parenteral	100 – 200mg every 2-4 wk or 50% per 1-2 wk	
Testosterone undecanoate	Parenteral	1000mg every 12 wk	
Testosterone gel 1.6%	Transdermal	50 – 100mg/d	
Testosterone patch	Transdermal	2.5 – 7.5mg/d	

SC, subcutaneous; WK, week.

risk of hyperprolactinemia with CPA use, the maximum recommended dose is 25mg daily (15, 16). The study by Kuijpers et al. has observed that the 10mg dose of CPA is as effective as higher doses but with fewer side effects (17). In UK, Italy, and Netherlands, transgender women are now treated with GnRH agonist (GnRHa) to lower testosterone levels (18). GnRHa substitutes the pulsatile physiological release of GnRH by a continued release of the GnRHa administrated and inhibits the secretion of FSH/LH from the pituitary to the testicle/ovary. Gosereline and leuprolide have been used instead of CPA to reduce testosterone levels and lower adverse reactions (7). Spironolactone lowers testosterone synthesis and action at the androgen receptor and is also an antagonist of the mineralocorticoid receptor and potassium-sparing diuretic. Flutamide also has antiandrogenic effects, but it is not recommended (7, 18, 19).

Progesterone therapies such as medroxyprogesterone have been used to reduce testosterone concentrations in transgender women (19). Some may ask for progesterone to enhance breast development; however, clinical evidence does not support this effect (20). Furthermore, there are concerns regarding the potential increased risk of thromboembolism and stroke found in cisgender women taking progesterone (21, 22).

Finasteride therapy (5α-reductase inhibitor) has effectively improved hair loss in transgender women with androgenic alopecia without significant side effects. Nevertheless, the routine use of 5α-reductase inhibitors has been limited over previous concerns of long-term sexual dysfunction and depression reported in cisgender men (23, 24).

Physical Changes in Transgender Women

The aim of GAHT in transgender women is to induce feminine and reduce masculine physical traits. The development of breast tissue is one of the most expected changes in transgender women and is associated with improvement in body discomfort score (25). However, less than 20% of transgender women reach Tanner breast stage 4 to 5 after 24 months of hormone therapy; therefore, mammoplasty is often requested. Several studies show that a plateau is achieved within the first 6-9 months of treatment (26–28). Recent data suggests that sustained breast development was observed during a period of three years of follow-up with a more lateral and caudal position compared to ciswomen (28). Testicular volume decreases ~ 60% after 24 months of GAHT is also observed, as well as reduction of erections and ejaculation (25). An increase in body weight was associated with an increase in body fat, specifically in the gynoid regions, and a reduction in lean body mass (25, 29–31). Facial hair diminished and the Ferriman-Gallwey scores improved after two years of GAHT, as well as the hair loss pattern (25). Voice changes are seldom observed; therefore, transgender women will have to look for voice therapy or phonosurgery (32, 33).

Metabolic Changes in Transgender Women

The metabolic effects of estrogen therapy are focused on liver function alterations and lipid parameters. Hepatic lipase activity decreases by 64% and lipoprotein lipase by 23%. Hepatic lipase decreases HDL-cholesterol (HDL-c) levels and increases the formation of small, dense LDL (LDL-c) highly atherogenic. Reduction of hepatic lipase levels with estrogen may reduce the formation of LDL-c (34). However, the use of estrogen therapy in transgender women showed no statistically significant difference in total cholesterol, serum LDL-c or HDL-c, but an increase in plasma triglyceride levels after 24 months was found (35). Conflicting data seem to show unmodified or reduced insulin sensitivity, with unaltered fasting glucose and stable or increased blood pressure (34). Estrogen therapy in transgender women has been associated with reduced plasma homocysteine levels, independently of the route of administration (36, 37). The impact of estrogens on prothrombotic status remains unclear. While some studies with EE in combination with CPA, but not with transdermal estradiol, show an increase in C-reactive protein (CRP) and decreased tissue plasminogen activator; other studies have shown no effect on CRP (38, 39).

Cardiovascular Outcomes in Transgender Women

Data in transgender women receiving estrogen therapy are limited to observational and cohort studies. However, retrospective studies have shown a higher incidence of thromboembolic events in transgender women with EE and CPA compared to a similar reference group of the population (40) and using equine estrogens compared to estrogen valerate or EE when mammoplasty was performed (16). **Table 2** shows the studies that evaluated CV outcomes and mortality associated with GAHT.

A systematic review and meta-analysis of CV outcomes in transgender people reported few cases of myocardial infarction (MI), stroke, or venous thrombosis; however, the incidence was higher in transgender women compared to transgender men (35, 40).

In 2018, a nationwide US survey was distributed across 22 states and included questions about the transgender condition; 0.6% of those surveyed identified as transgender people. The study found that transgender women reported higher MI than cisgender women (OR 2.9; 95% CI, 1.6 to 5.3; $p<0.001$) but with no differences when compared to cisgender men (44).

In addition, a survey conducted by the Centers for Disease Control and Prevention, with 1.8 million participants, also observed that all transgender individuals receiving GAHT had significantly higher rates of MI compared to their cisgender counterparts; after adjusting for CVD risk factors, transgender women had more than a two-fold increased risk in MI compared to cisgender women. Transgender women had no significant difference in MI risk compared to cisgender men (45).

A Dutch study of 2517 transgender women using estrogen followed for an average of 9 years found twice as many strokes and MIs as in cisgender women and almost twice as many strokes and no difference in MIs compared to cisgender men; also a five-fold and four-fold increase risk in thromboembolic events compared to both ciswomen and cismen, respectively (46).

TABLE 2 | Cardiovascular outcomes.

Author	Country/Year	n	Study	GAHT	Follow-up	Time of GAHT	CV Outcomes (n)	Mortality
Asscheman H et al. (40)	Netherlands 1989	303 TW 122 TM	Retrospective study	EE 100ug+ CPA 100mg Long-acting testosterone ester 250mg/2wk Oral testosterone undecanoate 120 -160mg/d	14 years	TW 4.4yr TM 3.6yr	VT/PE (29) TW Weight ↑ >10% MI (2) TW	No CV mortality
Van Kesteren PJ et al. (41)	Netherlands 1997	816 TW 293 TM	Retrospective study	Oral estrogens + anti-androgens. Transdermal estradiol			VT/PE TW No morbidity in TM	Total mortality was no higher than in the general population
Asscheman H et al. (4)	Netherlands 2011	966 TW 365 TM	Cohort study	EE or conjugated estrogens (until 1989). Transdermal E2 Estradiol valerate 2-4mg/d CPA 100mg/d Spironolactone 100 – 200mg/d. Testosterone ester IM 250mg/2wk Oral testosterone undecanoate 160-240mg/d Transdermal testosterone 50mg/d Lynestrenol (uterine bleeding persisted)	18.5 years	TW 19.4yr TM 18.8yr	Ischemic heart disease 18 TW 1 TM Cerebrovascular accidents 5 TW 0 TM	SMR TW 1.51 (1.47-1.55) SMR TM 1.12 (0.89-1.59)
Dhejne C et al. (3)	Sweden 2011	191 TW 133 TM	Cohort study	Not available	30 years		Not available	HR 2.5 (1.2-5.3)
Wierckx K et al. (42)	Belgium 2012	50 TW 50 TM	Cross-sectional study	Estrogen + CPA Testosterone	10 years	TW 6.3yr TM 8.7yr TM	Only TW TV/PE (4) TIA (1) MI (1)	Not available
Wierckx K et al. (43)	Belgium 2013	214 TW 138 TM	Case-control study	Transdermal estradiol gel/patch Estradiol valerate 2mg EE 50mcg Oral contraceptive Testosterone ester IM 2-3wk Transdermal testosterone 50mg/d Oral testosterone undecanoate	22 years	TW 7.7yr TM 9.7yr	TW VT/PE MI TIA/CVD T2DM TM VT/PE T2DM	9 TW 1 TM
Wierckx K et al. (5)	Belgium 2014	53 TW 53 TM	Prospective multicenter study	<45yr 50mg CPA + 4mg valerate estradiol >45yr 50mg CPA + 100ug/24hs transdermal 17B estradiol IM Testosterone undecanoate every 3 months	12 months	12 months	No severe adverse events	No deaths were observed

(Continued)

TABLE 2 | Continued

Author	Country/Year	n	Study	GAHT	Follow-up	Time of GAHT	CV Outcomes (n)	Mortality
Nokoff N et al. (44)	USA 2018	369 TW 239 TM 156 GNC	Secondary Data analysis based on 2015 Behaviors Risk Factor Surveillance System Survey (CDC) Cross-sectional study	Not available	–	–	TW HTA 29.2% MI 5.5% Angina or CHD 3.5% Stroke 2.6% TM HTA 25.2% MI 2% Angina or CHD 3.1% Stroke 2.3% No differences in GNC between cisgender men and women	
Alzahrani T et al. (45)	USA 2019	1842439 1267 TM 1788 TW	Combined data of Behavioral Risk Factor Surveillance System (BRFSS) (CDC) Cross-sectional study	Not available	-	-	MI TW OR 2.56 vs CIS W No increase vs CIS M TM OR 2.53 vs CIS M OR 4.90 vs CIS W	Not available
Nota NM et al. (46)	Netherlands 2019	2517 TW 1358 TM	Retrospective study	Not available	43 years	TW: 22.83yr TM: 11.03yr	TW Stroke (29) MI (30) VT (73) TM Stroke (6) MI (11) VT (2)	Not available
Scheres JLL et al. (47)	European Network for the Investigation of Gender Incongruence (ENIGI) 2021	92 TW 100 TM	Longitudinal study Baseline and 12 months after GAHT	Oral estrogen Transdermal estrogen Anti-androgen therapy + estrogen oral Anti-androgen monotherapy Transdermal testosterone IM testosterone	12 months	12 months	TW ↑FIX ↑FXI ↓pC ↓ activated protein C resistance	Not available

CHD, Coronary heart disease; CIS M, cisgender men; CIS W, cisgender women; CPA, Cyproterone acetate; CV, cardiovascular; EE, Ethinyl Estradiol; FIX, Factor IX; FXI, Factor XI; GAHT, gender affirming hormone therapy; HR, Hazard ratio; MI, Myocardial Infarction; pC, C protein; TIA, Transient ischemic attack; TM, Transgender men;TW, Transgender women; VT/PE, venous thrombosis, embolism pulmonary; ↑: increased; ↓: decreased.

MASCULINIZING HORMONE THERAPY IN TRANSGENDER MEN

Masculinizing GAHT in transgender men favors male secondary sex characteristics and minimizes feminine traits. Transgender men must be informed of the necessity of lifelong therapy with testosterone, its possibilities, risks, consequences, and limitations. Information related to options for the preservation of fertility should be provided before starting GAHT (7). Pregnancy contraindicates testosterone therapy, and relative contraindications include severe hypertension, sleep apnea, and polycythemia. Erythrocytosis, sleep apnea, and congestive heart failure can be exacerbated by testosterone therapy (7).

Types of Hormonal Therapy in Transgender Men

The main GAHT used to induce virilization is testosterone. Different testosterone formulations may be available depending on geographical location (**Table 1**). Most prescribed are injectable testosterone esters. Both parenteral and transdermal administration of testosterone are equally effective to achieve masculinization and serum testosterone values in the range of 300 - 1000ng/dl (10.4–37.4 nmol/l) in transgender men. Serum testosterone levels in injectable formulations are measured between administrations, although clinicians may choose to measure serum testosterone 24hs after injection and prior to the next dose (7).

More recently, the subcutaneous administration of testosterone was shown to be effective and preferred by transgender men at a median dosage of 75mg weekly (48). In Pelusi et al. study, the effects of three different testosterone formulations (gel, cypionate, and undecanoate) were evaluated at baseline. After 12 months of treatment, no differences were found regarding short-term safety, compliance, body composition, or metabolic parameters (49).

If menstrual bleeding does not stop after initiation of testosterone, a progestational agent, such as oral lynestrenol at 5 to 10mg daily or medroxyprogesterone at 5 to 10mg, might be considered (7, 50).

Physical Changes in Transgender Men

Menses discontinuation, clitoris enlargement, and lower-pitched voice are some of the changes aimed by transgender men (7, 51, 52). In addition, therapy will enhance a more masculine musculature, body shape with an increase in body weight, a decrease in body fat, and an increase in lean mass as well as grip strength (51–55). Testosterone therapy has been associated with increases in the Ferryman-Gallwey hirsutism scores. However, after 12 months, facial and abdominal hair do not reach diameters found in cisgender males. An increase in acne and alopecia is often observed as some of the side effects (51, 52, 54).

Metabolic Changes in Transgender Men

Lipid parameters are adversely modified by testosterone therapy in transgender men. A recent meta-analysis of the available data demonstrated no change in total cholesterol or LDL-c. Still, there was a minor increase in triglyceride and a decrease in plasma HDL-c levels, both of which are pro-atherogenic (55, 56). Another meta-analysis showed a progressive change in lipid parameters over 24 months with higher triglycerides levels compared with baseline; statistically significant serum LDL-c increase and HDL-c decrease were also observed, with no statistically significant differences in total serum cholesterol level (56). Testosterone therapy has no effect on fasting glucose, fasting insulin, or glucose utilization. However, transgender men were found to have decreased adiponectin, which is associated with insulin resistance and higher CV risk (34). Regarding blood pressure, the results of various studies are contradictory (57). Testosterone therapy increases plasma homocysteine levels in transgender men, which could have a negative impact on CV risk. After one year of hormonal treatment, transgender men presented increased homocysteine and leucocytes levels, with an increase in mean maximum carotid intimal media thickness (36, 54).

Cardiovascular Outcomes in Transgender Men

Present evidence regarding testosterone therapy and CV disease risk in transgender men is controversial. Several studies have observed that despite the negative effects of testosterone therapy on surrogate risks factors of CV disease, these do not translate into a significant effect on CV outcomes (**Table 2**). Furthermore, no elevated rates of CV deaths have been observed when compared with cisgender men and women at short and medium follow-up (30, 35, 40, 56).

In a cross-sectional study of 50 transgender men on testosterone therapy during an average of 10 years, no subject had experienced MI, stroke, or deep venous thrombosis (57). In a similar case-control study, 138 transgender men on testosterone therapy for an average of 7.4 years showed low CV morbidity. In transgender men, MI was higher when compared to cisgender women, but there was no difference when compared to cisgender men. After adjustment for CV risk factors, however, the study demonstrated that transgender men had an increased risk for MI compared to both cisgender populations. This study emphasizes the importance of additional CV risk factors such as smoking, reduced exercise, diabetes, and non-Caucasian ethnic origin, all of which were seen in higher numbers in the transgender population (35, 43). Similar data in the Dutch analysis of 1358 transgender men using testosterone followed for an average of 8 years, which found three times more MIs as in cisgender women with no differences compared to cisgender men and no differences in stroke compared to cisgender women or men (46).

There have been reports of a possible link between testosterone replacement therapy use and increased venous thromboembolism risk; however, these studies were criticized for including data on avascular necrosis of the femoral head, which are not classically viewed as venous thromboembolic events. Extensive epidemiological studies have demonstrated that there is no link between testosterone therapy and thromboembolism risk (46, 58).

HORMONAL TREATMENT IN ADOLESCENTS

The treatment in adolescents is generally based on two phases: the first phase consists of suppressing the gonadal axis once puberty has commenced (Tanner 2-3); The second phase is the introduction of the GAHT. Gonadal suppression is generally performed with GnRHa such as gosereline, leuprolide, triptoreline, and histrelin; it provides the adolescent and its family with the time and the space to explore the gender identity before the treatments starts which can imply irreversible changes. Suppression also improves the anxiety for developing the secondary sexual characters associated with the gender assigned at birth (7, 59). Scarce information is available concerning the metabolic effects of suppressive treatment; however, a decrease in height velocity in both transgender girls and boys is observed, as well as an increase in body mass index in comparison to the gender assigned at birth, with an increase in fat body mass and a decrease in lean body mass during the first year, that is stabilized afterwards. No effects on lipid or carbohydrate metabolism have been described (59). However, long-term CV effects are still unknown, so a healthy lifestyle and no smoking are encouraged. The effects of GnRHa on bone structure are still in debate. Data suggest that bone mineral density is preserved, but z-score decreases (more in transgender girls than boys) with improvement after GAHT introduction (60). Some side effects must be monitored, such as flushing, headache, mood changes, and hypertension (triptoreline) or intracranial hypertension (rare and associated to leuprolide). Other less effective alternatives used as a suppressive treatment are CPA in transgender girls or medroxyprogesterone to suppress menses in transgender boys (59–63).

GAHT introduction is generally recommended around the age of 16, although it can be considered around the ages of 14-16, even though there are very few published studies of being administered between the ages of 13.5 and 14 (7). There are two treatment regimens. In the case that GnRHa was introduced early in pubertal development, the puberty of the desired gender is induced by slow increasing doses of testosterone or estradiol, that are modified every six months. In the case that GnRHa began late in puberty, the suppression lasts about 3-6 months and GAHT begins at higher doses, with a faster increase to achieve maintenance dose (7, 62).

Since the long-term effects of both suppressive and GAHT treatment are uncertain, adolescents must be encouraged to adopt a healthy lifestyle, increase exercise, avoid tobacco, and keep regular check-ups with the endocrinologist for the monitorization of liver and renal function, lipids, and glucose.

MOLECULAR EFFECTS OF GENDER AFFIRMING HORMONE THERAPY

Basic research has found Androgen Receptors (AR) and Estrogen Receptors (ERs) in endothelial cells, suggesting that masculinizing and feminizing hormones have a direct impact on the vascular endothelium. Testosterone and estrogen bind to these receptors producing an increase in transcription of athero-protective genes and a downregulation of pro-atherogenic genes, which could be associated with a decrease in CV risk (64). In the work carried out by our group, we observed an increase in the ER methylation pattern in transgender men after 12 months of GAHT, an increase in AR methylation pattern in transgender women after 12 months of estrogenic treatment. Regarding the expression analysis, AR expression was significantly decreased in transgender men. AR, ER methylation were correlated with anthropometric, metabolic, and hormonal parameters, supporting that GAHT is associated with epigenetic changes that might affect the response to treatment with sex steroids (65). More recent data has also shown that GAHT modified the methylation pattern of ER, more similar to their gender (66). Therefore, research in methylation and expression of AR and ER may help to understand the different effects of GAHT in physical, metabolic, and CV outcomes in transgender people.

DISCUSSION

Several studies have been published concerning the metabolic effects of GAHT in both transgender women and men. However, data for metabolic effects are often contradictory and inconclusive. The main reasons are the observational and retrospective nature of studies, including populations with diverse hormonal regimens without medical supervision; most of them include EE at high doses in transgender women; the influence of possible bias by confounding factors (comorbidities, smoking, alcohol abuse, or HIV infection) (2–5, 9, 35, 40, 46).

In transgender women, recent regimens have excluded EE and suse oral and transdermal estradiol associated to CPA or other androgen-lowering agents under endocrinological control. Unfortunately, prospective data are still limited, and it is uncertain if these changes may improve CV and thromboembolic risk (2–7, 35, 40, 45). However, recent data suggest a higher risk of MI compared with ciswomen with no differences in comparison to cismen (44–46).

In transgender men, previous data suggest a lower risk of CV events in comparison to birth–assigned males, probably due to testosterone introduction at later ages and the possible protective effect of endogen estrogens before GAHT (2–7, 35, 40, 44–46). A prospective study performed by our group showed an impairment of lipid profile and an increase of homocysteine and leucocytes count, as well as a higher mean-maximum common intima-media thickness after 12 months of GATH (55). Recent data suggest a higher risk of MI in transgender men in comparison with ciswomen.

The introduction of GAHT at increasingly earlier ages, even before the full development of the secondary sexual traits, could lead to long-term changes in CV risk compared to current data and bring it closer or higher to the known risk of the identified gender (59–63). Future research is essential to find out this risk.

Moreover, non-binary transgender population may yearn for a more personalized treatment with a partial suppression of the traits associated with the gender assigned at birth and the development of some of the traits of the other gender. No clear regimens have been established, and no information is available concerning the effects on CV risk. Hence, future research in this group is necessary to ascertain their risk of CV illness.

In conclusion, future research should join forces to obtain data from prospective controlled studies, including larger samples. Therefore, studies should consider the introduction of GATH at progressively younger ages as well as the voice of non-binary transgender population in order to improve the knowledge of the CV effects of hormone therapy in these situations.

AUTHOR CONTRIBUTIONS

Conceived and designed the review: MM. Writing the manuscript: GA, MM, IH and NS. Revised the manuscript and contributed significantly to the intellectual value of the paper: IH, FH, EG-G, and AG. All authors contributed to the article and approved the submitted version.

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Metoidioplasty: Surgical Options and Outcomes in 813 Cases

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Introduction: Metoidioplasty is a variant of phalloplasty for transmen that includes the creation of the neophallus from a hormonally enlarged clitoris, urethral lengthening and scrotoplasty. The procedure results in male appearance of genitalia, voiding in standing position and preserved sexual arousal, but without possibility for penetrative intercourse. We evaluated outcomes of metoidioplasty at our center, based on latest surgical refinements.

Methods: During the period of 14 years (from February 2006 to April 2020), 813 transmen with mean age of 24.4 years and mean body mass index of 24.6, underwent one stage metoidioplasty. Hysterectomy was simultaneously performed in 156, and mastectomy in 58 cases. Hysterectomy, mastectomy and metoidioplasty were done as a one-stage procedure in 46 transmen. Patients are divided in 5 groups, depending on the type of urethroplasty. Postoperative questionnaires were used to evaluate cosmetic and functional outcomes, as well as patients' satisfaction.

Results: Follow-up ranged from 16 to 180 months (mean 94 months). Mean surgery time was 170 minutes and mean hospital stay was 3 days. Length of the neophallus ranged from 4.8 cm to 10.2 cm (mean 5.6 cm). Urethroplasty was complication-free in 89.5% of cases, and ranged between 81% to 90.3% in different groups. Urethral fistula and stricture occured in 8.85% and 1.70% of cases, respectively. Other complications included testicular implant rejection in 2%, testicular displacement in 3.20% and vaginal remnant in 9.60% of cases. From 655 patients who answered the questionnaire, 79% were totally satisfied and 20% mainly satisfied with the result of surgery. All patients reported voiding in standing position and good sexual arousal of the neophallus, without possibility for penetrative intercourse due to small size of the neophallus.

Conclusion: Metoidioplasty has good cosmetic and functional outcomes, with low complication rate and high level of patients' satisfaction. In transmen who request total phalloplasty after metoidioplasty, all available phalloplasty techniques are feasable.

Keywords: gender affirmation surgery, metoidioplasty, phalloplasty, urethroplasty, phalloplasty complications

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INTRODUCTION

The prevalence rate of gender dysphoria can not be determined precisely, but estimations range between 5-14 per 100.000 and 2-3 per 100.000 for persons assigned male at birth (AMAB) and assigned female at birth (AFAB), respectively. Recent studies suggest that the prevalence of a self-reported transgender identity in children, adolescents and adults ranges from 500 to 1300 per 100.000, markedly higher than prevalence rates based on clinic-referred samples of adults (1–3). The World Professional Association for Transgender Health (WPATH) has established the Standards of Care (SOC), the guidelines for the multidisciplinary treatment of such individuals (4). The treatment consists of diagnostic assessment, psychotherapy, hormonal therapy, and surgical therapy. Psychiatric assessment is the first step and is very complex because it is necessary to confirm gender dysphoria. The next step is hormonal therapy, which evidently improves emotional well-being and social functioning (5). Some individuals decide to continue to gender affirmation surgery (GAS), which presents the last and irreversible step in a transition process. According to SoC guideliness, individuals who wish to undergo GAS are required to provide two recommendation letters from certified psychiatrists and a gender specialist, as well as a confirmation of having been on hormonal therapy prescribed by an endocrinologist for a period of a minimum of one year.

With the increasing prevalence of transgender adults, including persons with binary and non-binary identities, the number of gender affirmation surgery (GAS) has also risen throughout the world. Heterogenity within the trans group and different requirements regarding the requests for GAS pose a question of additional analysis regarding the patient wishes, expectations and limitations of available surgical approaches. Non-binary transgenders may require only partial genital reconstruction that will satisfy their needs of standing micturition, sexual function and esthetic appearance (6, 7). After 50 years of surgical experience and numerous modifications of techniques, neophalloplasty still presents a greatest challenge in GAS (8). Metoidioplasty is a variant of phalloplasty that includes the creation of the small-sized neophallus from a hormonally hypetrophied clitoris, urethral lenthening, perineoplasty and scrotoplasty. The results are male-like genitalia, voiding in standing position and preserved sexual arousal, but without possibility for penetrative intercourse. Additional advantages are full erogenous sensation, the ability of erection and minimal scarring of the donor-site in a single stage procedure. Clear understanding of the anatomy of the clitoris and its surrounding tissues are essential for performing successful metoidioplasty in transmen (9). Several authors have attempted to use the hypertrophied clitoris for neophalloplasty, along with urethroplasty in the past decades (10–16). The technique was first mentioned in 1973 by Durfee and Rowland, while the term metoidioplasty was used first by Laub (10, 11). Laub performed a staged procedure, but voiding in a standing position was not achieved after the first stage. Bouman reconstructed the urethra until the tip of the neoglans without ventral chordee release (12). Hage combined the techniques of Bouman and Laub to obtain voiding while standing (13). Lebovic and Laub in 1999 reported good results in the appearance of external genitalia, but the neophallus was usually small and curved because of the intact urethral plate (14). Later in a long-term follow-up study Hage and van Turnhout stated that an average 2.6 procedures are needed to achieve satisfying outcomes after metoidioplasty (15). A new modification was published by Perovic and Djordjevic with higher success rates using tubularized flaps for urethral reconstruction in 2003 (16). Wide experience with surgical reconstruction of hypospadias improved the technique futher which resulted in the latest use of combined buccal mucosa or genital skin grafts with different local genital flaps to achieve a successful result. Latest advances in surgical techniques and perioperative care enabled safe one-stage gender affirmation surgery, metoidioplasty with hysterectomy and mastectomy (17, 18).

The aim of this study is to evaluate outcomes and complications in 813 cases of metoidioplasty in transmen, focusing on our modifications of urethroplasty techniques.

MATERIALS AND METHODS

Between February 2006 and April 2020, a total of 813 transmen with a mean age of 24.4 years (range from 18 to 58 years) underwent single stage metoidioplasty with urethral lengthening. Mean body mass index in this group was 24.6 (ranged from 16.4 to 32.8). Inclusion criteria of latest version of WPATH Standards of Care were used in this study (4). The study protocol was approved by the Ethics Committee of Belgrade Center for Urogenital Reconstructive Surgery (approval number: 2020/11). Surgery was planned following cross-hormonal therapy, lasting a minimum of one year. Mean period of preoperative hormonal therapy was 32 months, and raged between 14 months and 24 years. Patients had undergone metoidioplasty simultaneously with hysterectomy and adnexectomy or mastectomy in 156 and 58 cases, respectively. Hysterectomy and mastectomy were performed simultaneously with metoidioplasty (one-stage GAS) in 46 out of 813 cases (5.6%) (**Table 1**). Complete metoidioplasty was performed in each case and included: vaginectomy, clitoral reconstruction, urethral lengthening, scrotoplasty and bilateral implantation of the testicular prostheses. Patients are divided in groups, depending on the type of urethroplasty: group A - tubularization urethroplasty (92 cases), group B - onlay flap urethroplasty either with dorsal clitoral or labia minora skin flaps (42 cases), group C - buccal mucosa graft (BMG) with clitoral skin flap urethroplasty (83 cases), group D - BMG with labia minora flap urethroplasty (537 cases), group E - labia minora skin graft with clitoral skin/labia minora flap urethroplasty (59 cases). An optimal technique of urethroplasty in each case depends on individual anatomical characteristics and patients' preference for buccal mucosa grafting. Patients were treated preoperatively with topical dihydrotestosterone gel twice daily for three months prior to surgery, in order to obtain clitoral enlargement. The same regimen as for hypospadias is used, with well defined benefits (19). The clitoris had been additionally

TABLE 1 | Patients' demographic characteristics and type of GAS.

Patients	Age (mean)	Follow-up (mean)	Preoperative hormonal th (mean)	Gender affirmation surgery (GAS)			
				Metoidioplasty	Metoidioplasty+ hysterectomy	Metoidioplasty+ mastectomy	One-stage GAS*
813	18-58y (24.4y)	16-180m (94m)	14m-24y (32m)	553	156	58	46

One-stage GAS: hysterectomy + mastectomy + metoidioplasty.

enlarged using vacuum device during the 6-month period leading up to surgery (**Figure 1**).

Operative Technique

The patient is placed in lithotomy position. Elasticated thigh-height stockings and low-molecular weight heparin are used to minimize the risk of deep vein thrombosis. Antibiotic prophylaxis (vancomycin) is administered after anesthesia is introduced. Complete vaginal mucosa is removed by colpocleisis, except one small portion close to the native urethral meatus, which is used later for bulbar urethroplasty. Vaginal vault is completely closed with circular reabsorbable sutures, and male-like perineum is created. A stay suture is placed through the clitoral glans and clitoral degloving is performed by circular incision between the inner and outer layer of clitoral prepuce downwards to the urethral plate, and continued with partial or complete dissection of the suspensory ligaments to lengthen and straighten the clitoris. Additional straightening and lengthening are obtained by urethral plate dissection and division to correct ventral chordee (**Figures 2**–**4**). Careful dissection is needed to prevent the injury of spongy tissue. The most difficult part of the surgery is the urethroplasty which starts with the reconstruction of the bulbar urethra by joining well-vacularized vaginal flap with the proximal part of the urethral plate (**Figure 5**). Additional reconstruction is performed by combining different available flaps and grafts. In cases with a well-developed and long urethral plate, urethral reconstruction can be performed with a simple tubularization of the wide plate, without affecting neophallic length. If a urethral plate was divided, the remaining defect is covered with buccal mucosa graft or labia minora skin graft. Buccal mucosa graft is harvested from the inner cheek by standard technique, with preserving integrity of the buccinator muscle, branches of facial nerve and external orifice of the parotid gland, as well as avoiding cosmetic problems of scarring. Buccal mucosal graft can be replaced by labia minora skin graft in case of good tissue quality or patients preference. This piece of hairless skin graft can be used as an equivalent of buccal mucosa graft (**Figure 6A**). The graft is fixed and quilted to the cavernosal bodies in the gap created after division of the short urethral plate, starting from the advanced urethral meatus to the tip of the glans, thus completing the dorsal aspect of the neourethra. Graft size depends on the neourethral length. Ventral part of the neourethra is created as an onlay flap from the labia minora or a dorsal clitoral skin flap buttonholed ventrally. The inner surface of one of the labia minora is dissected with the dimensions appropriate to cover the dorsal part of the neourethra (**Figure 6B**). Its dissection starts from the vaginal vestibulum and runs upward to the clitoral glans. The lateral wedge of the flap is defined as the border between the inner and outer labial surface. Finally, the flap is harvested by simple de-epithelialization of the outer labial skin, thus perfect vascularization of the flap can be preserved. Then the pedicle is additionally mobilized and lengthened from the subcutaneous tissue of the appropriate labia majora to enable suturing with the dorsal part of the neourethra without tension. The urethra is calibrated to be at least 16 Fr. A 12-14 Fr silicone stent is placed into the neourethra to moisturize inner surface and maintain the urethral lumen. The glans is opened in the midline by two parallel incisions, and both glans wings are dissected extensively to enable glans approxiamtion without tension after urethral reconstruction. Using this technique the neourethral meatus is placed at the tip of the glans (**Figure 7**). Remaining clitoral and labia minora skin is used to cover the penile shaft. It is mandatory to cover all suture lines in order to

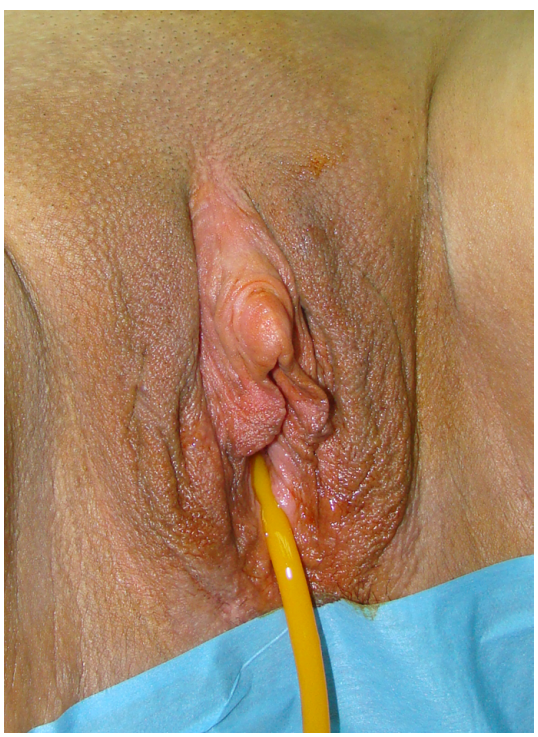

FIGURE 1 | Preoperative appearance of female genitalia after topical testosterone treatment.

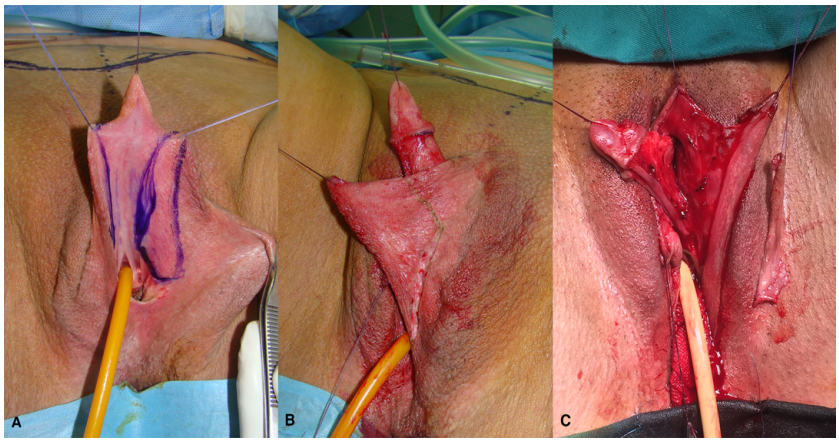

FIGURE 2 | Drawings of the urethral plate and inner side of labia minora, which is planned for urethral reconstruction **(A)**; outer layer of the labia minora is planned for skin graft harvesting **(B)**; labia minora flap and skin graft are created for urethral lengthening **(C)**.

prevent postoperative urethral complications. Scrotoplasty is done by joining the two labia majora in the midline and silicone testicular prostheses are implanted through bilateral incisions above the labia majora (**Figure 8**). It is very important to create a well-defined penoscrotal angle and male-looking external genitalia (**Figure 9**). In patients with developed mons pubis, additional monsplasty with the resection of the adipose tissue is performed to secure voiding in standing position and create better cosmesis. At the end of the surgery self-adherent dressing is used on the neophallus for 10 days. Suprapubic urinary catheter is maintained for 3 weeks, the urethral stent is removed 7 days after surgery. Vacuum device

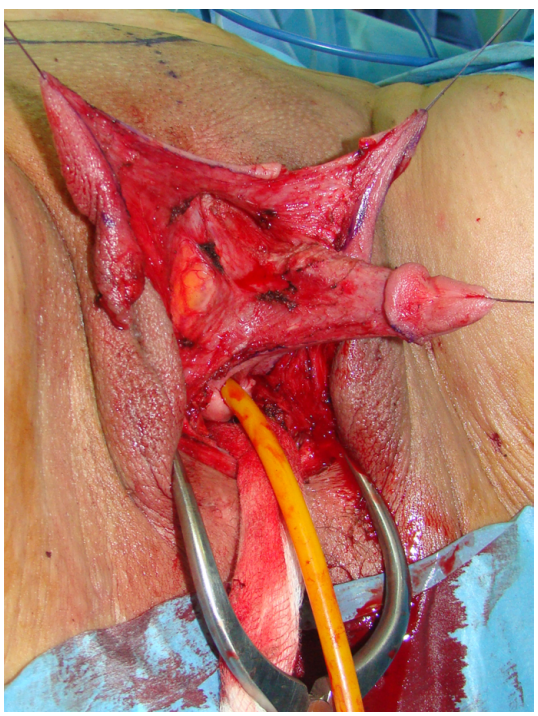

FIGURE 3 | Clitoris is maximally lengthened by dividing of suspensory ligaments, dorsally.

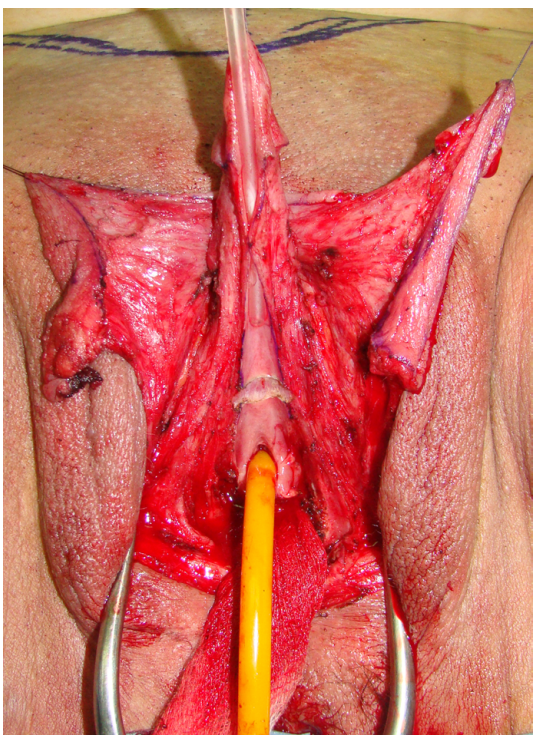

FIGURE 4 | Additional lengthening is achieved by dividing of short urethral plate, ventrally.

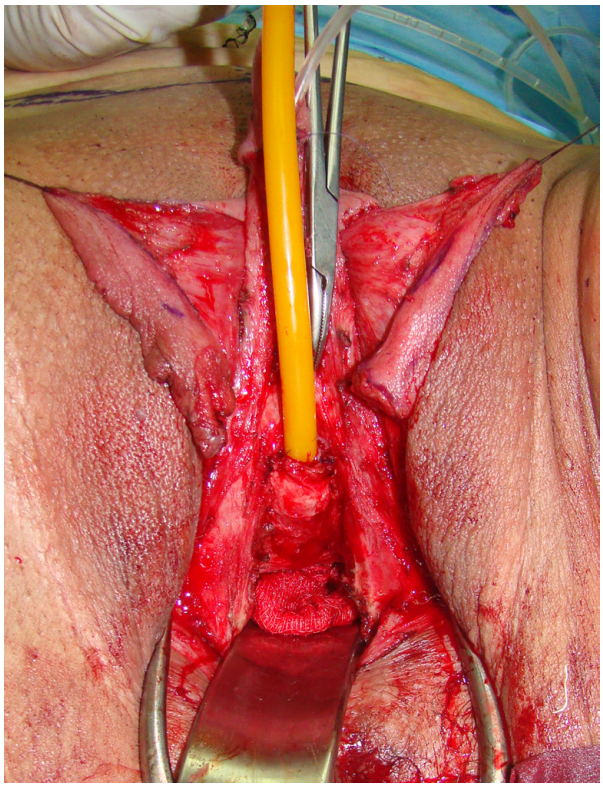

FIGURE 5 | Bulbar part of the neourethra is created and covered with bulbar muscles.

is recommended for 6 months postoperatively, starting 4 weeks after surgery to prevent adhesions and retraction or shortening of the neophallus.

All patients had a check-up at 3, 6 and 12 months postoperatively. Postoperatively, questionnaires were sent by e-mail including questions about functioning (voiding while standing, erection quality, sensation, possibility of penetration), cosmesis and patients' satisfaction (18). Satisfaction rates were measured using a 5-point scale (1: totally dissatisfied, 2: mainly dissatisfied, 3: neither dissatisfied nor satisfied, 4: mainly satisfied, 5: totally satisfied).

RESULTS

The mean follow-up was 94 months (ranged from 16 to 180 months). Mean length of the neophallus was measured 3-6 months after surgery and ranged from 4.8 cm to 10.2 cm (mean 5.6 cm). Mean operative time of metoidioplasty, without additional procedures, was 170 minutes (ranged from 112 to 217 minutes). Mean hospitalization was 3 days (ranged from 1 to 5 days).

Most common complications were related to urethral lengthening (urethral fistula and stricture), and occured in 86 out of total 813 cases (10,55%). Successful result at the 12-month check up was achieved in 86-90% of cases, depending on the type of urethroplasty (**Table 2**). Optimal urethral diameter was confirmed by urethrocystography and uroflowmetry, with an average flow rate of 21.6 ml/sec (ranged from 16.9 to 27.2 ml/sec).

Other complications included testicular implant rejection in 17 cases (2%) and testicular displacement in 26 cases (3.20%),

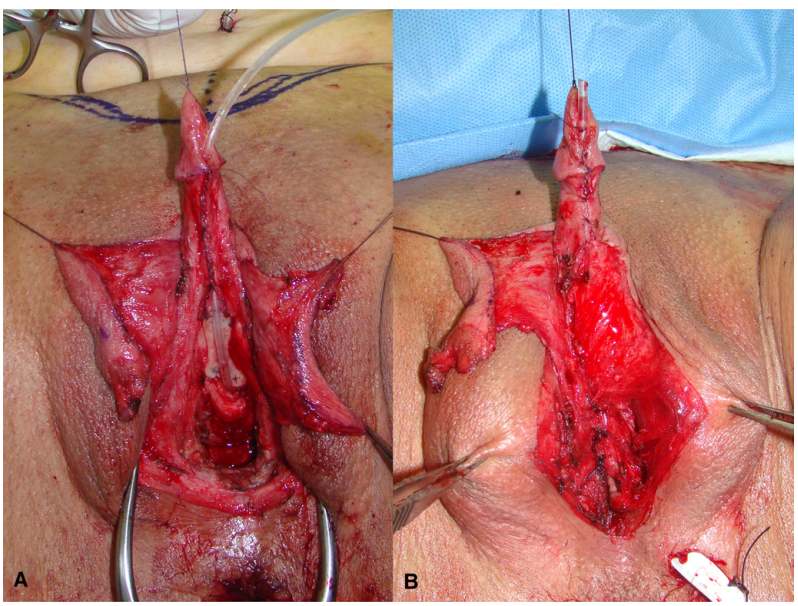

FIGURE 6 | Labia skin graft is fixed in the gap between divided urethral plate **(A)**; left labial skin flap is used for urethral tubularization, while flap pedicle is used for covering of the suture lines; distal urethra is created by simple urethral plate tubularization and glans closure **(B)**.

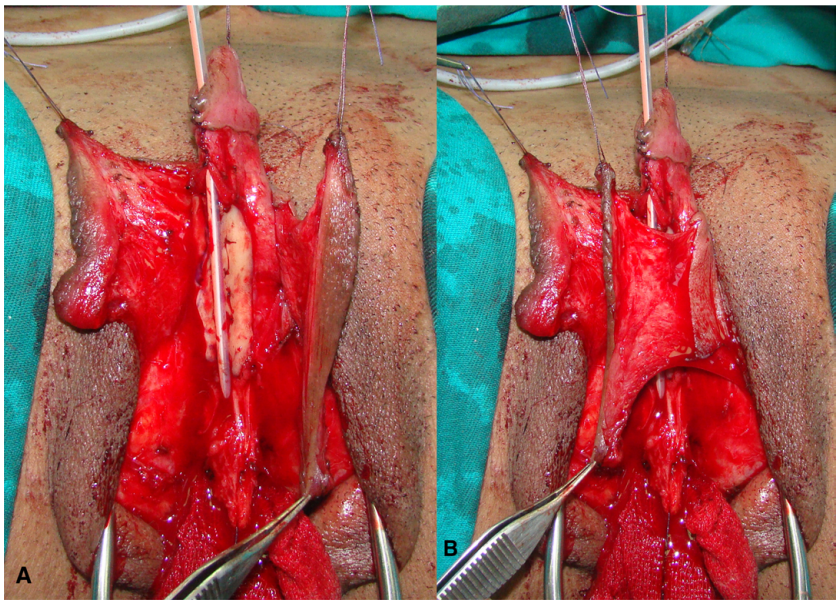

FIGURE 7 | Buccal mucosa graft is used for urethral substitute **(A)**; left labial flap with abundant vascular pedicle is used as an anterior part of the neourethra **(B)**.

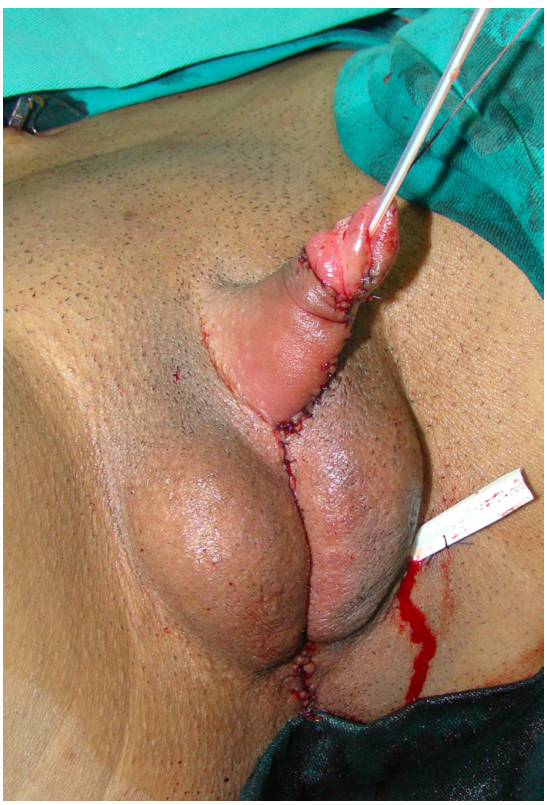

FIGURE 8 | Appearance at the end of surgery. Good size of the neophallus is achieved. Scrotums are created from labia majora and testicular prostheses are inserted.

which was corrected by new implantation or a repositioning of the implant into a proper scrotal pocket. A perineal cyst and/or perineal discharge due to remnant of vaginal mucosa was observed in 78 cases (9.60%). All complications were sucessfully managed with revision surgery 6-12 months after the primary procedure.

Totally 655 patients (80%) answered the questionnaire. According to self-report analysis, majority were satisfied with the cosmetic appearance of their new genitalia (79% totally satisfied, 20% mainly satisfied and 1% dissatisfied). All patients reported good tactile and erogenous sensation of the neophallus. The length of neophallus was inadequate for full penetration in those who reported sexual intercourse. Nevertheless, erection of the clitoris with its completely preserved sensation was reported by all of them. None of the patients reported problems with arousal, masturbation or orgasm.

Seventy six patients (9.35%) required total phalloplasty at our center after metoidioplasty. Musculocutaneous latissimus dorsi free flap phalloplasty was performed in 62, and abdominal phalloplasty in 14 cases.

DISCUSSION

Neophalloplasty in transmen is one of the most challenging parts of gender affirmation surgery. Since surgical results depend on many factors, including anatomical characteristics, tissue quality and patients' preference, there is no single and perfect solution for everyone (20). Detailed preoperative consultation with the surgeon and the mental health specialist is necessary in order to discuss the patient's expectations, available surgical options and

FIGURE 9 | Two years later, good penile length and aestetical outcome are achieved.

complications, moreover to prevent postoperative disappointment and additional surgeries.

Advantages and disadvantages of metoidioplasty, including each technique, have already been well defined (16). Among the currently used metoidioplasty techniques, complete (Belgrade) metoidioplasty is the only one that would enable complete reconstruction and voiding in standing position in a single stage procedure (21). This series presents one of the largest reported series in metoidioplasty, with long-term follow-up. Outcomes regarding cosmetic as well as functional aspect after metoidioplasty are good and quite similar to previous reports (22). However, this study provides a new insight in metoidioplasty from a high-volume center, based on large number of patients, long term follow-up and new urethroplasty modifications.

Reconstruction (straightening and lengthening) of the clitoris remains one of the crucial steps for successful outcome, and is provided by complete division of the suspensory ligaments and urethral plate. Straightening and lengthening of the clitoris significantly increase the length of the neophallus, but its final size depends on the clitoral size before surgery (23). However, this radical approach is not always necessary because it may not result in real or functional gain in penile length, but leads to unpleasing cosmetic results due to the scrotalization of the neophallus. Good appearance of the external genitalia can be achieved by creation of the penoscrotal angle as in a male by using rotational flaps from the remaining clitoral and labia minora skin. This way, residual skin scars are also prevented. Retraction of the neophallus due to scar formation is prevented by postoperative treatment with vacuum pump. For this reason, it is recommended for 6-12 months postoperatively, as an additional factor of good cosmetic outcome. An average neophallic length of 5.6 cm is not different from previous reports. The same is for hospital stay and operative time (18, 23, 24).

Urethral lengthening is the most challenging part of metoidioplasty, with many modifications reported in literature, to achieve better results. Significant improvements were made with using of dorsal clitoral skin flap, button-holed ventrally (17). After division of short urethral plate for clitoral lengthening, incorporating of buccal mucosa graft (BMG) for reconstruction of dorsal urethra became a gold standard, and covered with clitoral skin or labi aminora flap. Combination of BMG and labia minora flap has been proved as the best possible option so far, with low complication rate (23). Lately we have been using labial skin graft instead of BMG, in selected cases. In this way, we preserve buccal mucosa for urethral reconstruction as a part of potential phalloplasty in the future. This urethroplasty technique has a high rate of success in our series (89.80%), almost the same as the technique with BMG (90.30%). Therefore, it can have an important role in patients who are possible candidates for total phalloplasty in the future. One more important issue is the reconstruction of the bulbar urethra. The urinary stream is strongest at the bulbar segment, which therefore is the site with a high risk of fistula formation. Joining the clitoral bulbs over the lengthened urethra, with additional covering using the remaining surrounding vascularized tissue, is considered key to successful fistula prevention (comparison and lengthening). All patients in this series were able to void in standing position, which is one of the main goals of metoidioplasty. This aim is achieved even in obese patients, since obesity is a limitation factor in accomplishing this goal of metoidioplasty. If the neophallus is not long enough, it will stay buried in excessive fatty tissue.

Out of 813 patients in this group, only 86 (10.5%) had urethroplasty complications. All techniques had a success rate of

TABLE 2 | Urethral lengthening: outcomes and complications.

Type of urethroplasty (groups)	No of cases	Fistula No (%)	Stricture No (%)	Successful cases (%)
Group A - Tubularization	92	8 (8.70)	1 (1.10)	83 (90.20)
Group B - Onlay flap	42	6 (14.30)	2 (4.75)	34 (80.95)
Group C - BMG with clitoral skin	83	9 (10.85)	2 (2.40)	72 (86.75)
Group D - BMG with labia minora flap	537	44 (8.20)	8 (1.50)	485 (90.30)
Group E - labial skin flap/graft	59	5 (8.50)	1 (1.70)	53 (89.80)
Total	813	72 (8.85)	14 (1.70)	727 (89.45)

more then 80%, but combination of buccal mucosa graft and labia minora flap should still be considered as the gold standard and primary option. Other complications were mostly cosmetic, and related to testicular implants and vaginal remnants. They are all successfuly treated with minor revision after 6-12 months. It is also important to point out that majority of patients (99%) reported high level of satisfaction with aesthetic appearance after metoidioplasty, without compromising sensitivity and sexual arousal, but without possibility for penetrative intercourse.

A limitations of the study could include a lack of detailed statistical analysis of the cohort, as well as a lack of similar studies in literature for adequate comparison.

The ideal technique for creating neophallus in transmen is still missing. Metoidioplasty, as a single-stage variant of phalloplasty in transmen, is a viable, safe, cost-effective and time-sparing surgical procedure. Good cosmetic and functional outcomes are achieved, with low rate of complications, short hospital stay and high rate of patients' satisfaction. In patients who present with the desire for total phalloplasty after metoidioplasty, neophallus can be created using either known technique of phalloplasty.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Belgrade Center for Urogenital Reconstructive Surgery, Approval number: 2020/11). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

NB and MD contributed to conception and design of the study, and NB wrote the first draft of the manuscript. BS and MB organized the database and wrote sections of the manuscript. AS organized the database and revised the final version. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Risk of Venous Thromboembolism in Transgender People Undergoing Hormone Feminizing Therapy: A Prevalence Meta-Analysis and Meta-Regression Study

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Background: Although venous thromboembolism (VTE) is a recognized side effect of some formulations of estrogen therapy, its impact in transgender people remains uncertain. The aim of this study was to define pooled prevalence estimate and correlates of VTE in Assigned Males at Birth (AMAB) trans people undergoing gender affirming hormone therapy.

Methods: A thorough search of MEDLINE, COCHRANE LIBRARY, SCOPUS and WEB OF SCIENCE databases was carried out to identify suitable studies. Quality of the articles was scored using the Assessment Tool for Prevalence Studies. Data were combined using random effects models and the between-study heterogeneity was assessed by the Cochrane's Q and I^2.

Results: The eighteen studies included gave information about 11,542 AMAB undergoing gender affirming hormone therapy. The pooled prevalence of VTE was 2% (95%CI:1-3%), with a large heterogeneity (I^2 = 89.18%, P<0.0001). Trim-and-fill adjustment for publication bias produced a negligible effect on the pooled estimate. At the meta-regression analysis, a higher prevalence of VTE was significantly associated with an older age (S=0.0063; 95%CI:0.0022,0.0104, P=0.0027) and a longer length of estrogen therapy (S=0.0011; 95%CI:0.0006,0.0016, P<0.0001). When, according to the meta-regression results, the analysis was restricted to series with a mean age ≥37.5 years, the prevalence estimate for VTE increased up to 3% (95%CI:0-5%), but with persistence of a large heterogeneity (I^2 = 88,2%, P<0.0001); studies on younger participants (<37.5 years) collectively produced a pooled VTE prevalence estimate of 0% (95%CI:0-2%) with no heterogeneity (I^2 = 0%, P=0.97). Prevalence estimate for VTE in series with a mean length of estrogen therapy ≥53 months was 1% (95%CI:0-3%), with persistent significant heterogeneity (I^2 = 84,8%, P=0.0006); studies on participants subjected to a shorter length of estrogen therapy (<53 months), collectively produced a pooled VTE prevalence estimate of 0% (95%CI:0-3%) with no heterogeneity (I^2 = 0%, P=0.76).

Conclusions: The overall rate of VTE in AMAB trans people undergoing gender affirming hormone therapy was 2%. In AMAB population with <37.5 years undergoing estrogen therapy for less than 53 months, the risk of VTE appears to be negligible. Further studies are warranted to assess whether different types and administration routes of estrogen therapy could decrease the VTE risk in AMAB trans people over 37.5 years subjected to long-term therapy.

Systematic Review Registration: [https://www.crd.york.ac.uk/PROSPERO/], identifier [CRD42021229916].

Keywords: gender dysphoria, gender affirming hormone therapy, venous thomboembolism, transgender, estrogen

INTRODUCTION

Transgender people do not experience gender as consistent with their birth sex. The non-correlation between experienced gender and biological sex, known as gender incongruence (1), can lead to stigma, depression, body uneasiness, social margination for Assigned Males at Birth (AMAB) and Assigned Females at Birth (AFAB) trans people ("gender dysphoria"). For those who want to change all/some physical features can start gender affirming care (hormonal and/or surgical treatment).

According to a meta-analysis by Collin and colleagues, the overall prevalence estimates for transgender diagnoses were 2.5 per 100,000 for AFAB and 5.8 per 100,000 for AMAB, although prevalence may vary based on different definitions (2).

World Professional Association for Transgender Health (WPATH) guidelines define hormone treatment as medically necessary for transgenders asking for medical interventions to affirm their gender (3). The Endocrine Society recommends oral, transdermal, or intramuscular 17β-estradiol for AMAB trans people (4). Oral administration includes micronized 17β-estradiol, conjugated estrogens and estradiol valerate, quickly cleaved to 17β-estradiol, while ethinyl estradiol is no longer recommend because of the poor safety profile (5). Co-administration with androgen inhibitors (cyproterone, spironolactone, progesterone) is often chosen to foster feminization (6, 7). However, the lack of international standardization of specific hormone regimens for gender affirming therapy in AMAB trans people hinders the knowledge of the side effects of hormone treatment, including venous thromboembolism (VTE).

The incidence rate of VTE in young women who do not use estrogens is about 1 in 10,000 per year (8–10). Much of the available data on thrombotic risk associated to estrogen treatment steams from studies on cisgender women treated with combined oral contraceptives (COCs), or with hormone replacement therapy (HRT) (11). In cisgender women, COC increases the risk of VTE by approximately 2–4 fold (10, 12) and a higher risk would result from increases in estrogen dose (13). Transdermal estrogen formulations used for HRT in postmenopausal women do not seem to be associated with a significant increase in the VTE risk (12, 14–16) and had showed a low thrombogenic profile in AMAB trans people, although there are no head-to-head studies with other estrogen formulations (17, 18). However, thrombophilia, smoking, obesity, age, major surgery and fractures are well-recognized risk factors in the general population and could contribute, alone or in combination, to promote VTE in COC and HRT users (19–22).

Results of studies on the thrombotic risk in cisgender women under COC or HRT should not be translated to AMAB trans people due to differences in age, estrogen formulations and doses (23); cisgender women and AMAB trans people do have genetic differences and AMAB usually also use androgen inhibitors. Furthermore, many data on the risk of VTE in AMAB trans people undergoing feminizing hormone treatment have been produced in case reports or small series, thus reaching a low statistical power (18). In a meta-analysis by Khan and colleagues (7), the incidence rate for VTE was 2.3 per 1,000 person-years (95% CI: 0.8 – 6.9). However, this estimate was burdened with a large and unexplained between-study heterogeneity ($I^2 = 74\%$; $P = 0.0039$). More recently, a systematic review pointed to a significantly higher incidence of VTE in treated AMAB compared to AFAB trans people (42.8 *vs* 10.8 VTE per 10,000 patient years; $P = 0.02$) (24). Again, a large between-study heterogeneity arose, and the qualitative approach did not allow the identification of covariates potentially able to influence the pooled data.

Given the large unexplained heterogeneity among the studies, the actual impact and risk of VTE in AMAB trans people receiving gender affirming hormone therapy remains uncertain. In this light, we aimed to perform a systematic review and a meta-analysis of available studies to define pooled prevalence estimate and correlates of VTE in AMAB undergoing hormone feminizing therapy.

MATERIALS AND METHODS

The study was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) (25). It also complies with the guidelines of Meta-Analyses and Systematic Reviews of Observational Studies (MOOSE) (26). The PRISMA-P and MOOSE checklists have been presented as **Supplementary Table 1** (A and B). The study is registered in the PROSPERO (International Prospective Register of Systematic Reviews) with the number CRD42021229916. (https://www.crd.york.ac.uk/PROSPERO/).

Systematic Search Strategy

A systematic search was performed in MEDLINE, Scopus, Cochrane Library and Web of Science, including the following free and vocabulary terms: 'gender dysphoria', 'gender identity', 'MTF', 'Assigned Males at Birth', 'AMAB', 'gender transition', 'transsexual', 'transfeminine', 'transwomen', 'gender affirmation', 'gender affirming hormone therapy', 'feminizing therapy', 'estrogen', 'hormone therapy', 'thrombosis', 'embolism', 'thromboembolism', using the Boolean functions AND/OR. The search was restricted to English-language studies enrolling human participants, published up to April 2021. If it was not clear from the abstract whether the study contained relevant data, the full text was retrieved. The reference lists of the identified articles were also scrutinized to find possible additional pertinent studies.

Inclusion and Exclusion Criteria

Eligible studies were identified according to a PECOS (Population, Exposure, Comparison/Comparator, Outcomes, Study design) model (**Supplementary Table 2**).

Studies were included in quantitative analysis if they reported the prevalence (or information for its calculation) of VTE events in AMAB trans people recruited from the general population or from cohorts of patients. Observational studies (case-control, cross-sectional, prospective and series of cases), as well as intervention studies were screened for eligibility. Only information about cases (AMAB trans people) were extracted from case-control studies. Duplicates were rigorously checked and removed.

Reviews, meta-analyses and studies lacking to assess the outcomes of interest were excluded. When the same population sample was used for multiple publications, only the study with the largest number of participants was included.

Two independent reviewers (M.T. and S.P.) evaluated the full text of all selected studies for eligibility, and, where disagreement occurred, a third reviewer (A.B.) took a decision after open discussion.

Data Extraction

Data were extracted from the selected studies by four independent reviewers (A.P., S.P., F.D.A. and D.T.) by including the first author, publication year, country/geographic region, study design, the total number of AMAB and the number of those who have experienced a VTE event. When available, the mean age and body mass index (BMI) of the participants, smoking habit, type, dosage and duration of hormone therapy, diagnosis of comorbidities, including type-2 diabetes mellitus (T2DM), dyslipidemia and hypertension, were also taken into account. When summary statistics were not fully reported, these were calculated, whenever possible (27). Where data were missing, incomplete or inconsistent, the authors were contacted to obtain necessary information.

Quality Assessment

Quality of the studies was assessed using an adapted Assessment Tool for Prevalence Studies (28). This tool, designed to assess the risk of bias in prevalence studies, considers 10 different items, including representativeness and selection of the study population, likelihood of non-response bias, process of data collection, appropriateness of the definition of cases (AMAB trans people) as well as of the measurement of the parameter of interest (prevalence of VTE). Response options for individual items were either low or high risk of bias and a summary assessment of the overall risk of bias was based on the subjective judgment attributed to the 10 items: 7-10 items with 'low risk' judgment indicated an overall low risk of bias; 4-6 items with 'low risk' judgment indicated an overall moderate risk of bias; 0-3 items with 'low risk' judgment indicated an overall high risk of bias.

Quality assessment was performed independently by 2 reviewers (M.T. and C.C.) and any disagreement was resolved by involving a third reviewer (A.B.) who re-evaluated the original study.

Statistical Analysis

The pooled prevalence of VTE was estimated by a random-effects model which assumes that the included studies have varying effect sizes, thus providing a conservative estimate of the overall effect. The 95% confidence intervals (CIs) of the prevalence reported in individual studies were estimated from the proportion of cases of VTE and the sample size, using the binomial Clopper-Pearson exact method. After ascertaining the non-normal distribution of the original data sets (by the Shapiro-Wilk test), the Freeman-Tukey double arcsine transformation was applied to the primary study data to approximate normality. The final pooled results and 95% CIs were back transformed and expressed as percentages for an easier interpretation. An inverse variance method was used for weighting each study in the pooled estimates. The Cochran's Chi square (Cochran's Q) test and the I^2 test were used to analyze the statistical heterogeneity between the results of different studies: an I^2 >50% and/or P <0.05 indicated substantial heterogeneity (29).

Publication bias was assessed through funnel plots (30) and the Begg adjusted rank correlation test (31). In case of asymmetric funnel shape, Duval and Tweedie's 'trim-and-fill' analysis was carried out to detect putative missing studies which could rebalance the distribution; the analysis provides an adjusted pooled estimate taking the additional studies into account, thus correcting the analysis for publication bias (32).

Covariates that could affect the estimates, such as the mean age and BMI of the participants, comorbidities, smoking habit, type, dosage and duration of hormone therapy were included in linear meta-regression models.

Data were analyzed and graphed using the packages 'metafor' and 'ggplot2' of the R statistical software (version 4.0.3, 2020; The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study Selection and Quality Assessment

From the electronic search we retrieved a total of 1039 studies and, after removal of duplicates, 910 studies were left. 811 papers were excluded as irrelevant, based on title and abstract reading. Hence, as shown in **Figure 1**, 99 studies were identified, of which 18 met

the inclusion criteria (17, 33–49). The studies by Asscheman and colleagues published in 1989 (50) and 2011 (51) were excluded since the population under investigation was already included in the paper by van Kesteren et al., 1997 (47). The studies by Wierckx et al., 2012 (52) and Wierckx et al., 2014 (53) were also excluded because subjects included were considered in the study by Wierckx et al., 2013 (48). Details of the selected articles are summarized in **Table 1** and **Supplementary Table 3**.

Table 2 showed quality assessment of the studies: 15 studies were considered at low/moderate risk of bias, whereas an overall high risk of bias was attributed to the remaining 3 studies.

Synthesis of Results and Publication Bias

As shown in **Figure 2**, the included studies collectively gave information about VTE in 11,542 AMAB trans people, resulting in a pooled VTE prevalence estimate of 2.0% (95% CI: 1.0 - 3.0%), with a large heterogeneity (I^2 = 89.2%, $P_{\text{for heterogeneity}}$ <0.0001).

Although the Begg's rank correlation test suggested a not significant asymmetry in funnel plot of VTE (Kendall's τ = 0.072, P = 0.71), the trim-and-fill analysis identified five putative 'missing study' on the right side of distribution (**Supplementary Figure 1**). Nevertheless, when the funnel plot distribution was rebalanced by including these putative additional studies, the adjustment for publication bias produced a negligible effect on the pooled prevalence estimate for VTE (adjusted pooled prevalence: 1.9%, 95% CI: 1.0 - 2.9%).

Analysis of the Between-Study Heterogeneity: Meta-Regression and Sub-Group Analyses

Meta-regression analyses were carried out to find out covariates that could affect the prevalence estimate. No significant relationship with VTE was found for BMI (S = -0.0021; 95% CI: -0.0199, 0.0158, P = 0.8), number of current smokers (S = -0.0017; 95% CI: -0.0041, 0.0007, P = 0.2), number of participants taking oral estrogen therapy (S = 0.0000; 95%CI: -0.0010, 0.0010, P = 0.9), number of participants taking estrogen valerate (S = 0.0008; 95% CI: -0.0003, 0.0019, P = 0.2), diagnosis of T2DM (S = -0.0018; 95% CI: -0.0220, 0.0183, P = 0.9), dyslipidemia (S = -0.0024; 95% CI: -0.0063, 0.0014, P = 0.2), and hypertension (S = -0.0029; 95% CI: -0.0103, 0.0044, P = 0.4).

Both an older age of the participants and a longer length of estrogen therapy were significantly associated with a higher prevalence of VTE (for mean age of participants: S = 0.0063;

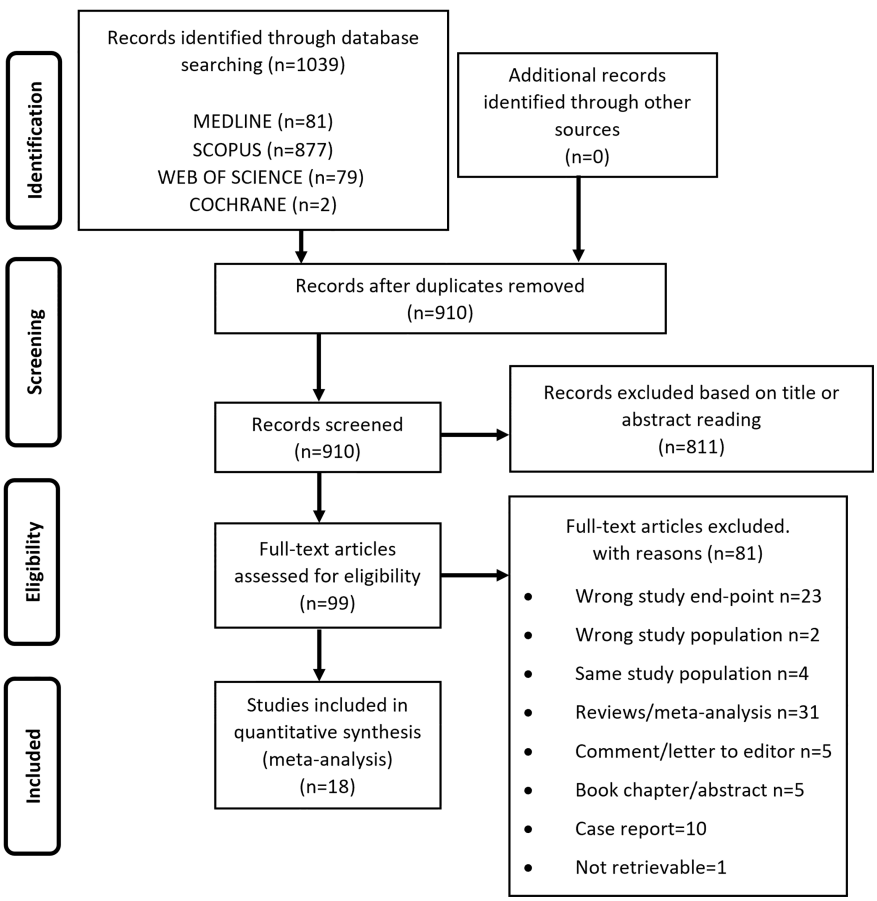

FIGURE 1 | Flow diagram showing an overview of the study selection process.

TABLE 1 | Characteristics of the included studies.

Study	AMAB (n)	VTE (n, %)	Thrombophilia inherited risk factors	Months of therapy (mean)	Mean age (years)	Mean BMI (kg/m^2)	Oral estrogen (n, %)	DM (n, %)	Hypertension (n, %)	Dyslipidemia (n, %)	Current smokers (n, %)	Estrogen valerate (n, %)
Arnold et al. (33)	676	1 (0.15%)	Case with VTE negative for anti-phospholipid Abs, factor V Leiden or PT gene mutations	22.8	33.2	26.6	676 (100%)	43 (6.4%)	88 (13.0%)	59 (8.7%)	143 (21.2%)	0 (0.0%)
Dittrich et al. (34)	60	1 (1.67%)	Case with VTE positive for a homozygous mutation in C677 T MTHFR	24.0	38.4	24.2	60 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NR	60 (100%)
Getahun et al. (35)	2842	61 (2.15%)	NR	NR	NR	NR	853 (30.0%)	NR	461 (16.0%)	170 (6.0%)	434 (15.0%)	NR
Jain et al. (36)	92	0 (0.00%)	NR	40.8	31.0	NR	0 (0.0%)	0 (0.0%)	NR	NR	NR	0 (0.0%)
Kozato et al. (37)	662	1 (0.20%)	NR	NR	35.6	25.7	210 (31.7%)	NR	NR	NR	NR	0 (0.0%)
Meyer et al. (38)	155	3 (1.90%)	One case with VTE positive for heterozygous PT mutation	NR	NR	NR	73 (47.1%)	NR	NR	NR	NR	155 (100%)
Mullins et al. (39)	182	0 (0.00%)	Thrombophilia screening: elevated PAI-1 levels, n = 5; PAI-1 gene polymorphism, n = 5; elevated factor VIII level, n = 4	20.8	18.0	NR	165 (90.7%)	NR	NR	NR	94 (51.6%)	0 (0.0%)
Nolan et al. (40)	178	1 (0.60%)	NR	67.2	36.2	25.2	NR	8 (4.5%)	15 (8.4%)	12 (6.7%)	NR	NR
Nota et al. (41)	2517	73 (2.90%)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Ott et al. (17)	162	0 (0.00%)	Thrombophilia screening: aPC resistance, n = 12; aPC resistance + homozygous factor V Leiden mutation, n = 1	64.8	36.6	22.7	0 (0.0%)	2 (1.2%)	35 (21.6%)	62 (38.3%)	96 (59.3%)	0 (0.0%)
Prior et al. (42)	50	0 (0.00%)	NR	12.0	32.7	NR	50 (100%)	NR	NR	NR	NR	0 (0.0%)
Pyra et al. (43)	2509	19 (0.80%)	NR	53.1	37.5	32.9	946 (37.7%)	83 (3.3%)	49 (1.9%)	NR	NR	NR
Schlatterer et al. (44)	46	0 (0.00%)	NR	NR	NR	NR	26 (56.5%)	NR	NR	NR	20 (43.5%)	0 (0.0%)
Seal et al. (45)	330	4 (1.20%)	NR	109.2	45.6	NR	330 (100%)	1 (0.3%)	2.6%	NR	NR	163 (49.4%)
Tack et al. (46)	21	0 (0.00%)	NR	15.6	17.6	NR	21 (100%)	NR	NR	NR	NR	21 (100%)
van Kesteren et al. (47)	816	45 (5.50%)	NR	NR	46.5	NR	NR	NR	61 (7.5%)	NR	NR	0 (0.0%)
Wierckx et al. (48)	214	11 (5.10%)	NR	152.0	43.7	24.7	99 (46.3%)	8 (3.7%)	NR	NR	NR	91 (42.5%)
Wilson et al. (49)	30	0 (0.00%)	NR	6.0	38.6	NR	23 (76.7%)	0 (0.0%)	NR	NR	2 (6.6%)	0 (0.0%)

Values are presented as mean or number (%). Abs, antibodies; AMAB, Assigned Males at Birth; BMI, body mass index; DM, diabetes mellitus; MTHFR, methylenetetrahydrofolate reductase; NR, not reported; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin; VTE, thromboembolic events.

95% CI: 0.0022, 0.0104, P = 0.0027, **Figure 3A**; for mean months of estrogen therapy: S = 0.0011; 95% CI: 0.0006, 0.0016, P <0.0001, **Figure 3B**).

To substantiate the impact of the age and therapy duration as sources of the between-study heterogeneity, in subsequent subgroup analyses, pooled estimates were calculated separately for studies enrolling AMAB trans people below and above 37.5 years of age and for those on participants under estrogen therapy for less or more than 53 months. Dichotomization values were chosen according to the distributions of meta-regression bubble plots (**Figure 3**).

When analysis was restricted to series with a mean age ≥37.5 years, the prevalence estimate for VTE increased up to 3.0% (95% CI: 0.0 - 5.0%), but with persistence of a large heterogeneity (I^2 = 88.2%, P < 0.0001; **Figure 4A**). On the contrary, studies on younger participants (mean age <37.5 years) collectively produced a pooled

TABLE 2 | Quality assessment of the included studies.

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	OVERALL
Arnold et al. (33)	L	L	H	H	L	L	L	L	L	L	Low risk of bias
Dittrich et al. (34)	H	H	H	L	L	L	L	L	L	L	Low risk of bias
Getahun et al. (35)	L	H	H	L	H	H	L	H	L	H	Moderate risk of bias
Jain et al. (36)	H	H	H	L	L	L	L	L	L	L	Low risk of bias
Kozato et al. (37)	H	H	H	H	H	L	H	L	L	H	High risk of bias
Meyer et al. (38)	H	H	H	H	H	L	L	L	L	L	Moderate risk of bias
Mullins et al. (39)	L	H	H	L	H	L	L	H	H	L	Moderate risk of bias
Nolan et al. (40)	H	H	H	L	H	L	L	L	L	H	Moderate risk of bias
Nota et al. (41)	H	H	H	H	H	L	L	L	L	L	Moderate risk of bias
Ott et al. (17)	H	H	H	H	H	L	L	L	L	L	Moderate risk of bias
Prior et al. (42)	H	L	H	L	H	L	L	L	L	L	Low risk of bias
Pyra et al. (43)	L	H	H	L	H	L	H	L	H	H	Moderate risk of bias
Schlatterer et al. (44)	H	H	H	H	L	L	L	H	H	L	Moderate risk of bias
Seal et al. (45)	H	H	H	H	H	H	L	H	L	L	High risk of bias
Tack et al. (46)	H	H	H	H	L	L	H	L	H	H	High risk of bias
van Kesteren et al. (47)	L	L	H	L	H	L	L	L	L	L	Low risk of bias
Wierckx et al. (48)	H	H	H	H	L	L	H	L	H	H	Moderate risk of bias
Wilson et al. (49)	H	L	H	H	L	L	L	L	L	L	Low risk of bias

H, High risk; L, Low risk.

Q1. Was the study's target population a close representation of the national population in relation to relevant variables?

Q2. Was the sampling frame a true or close representation of the target population?

Q3. Was some form of random selection used to select the sample, OR was a census undertaken?

Q4. Was the likelihood of non-response bias minimal?

Q5. Were data collected directly from the subjects (as opposed to a proxy)?

Q6. Was an acceptable case definition used in the study?

Q7. Was the study instrument that measured the parameter of interest (prevalence of thromboembolic events) shown to have reliability and validity?

Q8. Was the same mode of data collection used for all subjects?

Q9. Was the length of the shortest prevalence period for the parameter of interest appropriate?

Q10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?

OVERALL. Summary item on the overall risk of study bias: 7-10 items with 'low risk' judgment = overall low risk of bias; 4-6 items with 'low risk' judgment = overall moderate risk of bias; 0-3 items with 'low risk' judgment = overall high risk of bias.

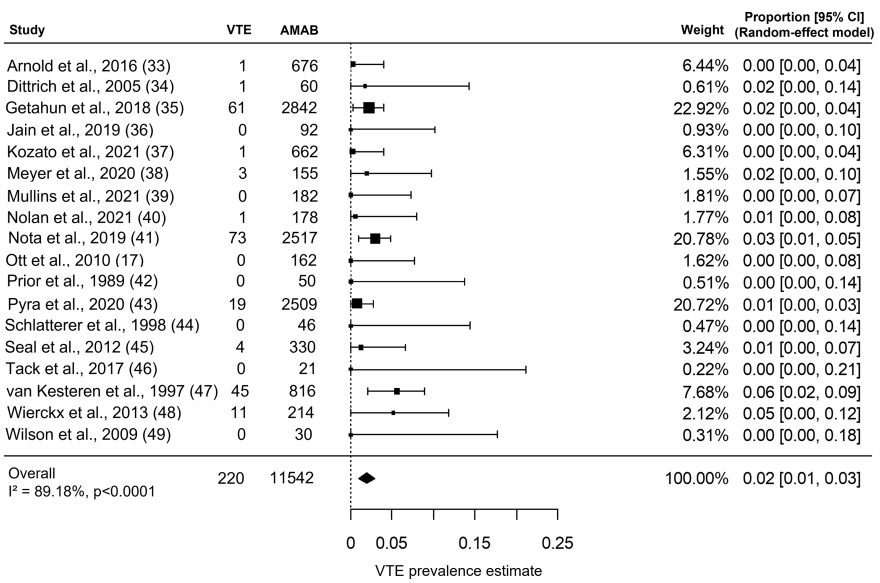

FIGURE 2 | Forest plot depicting the pooled prevalence estimate for venous thromboembolism (VTE) in Assigned Males at Birth (AMAB) trans people. Diamond indicates the overall summary estimate and the width of the diamond represents the 95% confidence interval (CI); boxes indicate the weight of individual studies in the pooled result.

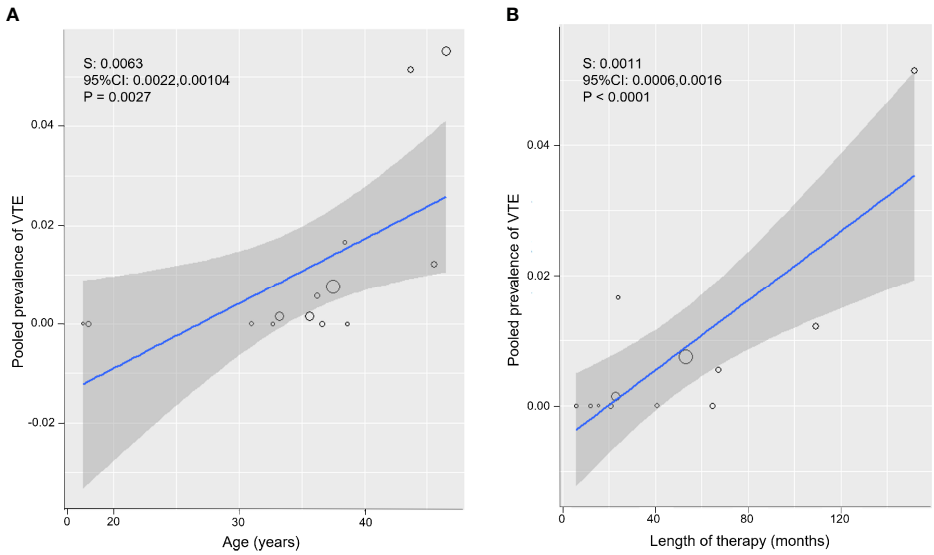

FIGURE 3 | Meta-regression bubble plots: prevalence of venous thromboembolism (VTE) in Assigned Males at Birth (AMAB) trans people as a function of the mean age **(A)** and mean length of estrogen therapy **(B)**. The predicted effects (solid line) with corresponding confidence intervals (gray range) are also shown. CI, confidence interval; S, slope.

VTE prevalence estimate of 0.0% (95% CI: 0.0 - 2.0%) with no heterogeneity (I^2 = 0.0%, P = 0.97; **Figure 4B**).

Estimate prevalence for VTE in series under estrogen therapy for more than 53 months was 1.0% (95% CI: 0.0 - 3.0%), with persistent significant heterogeneity (I^2 = 84.8%, P = 0.0006, **Figure 5A**). As shown in **Figure 5B**, studies on participants under estrogen therapy for less than 53 months, instead, produced a pooled VTE prevalence estimate of 0.0% (95% CI: 0.0 - 3.0%) with no heterogeneity (I^2 = 0.0%, P = 0.77).

DISCUSSION

To our knowledge, this is the largest meta-analysis performed on VTE risk in AMAB trans people under gender-affirming hormone therapy: it included 18 studies, collectively giving information about 11,542 AMAB trans people. The overall pooled VTE prevalence estimate was 2%, but with large heterogeneity. Our results were similar to those of Khan and colleagues (7), who reported an incidence rate for VTE in AMAB treated with estrogens of 2.3 per 1,000 person-years, resulting from the analysis of a smaller number of studies. According to a recent systematic review by Kotamarti et al. (24), the incidence of VTE in AMAB would be significantly higher than in AFAB trans people.

A number of factors could contribute to the variable VTE risk in transgender people undergoing gender affirming treatment, including the type of estrogen and the route of administration, age at the estrogen therapy onset, length of therapy, concomitant conditions such as smoking, obesity, thrombophilia and comorbidities (16, 20–22, 54–56). In the present study, meta-regression analyses showed no significant relationship of VTE with BMI, smoking, diagnosis of T2DM, dyslipidemia and hypertension, albeit with the due caution this subject deserves due to the lack of information about these variables in many studies (**Table 1** and **Supplementary Table 3**). Indeed, it is known that obesity increases the risk of VTE in cisgender women using COCs (21) and the combination of COCs and smoking could exert a synergistic effect (22). Interestingly, consistent with our findings, in the recent systematic review by Kotamarty et al. (24), although AMAB trans people exhibited a lower BMI and an almost 2-fold higher prevalence of smoking compared to cisgender women, these variables were not correlated with the risk of VTE. Moreover, in the present meta-analysis, neither estrogen valerate nor oral estrogen use was related to VTE risk. Unfortunately, the dearth of information about type of estrogen and route of administration (**Supplementary Table 3**) did not allow us to carry out sub-group analyses with these variables.

Worth noting and previously not reported was the here revealed significant association of VTE with an older age of participants and a longer length of estrogen therapy, thus indicating that the longer the exposure time to therapy, the greater the rate of thromboembolic complications for AMAB trans people. Accordingly, when we restricted the analyses to younger series (mean age <37.5 years) and those under estrogen therapy for less than 53 months, the risk was wiped out with no between-study heterogeneity. Therefore, we surmise that the enrollment of series with different mean age and different length of estrogen therapy contributed to the large between-study heterogeneity. Although statistical analyses produced an overall VTE prevalence of 0% in sub-groups younger than 37.5 years and under estrogen therapy for less than 53 months, a complete absence of risk would be unrealistic in these populations. Our

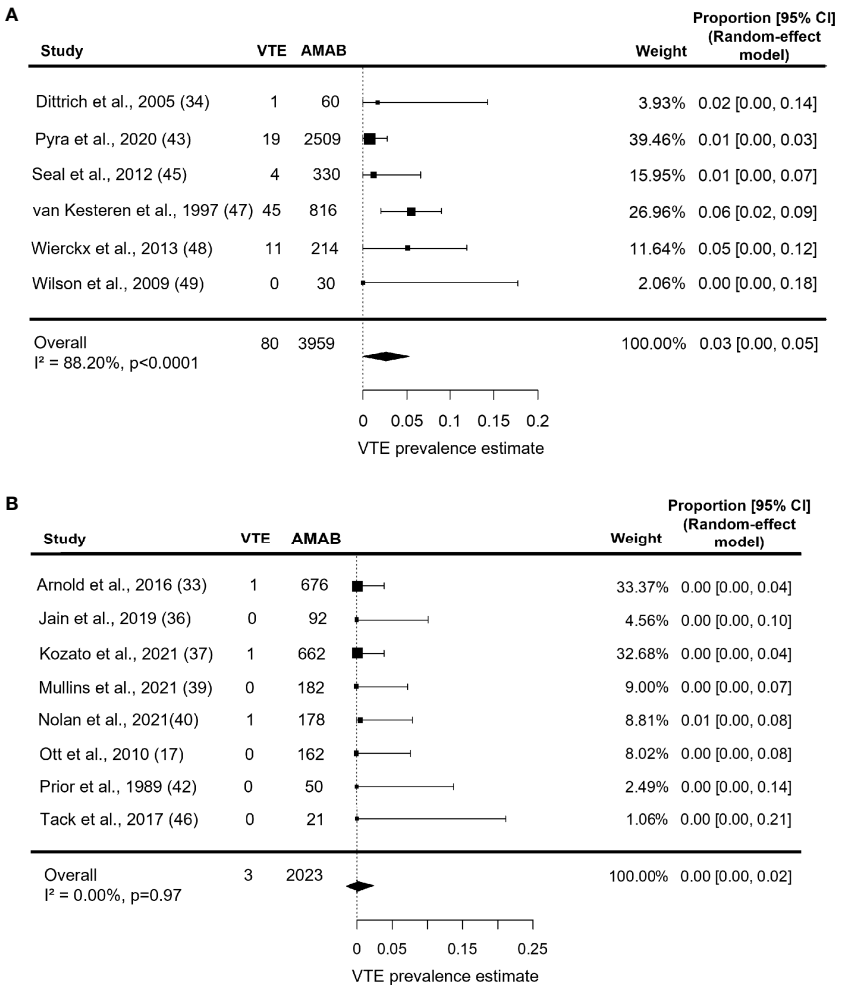

FIGURE 4 | Forest plots depicting the results of the subgroup analysis of the prevalence of venous thromboembolism (VTE) in Assigned Males at Birth (AMAB) trans people by mean age. The pooled prevalence estimate was calculated separately for studies enrolling AMAB **(A)** above and **(B)** below 37.5 years of age. Diamonds indicate the overall summary estimates and width of the diamonds represents the 95% confidence interval (CI); boxes indicate the weight of individual studies in the pooled results.

results should instead suggest that a not negligible rate of VTE, albeit low, has to be taken into account after 4-5 years of estrogen therapy, especially in older AMAB trans people. This latter finding, if on one hand could be due to an actual thrombophilic effect of prolonged estrogen treatment regimens (54), on the other, it could reflect a higher number of VTE diagnoses arising over time from regular control visits which usually trans people undergo in clinical settings. The persistence of a large between-study heterogeneity in these sub-analyses might be related to the different prevalence of comorbidities in the study populations, as well as to other variables, including the possible influence of different regimens of antiandrogens.

This study has some limitations. First, as mentioned above, the paucity of available data did not allow to perform reliable quantitative analyses to assess the best estrogen treatment regimen associated with the lowest risk for VTE in this population. However, transdermal estrogens and oral estradiol valerate are recommended by the current WPATH guidelines in people with VTE risk factors (3) while the use of ethinyl estradiol formulation is not recommended (18). Furthermore, the impact of inherited risk factors for VTE in AMAB trans people undergoing gender affirming hormone therapy remains uncertain as only five studies reported results of thrombophilia screening (**Table 1**). Finally, the trim-and-fill analysis revealed a possible publication bias, suggesting that published studies might be not fully a representative sample of the available evidence. Nevertheless, the corrected pooled OR, taking into account the putative missing studies, demonstrated that publication bias did not substantially affect the overall estimate.

In conclusion, the overall rate of VTE in AMAB trans people undergoing gender-affirming hormone therapy was 2%. Our analyses revealed that in AMAB series with younger age (<37.5

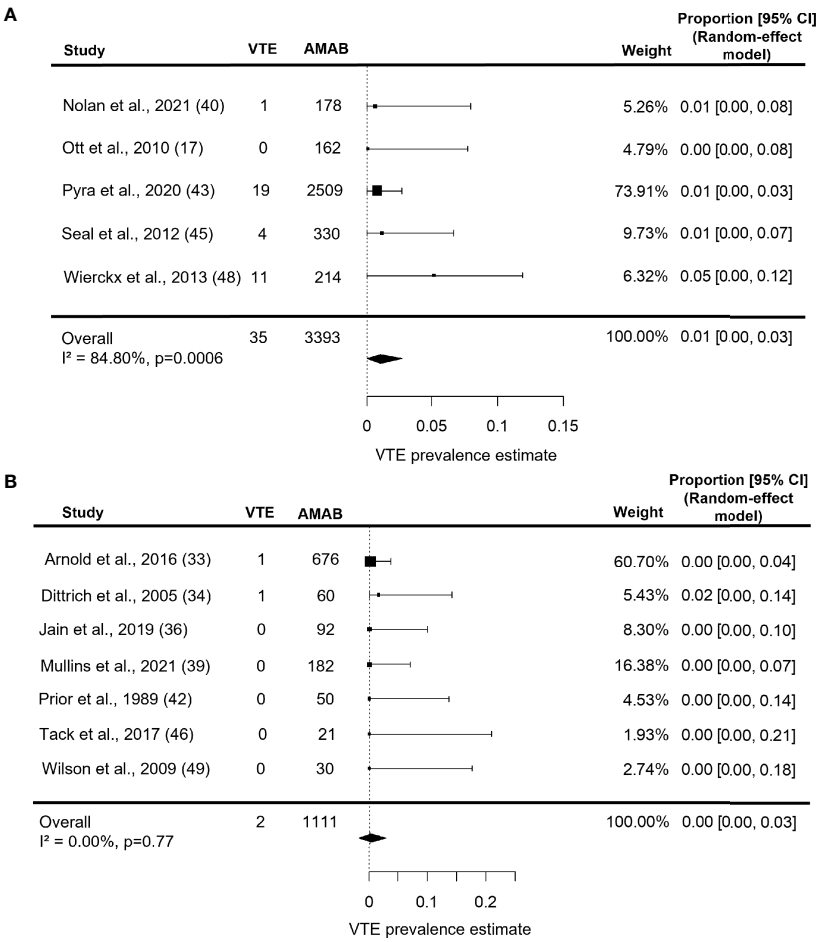

FIGURE 5 | Forest plots depicting the results of the subgroup analysis of the prevalence of venous thromboembolism (VTE) in Assigned Males at Birth (AMAB) trans people by length of estrogen therapy. The pooled prevalence estimate was calculated separately for studies enrolling AMAB under estrogen therapy for **(A)** more and **(B)** less than 53 months. Diamonds indicate the overall summary estimates and width of the diamonds represents the 95% confidence interval (CI); boxes indicate the weight of individual studies in the pooled results.

years) and under estrogen therapy for less than 53 months the risk of VTE appears to be negligible. Further studies investigating type and modalities of estrogen therapy are warranted to better manage the risk of VTE in this population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MT and AB conceived the concept and design. MT, SP, and AB evaluated the full text of all selected studies for eligibility. AP, SP, FD'A, and DT were involved in the acquisition of the data. MT, AB, and CC were involved in evaluation of quality assessment. MT and AB were involved in the statistical analysis and interpretation of the data. MT wrote the article under SF supervision. MB and SF critically reviewed the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.741866/full#supplementary-material

Supplementary Figure 1 | Funnel plots of results from studies assessing the prevalence of venous thromboembolism (VTE). The trim-and-fill analysis identified five putative missing studies (white circle) on the right side of distribution.

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SYSTEMATIC REVIEW
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Barriers to Accessing Health Care in Rural Regions by Transgender, Non-Binary, and Gender Diverse People: A Case-Based Scoping Review

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Research shows an overrepresentation of trans people in vulnerable socioeconomic situations, primarily due to experiences of discrimination. At the same time, rural or suburban living areas often lack specialized trans-related health care, which a majority of trans people rely on to some extent. Taken together, the lack of both socioeconomic resources and access to trans-related health care can exacerbate health-related distress and impairment for trans people. We illustrate this problem using case vignettes of trans people from rural and suburban areas in (Northern) Germany. They are currently participating in an e-health intervention and randomized controlled trial (RCT) called *I²TransHealth*, whose case vignettes provided the impetus for the scoping review. The scoping review analyzes the impact of place of residence and its intersection with barriers to accessing trans-related health care. PubMed and Web of Science Data bases were searched for relevant studies using a search strategy related to trans people and remote, rural, or suburban residences. 33 studies were selected after full-text screening and supplemented *via* reference list checks and study team expertise by 12 articles addressing the living conditions of remotely living trans people and describing requirements for trans-related health care. The literature on trans people living remotely reveals intersections of trans mental health with age, race, gender expression, geographic location, community size, socioeconomic status, discrimination experiences, and attitudes towards health care providers. Several structural health care barriers are identified. The role of health care professionals (HCPs) for remotely living trans people is discussed. There is no need assuming that rural life for trans people is inevitably worse for health and well-being than urban life. Nevertheless, some clear barriers and health disparities exist for trans people in remote settings. Empowering trans groups and diversity-sensitive education of remote communities in private and institutional settings are needed for respectful inclusion of trans people. Facilitating access to trans-related health care, such as through video-based e-health programs with HCPs, can improve both the health and socioeconomic situation of trans people.

Keywords: trans health care, barriers accessing health services, transgender mental health, geographic location, urban-rural divide, remoteness, e-health

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INTRODUCTION

Living outside of metropolitan areas often presents a significant challenge when trying to access specialized health care. In particular, health care for trans people (the term "trans" includes but is not limited to transgender, non-binary, or gender diverse people) remains a service provided mainly in larger cities. Thus, finding health care that meets their needs can often prove difficult for trans people living in rural settings since trans-informed health care professionals (HCPs; e.g., mental health professionals, in short MHPs, or physicians) are unlikely to be present in the area (1). Accessing health care becomes more complicated when certain factors, such as age, financial insecurity, or lack of education, are added to the difficulties inherent in rural areas. Improving this situation could be beneficial for trans people, as research has shown that good experiences with HCPs are positively associated with both general and mental health (2).

E-health approaches are being considered as a possible solution for access barriers to trans-related health care (3, 4). They can provide appropriate mental and physical health services to a wider range of people (5, 6). As a broad application area, e-health means a range of technologies to promote health and well-being. It ranges from electronic patient records and online consultations to mobile devices or apps. In short, e-health functions as a collective term for electronic information and communication systems in the health care system (7, 8). HCPs offer services using digital software with the aim of supplementing and improving their services. E-health approaches specific to mental health are often discussed interchangeably with the terms online therapy or distance counseling, implying treatment despite physical distance. Here, HCPs use electronic media enabling digital exchange with their patients, whether through e-mail, chat or video consultations (4). While digital care was long understood to mean primarily electronic patient records (7), newer services such as video consultations are gradually gaining ground in the course of digitalization and the legalization of communication media for patient treatment. E-health platforms that include video consultations still require intensive evaluation because they are a relatively novel tool (8). However, e-health approaches could be particularly helpful for hard-to-reach groups. Specifically, e-health approaches including video consultations are meant to help trans people accessing trans-informed HCPs regardless of their place of residence. In order to further investigate the potential of e-health approaches, we are currently conducting a randomized controlled trial (RCT) that allows trans people in remote, rural, or suburban areas in the early phase of transition or exploration of gender-related issues to participate in our internet-based health care program *i²TransHealth* (https://www.i2transhealth.de/english-landing-page/), which provides video consultations by trans-informed HCPs and local crisis interventions by general practitioners (GPs) and psychiatrists.

In the course of clinical work with service users of our internet-based health care program, *i²TransHealth*, it has become clear that trans people away from metropolitan areas experience disadvantages due to where they live. They experience marginalization through close-knit, prejudiced rural or small-town communities, they distrust their surroundings, and some see trans-related health care in their area as critical or nonexistent. We sought to examine this clinical impression and identify evidence-based studies that allow meaningful judgments about the additional burden of rural socialization of trans people on access to trans-related health care. A 2016 systematic review generally addressed primary health care for trans people with almost exclusively urban samples (9). A 2017 systematic review summarized rural sexual and gender minority health and health care generally without a trans-specific focus (10). Both systematic reviews examined U.S. studies exclusively (9, 10). Thus, we analyzed specific barriers to accessing trans-related health care of trans people with rural residential experiences. We collected clinical impressions in case vignettes and used this as a basis to develop a case-based scoping review that specifically addresses rural socialization of trans people, including those outside the United States.

This article reviews the existing literature on the lives of trans people in relation to their place of residence (e.g., remote, rural, and suburban areas) and how this, in addition to other sociodemographic factors and multiple lived experiences of discrimination in the community at home and among HCPs, affects access to trans-related health care. If studies permit a statement, differences and similarities between rural areas and big cities are outlined. First, we introduce case vignettes that aim at illustrating the situation of three trans people living in rural or suburban areas and are currently participating in *i²TransHealth*. The case vignettes appear different at first glance, but their shared problems stem from their remote living situation and have thus inspired this paper. As the case vignettes reveal intersecting barriers which require closer examination, they served as a guiding lens through which we reviewed the recent research on the topic.

The first case vignette of a trans woman illustrates that disclosure of her female gender in late adulthood life can be difficult because small-town, close-knit social structures can be rigidly attached to one form of living together:

> *M., a 60-year-old Caucasian trans woman with a middle school education, does the domestic work in her small family and lives in a small town. Encouraged by the emerging social liberalization, she no longer wanted to hide and, after consulting her wife, occasionally put on her "feminine" clothes when alone with her. She now increasingly expresses her femininity in everyday life at home, but since she doesn't want other people in town to see her this way, she changes clothes and removes make-up several times a day. The increased female gender expression has led to resistance from M.'s wife, who has said "I married a man" and has threatened to move out. She currently has no contact with other trans people nor is she trying to engage with the trans community. M. understands how overwhelming her transition might be for her family. In order to make them less uncomfortable, she wears*

less explicitly feminine clothes or does so only during the video consultations conversations. At one point, she dared to go to the nearby city dressed in feminine clothes but disguised by a face mask (due to COVID-19). She wishes she could go outside like this with her wife but can currently only express her female gender at home and even there it is limited. Thus, the current goal of treatment remains searching for ways, places and the right pace at which M. can live as the woman she is, while at the same time not threatening her family's cohesion.

Another aspect of a remote life may be a lack of knowledge about gender dysphoria, and thus an awareness of treatment options and potential pathways must first be acquired:

L. is a 26-year-old Caucasian trans man living in a village in Northern Germany. As a child, L. always insisted on short hairstyles and displayed several traditionally male-associated interests and hobbies. For a long time, L. did not understand why he felt such intense discomfort in respect to his body. Only three years ago did he stumble onto a YouTube video by a fellow trans man and learned about the concept of being transgender. Few MHPs work within L.'s local area and even fewer have sufficient knowledge about gender dysphoria. Prior to joining the i²TransHealth project, L. had one initial session with a MHP but didn't feel comfortable there. The internet has been L.'s main source of information about trans issues, but he doesn't like posting about his personal matters in online forums or on social media. Given the lack of local in-person opportunities, his options of meeting and talking to people who share some of his experiences remain very limited.

The third case vignette shows that the consequences of discriminatory experiences in health care can lead to significant impairment of one's health:

R., a 21-year-old Caucasian trans man, lives on a farm in a small town. From early childhood he has felt "more like a boy than a girl". Several years ago, R. sought out a MHP to move forward with his medical transition. However, he felt extremely uncomfortable with this MHP who frequently invalidated his experience. R. felt patronized and belittled. He says the MHP simply didn't believe him when he told him about his gender experience. Consequently, he also did not receive hormonal treatment. After a year, R. quit therapy. He says that this MHP was more harmful than helpful and that this experience put him off seeking further medical or mental health care for a long time. It took R. two years to recover from this. Only then did he find the courage to try again by reaching out to the i²TransHealth project. Having to live in a body widely perceived as female severely limits R.'s self-expression. He is constantly self-monitoring his appearance and his effect on other people and feels forced to "perform" his masculinity much more stereotypically than a cis man would have to. R. does not want to start his professional training by having to out himself in front of so many people (again) and is thus waiting until hormone treatment has begun and shown some effects.

As illustrated by the case vignettes, barriers to care are a frequently discussed problem in trans health services (5, 11). However, the actual characteristics of, and differences between, trans-related health care in urban and remote settings have rarely been researched (12). With this in mind, we aim to identify and reduce research gaps concerning the remote, rural, or suburban situation of trans people. Therefore, we review and evaluate previous research on the impact of rural or suburban living on access to general and trans-related health care, and identify research questions to be investigated in future studies. Additionally, we discuss how to better address trans people's needs in rural areas by taking an intersectional view of their experiences within the health care system. Thus, we aim to answer the following research questions:

1. How does living in rural areas affect access to specialized trans-related health care services?
2. How do health burdens of trans people in rural areas intersect with other barriers and risk factors in health care?
3. What possible solutions have been identified to address the problem of health burdens of trans people in rural areas?

MATERIALS AND METHODS

Scoping reviews have proven useful for research questions for which large research gaps and sparse literature available for systematic analysis (13, 14). The methodology of a scoping review is appropriate for providing guidance on the current state of the research literature in the case of a paucity of research on a topic and for making recommendations for future research (14). A scoping review aims to delineate previous concepts and substantive boundaries on an area of research by including studies regardless of their quality and allowing for an up-to-date assessment of the evidence (14).

For the present scoping review, the objective was to examine the significance of the urban-rural divide for trans-related health care. The overarching lens culminates in the three research questions of whether rural environments impose health burdens on trans people, whether these potential health burdens overlap with barriers to health care access that have already been studied in more detail, and whether health burdens can be prevented or reduced in non-urban communities. As introduced by the case vignettes, the three research questions of the scoping review address the impact of trans people's remote, rural, or suburban socialization experience on their barriers to accessing trans-related health care and the interrelated

aggravating factors. Against the background of the case vignettes, we analyze the evidence on residence as an aggravating factor combined with other barriers to care. This case-based scoping review concentrates on research investigating the benefits and drawbacks of rural living for trans people and/or their specific barriers to care. More general research on the lives of trans people is also discussed.

Within the present scoping review, the target population are trans people regardless of age or identity, although combined LGBTQ+ samples with trans people (i.e., people who identify as lesbian, gay, bisexual, transgender, or queer; the + stands for the inclusive representation of all identities and expressions) were allowed in the absence of comprehensive research on the topic area. Additionally, HCPs were included in the target population if they provided information about their work with trans people. Articles were excluded if they generally lacked any meaningful findings on non-urban residences or specifically failed to provide more in-depth analyses to trans people in combined samples. The core concept examined was remote, rural, or suburban regions and their intersection with barriers to accessing trans-related health care. The specific context was explicitly geographic location and thus the extent to which remote, rural, or suburban socialization influences trans health. Studies should consider perspectives of trans people with past or current rural residential experiences.

The search strategy followed a 3-step process recommended for a scoping review (14). First, a non-systematic search of databases was conducted to determine if studies existed on the topic under investigation. Based on the key terms found in abstract or full text, we found that an overly differentiated, potentially limiting search strategy was not indicated given the paucity of research. Second, a search was performed in the PubMed and Web of Science databases using the terms "transgender" AND "rural". During the review process, the search string was adapted to include synonyms related to transgender and rural areas. As we assumed the evidence base to be weak, no further restrictions were made. Third, the reference lists of the selected papers in the full-text review were scanned for additional potentially relevant studies. The approach to the search strategy was iterative in design, thus ensured an overview of the literature, a critical review of the search strategy, and supplementation of the included study through repeated searches. The full search strategy can be found in **Appendix 3** (**Supplementary Material**).

Both first and last author (JR, TN) elaborated on the search strategy and made considerations about specificity and sensitivity of the search. Due to the sparse literature and in order not to exclude relevant studies, we applied a high sensitivity to the search strategy, i.e., including false positive hits irrelevant to the research question was preferred over excluding relevant papers. JR exported the citations, pre-selected relevant studies in Rayyan (15), and performed full-text screening. The search included empirical qualitative and quantitative articles as well as theoretical reviews on the general situation and mental health of trans people in rural areas. Based on the research group's expertise on barriers to accessing trans-related health care, six other empirical studies and reports relevant to the research question were included. JR and TN discussed the reasonable inclusion of further studies for this purpose. In the course of this, included information sources went beyond empirical studies. Thus, reviews, reports, and gray literature are also found in the present scoping review (14).

The combined database searches from PubMed and Web of Science yielded 497 records (see **Appendix 1 Figure 1, Supplementary Material**). Of these, 133 duplicate records were removed before review. JR reviewed the papers according to the following inclusion criteria: peer-reviewed publication in English by mid-August 2021, study participants included HCPs or trans people, possibly also as a subgroup of the LGBTQ+ community, and non-urban place of residences were explicitly addressed. Research group members read articles in full if these criteria were met. 77 citations were included in the full-text review and checked for eligibility. The present work complies with the extension of the PRISMA Statement for Scoping Reviews (PRISMA-ScR) (16).

RESULTS

For the scoping review, 33 records were identified, to which additional literature was added. Supplemented by studies taken from reference lists and papers known to the research group, a total of 45 sources (43 empirical studies, 2 reviews) were selected for the scoping review and are presented in **Table 1** (**Appendix 2, Supplementary Material**). Among the 43 empirical studies, we found two types of articles: 31 articles dealing explicitly with trans people and 12 articles dealing with trans people within the general or broader LGBTQ+ population. The included studies are divided by study type into 28 quantitative studies (including 23 original papers, 4 reports, 1 poster presentation), 13 qualitative studies, 2 mixed method studies, and 2 systematic reviews. A flowchart of the study selection process is shown in **Figure 1** (see **Supplementary Material**). The final 45 articles included in the review describe studies from the United States, Canada, Australia, Germany, Georgia, Poland, Serbia, Spain, and Sweden, as well as an overall report on the situation in the European Union including the United Kingdom.

The few studies of trans people who have had experiences living in rural areas generally show mixed results such as influences from rural socialization experienced to varying degrees by trans people. To further organize the findings, we also looked at studies that consider trans people in the larger context of access barriers to trans-related health care, where the variable of place of residence is described but not always one of the main factors discussed.

Given the three research questions of the scoping review, we illustrate the results to clarify the influence of the urban-rural divide on access to trans-related health care. We begin with the sociodemographics of a trans person that regulate their access to health care (research question 1). We follow this with intersecting factors such as discrimination in the community

and highlight the consequences of negative or inadequate care situations as a health risk (research question 1 and 2). Previous approaches to addressing existing problems, such as support persons or groups and training for HCPs and caregivers, are listed in the following sections (research question 3).

Sociodemographic Aspects Concerning Trans People

In order to answer the first research question, sociodemographic data can provide an initial overview of possible health burdens for trans people due to their place of residence. The literature search detected several sociodemographic aspects that shape the lives of trans individuals and their access to trans-related health care. In terms of socioeconomic status, trans people are as likely to be married, employed and living in a rural area as the rest of the population, but are more likely to be People of Color, below the poverty line, and without a college degree according to a household probability sample of U.S. adults with 691 trans adults compared to 150,765 cis adults across all age groups (17). Several U.S. surveys confirm that many trans people, as well as other members of LGBTQ+ communities, do not have health insurance given their financial challenges and lack of mandatory coverage (18, 19). In terms of housing, a substantial number experience homelessness; in the U.S. National Transgender Survey of 27,715 respondents of all ages 18 and older, 30% ever experienced homelessness and 12% experienced homelessness in the past year (19). The LGBTI II report of the European Union member states and United Kingdom with over 139,799 people (mean age 29, age range 15 to 55+; 14% of the sample are trans people) shows 7% of European trans people are unemployed, 5% are unable to work due to health reasons, 46% have some to great difficulty making ends meet in their households, and a total of 48% report their place of residence as small town, village, or home in the countryside (11).

Problems stemming from sociodemographics come to a head for People of Color in limited access to health care in the U.S. According to a large quantitative study of 5,135 US transgender veterans (mean age 51.21), Black compared to White transgender veterans delayed or did not use mental health services even when they existed (20). At the same time, Black transgender veterans are 65% less likely to live in rural areas (20). But they have greater social disadvantages, increased health risk, be it alcohol abuse, heart problems, high blood pressure or depression (20).

Age is also a critical variable for trans patients seeking treatment. Given an online survey of 252 respondents (mean age 47.9), rural residing trans and LGBTQ+ US veterans face longer travel times to HCPs than their suburban or urban counterparts (21). According to the Canada Trans Health Survey, for rural and remote residing trans youth in particular, who comprised 9.3% of the total sample, aged 14 to 25, transportation presents significant obstacles for accessing urban health care (2). Samples often focus on middle-aged participants, neglecting trans youth in rural or suburban areas, where sparse research exists (22). However, trans people at the lower and higher ends of the age spectrum are particularly isolated in regards to health care (2, 21).

Growing up and Living With Bullying and Discrimination in the Community

The first two research questions require data on health burdens of rural residential experiences and how these, along with other barriers to trans-related health care, contribute to the extent to which help is sought and trusted by HCPs. The included studies reveal that the social climate of a rural or suburban community in which a person grows up or lives is formative in one's life, making sexual and gender minorities (SGM) vulnerable to mental health issues and less likely to seek help in their community or health care. A systematic review highlights that isolation in rural areas and low levels of social support have a negative impact on the health of SGM, and discusses the sparse health data and lack of tailored interventions to address existing disadvantages for LGBTQ+ people in remote, rural areas (10).

A school-based survey in the US state of Minnesota with 2,168 trans youth in 9th and 11th grade, representing middle adolescent age groups, found that those living in rural areas reported the highest levels of bullying and victimization compared to urban areas, while emotional distress was highest among people living in suburban areas (22). For LGBTQ+ people, an online survey in the US state of Nebraska with 770 respondents (aged 19 to 60 years or older; 10.9% of respondents identified as transgender) showed that rural LGBTQ+ people engaged less with others socially, came out to fewer people, and showed lower self-acceptance compared to their urban counterparts (18). A U.S.-wide survey of 5,420 LGBTQ+ secondary students (mean age 15.9 years; of whom 4.5% identified as transgender and 4.0% with a different gender identity) revealed that, as a subgroup, transgender youth are more likely to be bullied for their gender expression or sexual orientation than gay or bisexual male youth (23). Although students in rural schools generally experience less violence and harassment than general population in urban schools, LGBTQ+ youth experience rural schools as extremely unsafe places due to not blending in with cis-heteronormative norms (23). Conversely, queer youth in urban regions are less likely to experience discrimination than their rural counterparts. Victimization at school is often associated with increased self-harming or risky health behaviors among LGBTQ+ youth (23), compounded by a lack of safe spaces in a more homogenous rural school community or an environment that is generally less diverse. According to qualitative interviews with 30 trans adults (mean age 36.0) in rural US state of Montana, rural residing trans people have to deal with bullying, discrimination and marginalization in their environment (24). In particular, suicidality rates are high there. 80.0% reported having had suicidal thoughts in the past, and 46.7% reported having attempted suicide in the past (24). According to qualitative in-depth interviews with 19 trans people between the ages of 15 and 22 (mean age 18) living in Midwestern U.S. states, they indicate that resources for trans people such as existing SGM community groups and previous support or external validation of sexual or gender identity make them feel comfortable in the social climate of their area (25). According to data from 14 to 18 year old trans and gender questioning youth from 7 qualitative in-person

interviews and a survey of 70 respondents in the Midwestern U.S., young trans people's estimations of rural community climates toward SGM range from unsupportive to hostile (26). In rural areas, such as the US state of Nebraska, trans people tend to be less involved in peer groups and less supported by family or friends compared to other groups within the LGBTQ+ spectrum (18). Primary caregiver support is critical to the experience of social support because it can foster appreciative, respectful interactions within the family and environment, as revealed by in-depth interviews about maternal support with 25 trans adults (mean age 34.48) in Central Appalachia (27). MHPs, the authors recommend, should include the closest caregivers *via* counseling or psychoeducational workshops in rural areas to address the potential for stigma and stress within small and insular social networks (27). According to qualitative interviews with US trans people (age range 25 to 61; a focus group of 6 persons and 1 individual interview), interrelated issues affecting their well-being that MHPs should address are vocation, personal change and coming-out, acceptance, and identity (28). In these aspects, trans individuals are highly dependent on the support of their family and environment, which has often experienced less education on gender fluidity than urban spaces (28). In a qualitative interview study of 25 trans women (mean age 27.56 years) from the U.S. state of Oregon, several trans women who moved to Oregon's metropolitan areas with rural residential experience in Oregon or neighboring states addressed prior victimization experiences in general or severe threats of violence specifically in the family (29). Later, the "family of choice" in adulthood is seen as playing an important role in empowerment, navigating health care systems, and arrival in a metropolitan trans-friendly community (29).

According to an intersectional analysis of 45 individual interviews with trans men from the U.S. Midwest and Southeast, trans people do not reject rural life per se, but many do not want to stay in their home region but move to a new rural area in order to avoid unpleasant encounters with former acquaintances (30). A qualitative study from a Canadian small city with 13 young LGBTQ+ participants (aged 15 to 25 years; 4 individual interviews and 2 focus groups), of which 5 were trans, indicated that LGBTQ+ people who had so far lived exclusively in rural areas considered small towns to be more restrictive than LGBTQ+ people who had experiences living in both rural and urban areas (12).

Rural Health Care Providers

Previous research on stakeholders' estimations of LGBTQ+ populations by 207 community members from various U.S. town hall dialogues and summits revealed knowledge deficits in HCPs and a lack of culturally sensitive expertise in rural areas (31). Past research examining treatment counselors' attitudes toward trans people and LGB people found equal deficits in knowledge or skills to provide competent help in rural and urban settings (32). In 2004, 109 counselors from urban Chicago (mean age 41.3) and 242 counselors from rural Iowa (mean age 40.9), were similar in terms of their increased negative attitudes toward trans people (32).

The unquestioned assumption that patients are cisgender and heterosexual create a discriminatory experience for many LGBTQ+ people (33). From various survey and interview data with HCPs and LGBTQ+ people from the United States, Canada, and Europe (33–35), it appears that various HCPs exhibit ambivalence toward LGBTQ+ patients, microaggressions, and microinvalidations, e.g., misgendering or deadnaming trans clients. Rural HCPs have fewer LGBTQ+ patients and less diversity-related training opportunities, making the conscious creation of an LGBTQ+ friendly environment more unlikely (31). This, combined with the close-knit nature of many rural communities, makes it difficult to be open about one's gender identity or sexual orientation to a rural HCP because they probably know one's relatives and friends, according to qualitative in-depth interviews with 16 LGBTQ+ youth (ages 15-24) and 21 LGBTQ+ adults (ages 25 or older), as well as 14 key informants with experience working with LGBTQ+ clients in the Northwest Territories, Canada (33).

Both the Trans Health Survey in Europe (surveyed 885 trans health care users ages 16-77 with a mean age of 27 and 888 HCPs with mean age 41.7 from Georgia, Poland, Serbia, Spain, and Sweden) and national data from 5,831 U.S. transgender adults (mean age 37.0) show that when the environment is perceived as discriminatory rather than inclusive, the odds of trans people believing in and seeking trans-related health care are low (35, 36). A German online survey with a non-clinical sample with 415 trans people aged between 16 and 76 revealed fewer treatment experiences, and fewer contacts with support groups or other trans people among persons from rural areas compared to persons from urban areas (5, 37).

An online survey of 208 health care providers in West Virginia found that although a majority of rural or suburban HCPs held generally positive attitudes about treating trans people, they admitted to assuming their patients to be cisgender and needing further training to effectively offer care to trans people (38). Critically, HCPs who held fewer positive attitudes – who were also shown to be disproportionately male – perceived less barriers to treatment but also showed less personal preference to treat (38). Similarly, a systematic review on health and health care on rural residing SGM have found a lack of favorable attitudes, training and desire to train in rural HCPs (10). According to the U.S. National Transgender Discrimination Survey of 6,436 respondents of all ages, 19% of trans people were ultimately denied treatment by HCPs and 50% had to educate their HCPs about trans-related health care (39). Qualitative interview data from trans women with prior rural residential experience report that in rural areas, trans people have to pay for their own trans-related medications, and hospitals near their homes refuse to care for trans people, even when urgent treatment is needed for self-destructive behavior (29). According to questionnaire data from 13 transgender and sex/gender diverse people (majority aged 25-44), homophobia and transphobia are a factor in health care in the largely rural Northern Territory of Australia (40). HCPs are seen as mostly unhelpful, so several trans people seek medical care in other Australian states (40, 41).

Taken together, this creates a situation in which trans people are faced with (un-)intentional microaggressions and stigma (10, 38) while barriers to care remain largely unaddressed.

Experiences and Consequences of Insufficient Health Care

As illustrated by case vignette 3 and an answer to the second research question, a negative health care experience can have long-lasting effects and deter trans people from seeking further support, making them more vulnerable to mental and physical problems (42). Results of a systematic review on LGBTQ+ health and health care show that people belonging to SGM and living in rural areas have come to anticipate discriminatory health care based on former experience and often do not trust HCPs enough to disclose their sexual orientation or gender identity (10). As a result, many trans people avoid health care services entirely, which is particularly evident in conservative regions, or are forced to accept what is offered due to a lack of options (10, 36). Often, trans people expect to be treated badly (35) or have already suffered from experiences of discrimination and violence in public institutions, e.g. a doctor's office or hospital, a mental health clinic or emergency room (39). Analyses of semi-structured interviews with Australian remotely living 15 trans clients aged 19-69 years and 8 HCPs revealed, expected discrimination for several trans people is also based on their conservative assessment of their housing and not necessarily on actual experience (41). Researchers of a U.S. interview study with 10 rural residing trans people (mean age 36.2 years) noted a fundamentally pronounced negative attitude of trans people toward rural HCPs (43).

In a survey of 1,014 U.S. rural residing LGBTQ+ individuals (169 of the respondents were trans with a mean age of 32.2), higher anticipated and experienced stigma correlated with poorer reported health among trans people (44). In particular, assigned female at birth (AFAB) trans people avoid important sexual health services (e.g., contraceptives, PAP tests) and accept health risks if they lack access to specific and trans-informed clinics (45, 46). An illustrative example is offered by HPV vaccination recommendations and HPV vaccination: rural residing US trans individuals are primarily offered treatments based on their sex assigned at birth according to an analysis with 660 LGBTQ+ respondents (ages 18-34; 7% identified as trans man, 4% as trans woman, 6% reported a non-binary gender identity), rather than providing the vaccination to everyone right away (47).

Given the U.S. Survey Behavioral Risk Factor Surveillance System, 237 trans men compared with 163,685 cis adults of all ages showed a reduced likelihood of having a personal family doctor or undergoing cholesterol screening (48). Care refusal is a critical issue, as trans people in rural areas show several health risk factors, such as binge drinking, smoking, substance abuse in general, and higher odds of posttraumatic stress disorder (3, 44, 49).

On average, trans people have significantly poorer mental health than the rest of the general population (39, 50). This is particularly true for residents in rural areas. Research comparing location categories found that trans high school students in rural areas show the highest level of self-injury and suicide attempts (22). However, members of the suburban trans population showed the highest levels of depressive symptoms and suicidal thoughts despite their proximity to larger cities and resources, suggesting that location categories need more differentiation than just "rural" and "urban" (22). As an online survey of 414 trans people (mean age 39.58) found, trans people living in more rural US states suffered more from anxiety and depression than trans peers in other regions (51). In the Trans Health Survey of 902 trans people from Canada and the U.S. (mean age 32.47), rurality correlated with higher social anxiety among trans people (52). In an online survey (mean age 36.0 years), 91 trans people in the U.S. state of Nebraska reported higher rates of discrimination, depressive symptoms, and suicide attempts compared to 676 lesbian, gay, and bisexual people (53). Further, there are strong differences in access to health care between urban and rural citizens according to a survey with 414 trans people aged 18 to 78 years across the U.S. (51). Suitable mental health services are hardly available for rural residing trans people. Even in urban areas, a bottleneck situation exists due to the low number of trans-informed HCPs. The majority of trans people from one region seek treatment from the same few highly specialized experts, most of whom are known by name in the community and play an important gatekeeping role in health care, as highlighted in the European Trans Health Survey and qualitative individual interviews with 10 U.S. MHPs (mean age 56.4) about the health care situation (35, 54).

In a recent qualitative interview study of 2021 with 61 adult transgender and gender diverse people and 23 HCPs of all ages 18 and older, all from 25 different rural U.S. counties, trans people cite urgent community mental health needs such as, in addition to moving away from binary settings, increased accessibility of MHPs through more flexible solutions such as e-health approaches (55). HCPs of the same study did not come up with this idea, focused on existing approaches and systems in ensuring mental health care, but criticize that rural care cannot keep up with urban centers for health care (55). Meanwhile, qualitative interview data from Australia and the United States show that the Internet has become an important resource of information and community building for trans people seeking help (41, 43).

DISCUSSION

Evaluation of the Scoping Review

The current scoping review focused on the issues facing trans people in remote, rural, and suburban areas concerning their specific barriers to accessing trans-related health care, and potential approaches to address issues related to place of residence. Several research gaps emerged regarding the impact of location on the health and quality of life of trans people. Through the initial search strategy, it became obvious that previous research has not collected sufficient data on the urban-rural divide in trans health. Many studies did not

include rural or suburban trans people at all and could therefore not be considered for the review. Nevertheless, it was possible to obtain an overview of the situation of remotely living trans people by means of a scoping review.

Related to the first research question, we found that living in remote, rural, or suburban areas can significantly impede access to specialized trans health services. Trans-informed HCPs are predominantly found in metropolitan areas, which means long, costly commutes for trans people (2, 21). In addition, many rural or suburban residing trans people experience discrimination from non-specialized or dismissive HCPs in their area (31–33). When we look at the aggravating socioeconomic factors such as financial insecurity, job insecurity, housing insecurity among several trans people, a very limited access to health care becomes apparent (11, 17–19). Thus, according to the first research question, the health burden of trans people in rural areas seems to lie in structural problems related to remoteness, education, housing, health insurance coverage or employment, and age-specific mobility problems that impede access to trans-related health care. However, when evaluating research on urban-rural disparities, it appears relevant not to ignore the substantial number of trans people who have ever experienced homelessness in their lives, and thus may not even be reached by residence-based studies.

We were able to observe this picture also in the answer to the second research question. Starting with even more pronounced bullying experiences at school and in the surrounding area compared to urban trans peers, rural and suburban residing trans people experience discrimination from an early point in their lives (22–24). Discrimination can persist in close-knit rural social structures, including through other forms of discrimination, such as racism (30). Also, cis-heteronormative attitudes and behaviors of HCPs can make goal-directed health care difficult (38, 39). Many rural and suburban residing trans people avoid social contact and do not seek help and support, which can then be reflected in a rejection or avoidance of health care (35, 36). According to the second research question, we conclude that negative experiences and confrontation with strong homophobia and transphobia can damage a trans person's trust in their own environment. This unfavorable starting point overlaps with already known barriers to accessing trans-related health care, such as long journeys to specialized transgender clinics, and exacerbates the difficult mental health situation of trans people. Trans-related health care is primarily located in metropolitan areas. However, if trans people generally do not expect support from their environment, they may be unaware of support services or avoid trans-related health care services. The isolation of trans people can therefore deepen in rural areas.

In the third research question, we also looked at possible solutions that could address the problems of trans people, especially in rural or suburban areas. We identified that supportive caregivers (27) or SGM community groups (25) are significant in helping a trans person feel safe and comfortable in their environment. Diversity-aware trainings for HCPs and other professionals such as teachers or social workers should also remain in focus to create an inclusive environment (27, 28). In response to the third research question, we identified the often-emerged importance of the "family of choice" for a positive self-image and being accepted in a community (29). Because families of choice often first emerge in adulthood, we as a study team see the need for support systems in general but specifically in rural areas, from early in life. Through LGBTQ+ empowerment groups, education of families of origin, educational and health submissions about diversity, a social climate can be created in which trans people feel accepted and respected. These are meaningful health prevention interventions. These are known aspects that can increase a trans person's confidence in others and could increase the possibility that they will seek help and support in trans-related health care. New opportunities such as e-health approaches by trans-informed HCPs could fill a gap in care. The literature has barely touched on this possibility, if at all. We would like to illustrate below the potential role of e-health in overcoming or reducing barriers to trans-related health care.

The Role of E-Health

With the advent of digitalization in private life and the health care system, a number of possibilities are opening up for trans people in terms of individual access to trans-related health care. Qualitative interview data from Australia, North Queensland, revealed that the internet is crucial for many trans people to share resources and compensate for low local networking and peer support. For trans people, the internet and social media play an important role in finding information about gender identity, transition, and trans-related health care, and in reducing isolation, making reliable internet access essential for mitigating or overcoming existing problems, particularly in rural areas (41). Therefore, in light of previous articles and assessments, expanding online (and offline) trans support groups (41) as well e-health approaches (3, 4) could have a beneficial effect on trans-related health care, reduce financial burdens, and optimize the internet's health care potential. Trans people themselves also expect e-health approaches to improve health care in terms of better accessibility and flexibility (55).

Yet, there is limited data on the use of e-health approaches for LGBTQ+ health care. A pilot study on an e-health program for trans women of color in Washington, DC, appears to be a promising effective and low-cost way to overcome multiple health care barriers and increase the intention to seek trans-related health care (56). Due to the COVID-19 pandemic, many health care services have had to rapidly implement e-health technologies to ensure continued treatment. One U.S. LGBTQ+ clinic has received positive reactions from patients for their video consultations and reports fewer cancellations and no-shows, presumably due to the increased flexibility and decreased effort of attending an appointment. Patients also seemed more comfortable and relaxed (57). Other research on HIV prevention in LGBTQ+ populations *via* e-health has shown promising results in terms of program adherence and satisfaction (58).

E-health in medical care has been used widely in cancer prevention and care, an area comparable to trans-related health

care in its specialization and individualization of treatment. A review of e-health programs for cancer care has shown numerous benefits such as increased access to specialist and multidisciplinary health care (59). E-health were as effective as in-person interventions in providing psychosocial support, increasing quality of life and ensuring patient satisfaction (59, 60). Such findings hint toward the potential of e-health for improving health disparities and removing access barriers like the ones discussed in this article. With this in mind, we have designed an e-health program *i²TransHealth*, for which we are currently evaluating its efficacy within an RCT (https://clinicaltrials.gov/ct2/show/NCT04290286).

Research Bias Towards Big Cities and Neglect of Remote Life

The idea that the city is more inclusive than the country is pervasive and most research generally assumes metropolitan areas to be more progressive and trans-inclusive. Some researchers refer to this as the "metronormativity bias" (30). Many trans people and other LGBTQ+ representatives also strive for a life in a big city (12). Trans people themselves expect rural regions to be more conservative and discriminatory, which they often base not on actual experience but on less queer and trans visibility in rural areas (41). However, rural communities are not necessarily negative for trans people, just different (26). Personal fit greatly affects the experience of trans people in rural areas: Some trans men easily find connection in these communities, but this mainly applies to those who fulfill the traditional role of working-class White men, which excludes People of Color and non-traditionally male presenting trans men (30). Perceptions of rural communities can shift depending on life experiences and possibility for comparison with urban areas, as illustrated by a Canadian qualitative study (12). However, the study also showed a strong consensus of almost unequivocal experience of rural socialization as limiting and conservative.

Unfortunately, differentiated group analysis by place of residence often fails because trans people from rural areas are more difficult to reach for studies (22). Due to this limited available data, some reviews only include data on trans people in urban spaces or combine samples from larger cities and suburban regions, which reinforces the already existing metronormativity bias (22, 30). To enable analyses, some studies also combine rural and suburban communities (52). Other researchers strongly advocate differentiating suburban from rural communities, which often results in a very small number of rural participants (12, 18). These varying definitions and operationalizations, or lack thereof, of location categories (e.g., metropolitan regions, non-metropolitan areas, small metropolitan areas, small towns, rural communities, etc.) limit the conclusions that can be drawn from the current research (26). Grouping study participants by postal code or self-classification seems to be common, but a closer look at population sizes could be beneficial for more complex analyses (10).

Thus, reliable comparisons between rural and urban health care conditions remain difficult, although the finding that trans people in rural areas are likely to suffer from greater health inequalities seems fairly robust (9, 10). These inequities are already impacting primary care treatment, disease prevention, and health-damaging behaviors (9), with far-reaching consequences for health care utilization.

Limitations

A major limitation of the scoping review is the overwhelming representation of North American studies (i.e., 39 studies), largely due to the general lack of research on remotely living trans people. This is also reflected in the fact that previous work from other geographic regions, such as Europe, Africa, Asia, Australia, or South America, has limited in-depth comparison of rural and urban structures for trans people's lives. The few reports from other regions were added by the study team itself based on its own expertise (i.e., 4 European studies). However, the initial search also yielded at least two Australian studies. Overall, sparse research in trans-related health care has addressed the impact of the variable of place of residence on trans people's life satisfaction and the quality of health care they receive. This reduces the number of potentially relevant studies and exposes other inequities, such as that trans people who live near larger cities or trans-affirming spaces are more likely to be researched because they are easier to reach for study recruitment. As a study team, we executed the search strategy with the goal of high sensitivity to capture relevant studies that advance transgender- and rural-related research. Therefore, the scoping review depends on the available research literature on the concept of remote, rural, or suburban regions and their intersection with barriers to accessing trans-related health care. The number of relevant studies is small but highlights all the more the urgent need for further empirical research on the marginalization of remotely living trans people.

The focus on rural socialization of currently or ever remotely living trans people varied across studies. With 21 studies, about half of the included studies adopted purely rural or suburban samples, while the other included studies analyzed mixed samples with individuals of different places of residence. In this regard, the mixed samples were unbalanced, considering individuals with rural or suburban residences with their partial low representation in the small percentage range. Generalizing statements across communities are therefore not applicable. An international in-depth comparison of the situation of remotely living trans people is also not applicable due to the divergent study designs and overrepresentation of U.S. studies. On a positive note, as far as we know our scoping review is the first to include Australian, European, and Canadian studies in the literature related to the specific topic of the review.

As a consequence of the concept of a scoping review compared with a systematic review, the results underwent a synthesis of content rather than a critical examination of study design and strength of evidence of the included papers. Because this is one of the first reviews in the context of remote, rural, and suburban areas to highlight potential new solutions such as e-health approaches as potentially improving care for hard-to-reach populations of trans people, a more in-depth look at the global situation of trans people was not possible. The results can be considered preliminary. They should be revised as the

implementation of trans-specific support groups and educational opportunities in rural areas, as well as the digitalization of health care, continues to change the influence of the variable of place of residence and allows remotely living trans people to not feel disconnected and to be well served by e-health approaches.

Research on the health of SGM, particularly trans people, specifically in rural areas is very limited and consists mainly of cross-sectional surveys and qualitative studies (10). The definition of "rural" varies and this lack of consistent differentiation between location categories (i.e., rural, suburban, small town, etc.) might disguise distinct aspects that affect health care. Although the particular circumstances in the presented case vignettes of *i²TransHealth* may differ, all three trans persons are united by their remote living situation and their difficulties in accessing trans-related health care. Although the access barriers to trans-related health care are well known, it is still difficult for trans-informed HCPs to address structural factors, such as place of residence (11, 19, 35, 39). Clearly, one necessary step is to improve the quality and options of treatment in rural areas by proliferating the expertise currently only available in a few specialized urban clinics.

Thus, competence training for (rural) HCPs and, ideally, also teachers and social workers is of high importance (22, 31, 41) and should cover an intersectional view of discrimination as well as the effects of microaggressions on the course of treatment (34). E-health could also prove a powerful tool for spreading expertise and making quality trans-related health care more widely available. E-health could help break down some of the reservations toward trans-related health care, which stem from the fear of encountering uninformed and even discriminatory HCPs (10, 35, 43). Anecdotal evidence – such as our third case vignette – as well as empirical evidence suggests that these fears are not unfounded: 33% of respondents in the U.S. Transgender Survey said they had had at least one negative experience with a HCP in the past year related to being trans (19).

CONCLUSION

Using three case vignettes as a starting point, the scoping review focused on the lives of trans people in remote, rural, or suburban areas. The interest in assessing previous research was how living in remote, rural, or suburban areas affects individual access to trans-related health care services. Having identified place of residence as a potential aggravating factor, we also strove to pinpoint other barriers and risk factors. During the review we discussed intersections of trans mental health and discrimination (11, 39) with age, race, gender expression, geographic location, community size, socioeconomic status, experiences and attitudes towards HCPs. Potential innovative solutions to reduce inequities in access to trans-related health care, in our view, lie in e-health approaches that require further evaluation. Alongside this, HCPs are encouraged to engage the immediate environment, such as family or friends of a trans person, in therapeutic approaches and educational programs on inclusion and diversity to change discriminatory attitudes in communities. This could reduce problems resulting from place of residence and empower individuals to seek help in trans-related health care.

Although research on currently or ever remotely living trans people to access the urban-rural divide in trans-related health care is still in its infancy, a growing field of research has gradually emerged over the past few years. Note that researchers should not merely look at the (un-)availability of resources – e.g., no visible representation of LGBTQ+ groups in rural schools (22) or cis-oriented sexual health services in rural areas (41, 46) – but assess whether they are appropriate and queer/trans-specific (25). Also, a rural setting should not be categorically viewed as a barrier, but as a potentially aggravating factor. For even in a supportive rural environment, long journeys to specialized transgender clinics remain. Therefore, e-health approaches such as our *i²TransHealth* project could be a way to address or mitigate structural barriers. HCPs must widen their scope in order to reach people regardless of their place of residence. This would be one critical step towards decreasing inequalities and reducing the mental, physical, social, and economic burden that trans people have to bear. Whether such approaches are effective in breaking down barriers thus remains a worthy and important topic that we ourselves and hopefully other researchers and HCPs will continue to investigate.

ETHICS STATEMENT

The study was conducted in accordance with the declaration of Helsinki and was approved by the ethics committee of the Hamburg Medical Association (PV7131). All three persons introduced by the case vignettes signed their informed consent. Written informed consent was obtained from the individuals in the case vignettes presented for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JR: conceptualization, methodology, investigation, writing – original draft, writing – review & editing. WB: investigation, writing – review & editing. LT: investigation, writing – review & editing. AD: writing – review & editing, supervision, project administration, funding acquisition. PB: writing – review & editing, supervision, project administration, funding acquisition. TN: conceptualization, methodology, resources, writing – review & editing, supervision, project administration, funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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