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Transition to adulthood in Turner syndrome

Edited by
Aneta Monika Gawlik-Starzyk, Malgorzata Ewa Wiecek
and Malcolm David Cairns Donaldson

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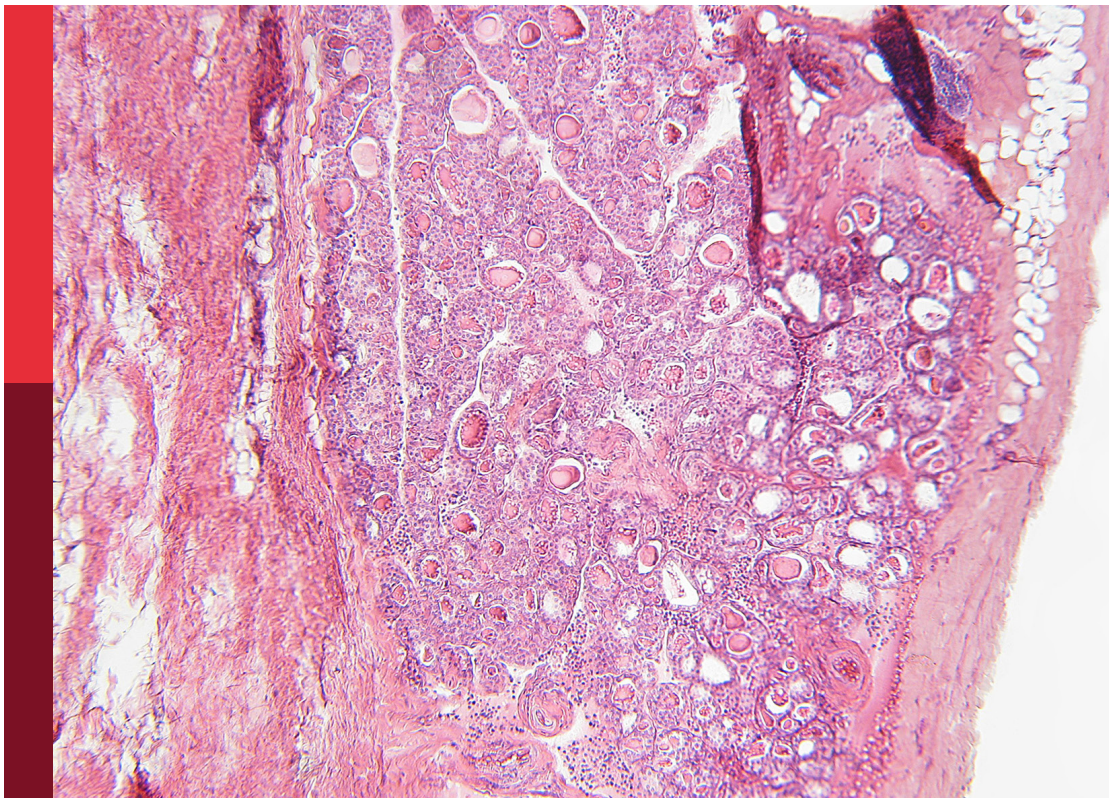

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Transition to adulthood in Turner syndrome

Topic editors

Aneta Monika Gawlik-Starzyk — Medical University of Silesia, Poland

Malgorzata Ewa Wiecek — Medical University of Silesia, Poland

Malcolm David Cairns Donaldson — University of Glasgow, United Kingdom

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EDITED AND REVIEWED BY
Sally Radovick,
Rutgers, The State University of New Jersey, United States

*CORRESPONDENCE
Małgorzata Więcek
mallgorzatawegiel@gmail.com

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Editorial: Transition to adulthood in Turner syndrome

Małgorzata Więcek[1]*, Malcolm Donaldson[2] and Aneta Gawlik-Starzyk[1]

[1]Department of Pediatrics and Pediatric Endocrinology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland, [2]Glasgow University School of Medicine, Glasgow, United Kingdom

KEYWORDS

Turner syndrome, transition, puberty induction, fertility preservation, multidisciplinary approach

Editorial on the Research Topic
Transition to adulthood in Turner syndrome

Turner syndrome (TS), defined as loss or abnormality of the second X chromosome, occurs in approximately 1:2000 – 1:2500 live-born female infants. Cardinal features are short stature, primary ovarian insufficiency, characteristic dysmorphic traits, and a range of associated abnormalities including congenital cardiovascular defects. Additionally, there is an increased risk of autoimmune diseases (e.g. autoimmune thyroiditis and coeliac disease). Other problems include renal anomalies, otological disease with hearing loss, visual problems, and mental health issues. Patients with TS therefore require a multidisciplinary approach with a team including endocrinologists, gynecologists, cardiologists, otolaryngologists and psychologists (1). Almost all patients require hormonal replacement therapy (HRT) to maintain satisfactory sex development and bone health (2, 3). Key issues encountered in adulthood, such as infertility, should be taken into consideration much earlier in life, to prepare the TS patient for further consequences and consider early strategies for fertility preservation (4).

For this Research Topic *Frontiers in Endocrinology* we have collected nine publications aimed at expanding our understanding of the health problems encountered in TS. This includes focus on a particularly critical phase – transition from pediatric to adult healthcare.

Improving the transition from pediatric to adult healthcare is crucial for effectively managing many chronic conditions, including TS. In this Research Topic, Zahra et al. investigated outcomes following transfer from the TS transition clinic in Glasgow to adult care. Their findings from 3- and 5-year follow-up were disappointing, with 44% of patients lost to follow-up at 5 years. This may be partly attributable to patients seeking care in other specialty clinics such as cardiology and otorhinolaryngology. The authors also highlight uncertainty as to whether these young women are receiving the recommended monitoring, hormonal replacement, and surveillance recommended.

Regarding short stature management, starting growth hormone (GH) therapy early yields the best outcomes in achieving adult height (AH). Another critical factor influencing AH is the timing of HRT for puberty induction. Kriström et al. examined AH in 132 patients with TS in relation to GH doses, age at GH and HRT. A clear dose-dependent effect on AH was found, and authors showed that starting GH treatment earlier permitted

pubertal induction at a normal age. This important study underscores the benefit of starting high dose GH treatment - 67 μg/kg/day – at an early age, to maximize prepubertal height gain and normalize childhood growth.

The spectrum of primary ovarian insufficiency from adolescence until menopause, and its impact on both puberty and fertility is discussed by Porcu et al. Most individuals will require HRT to promote pubertal development, menstruation, uterine growth, and bone health. In some patients, ovarian function is preserved but tends to diminish over the lifespan, usually during adolescence. It is during this critical period when the ovaries are still functioning optimally, that fertility preservation should be considered. Factors predicting ovarian reserve include mosaic karyotype (45,X/46,XX), spontaneous puberty, gonadotropin and anti-Müllerian hormone (AMH) levels. Related to this, an article by Hagen et al. broadly addresses AMH and other factors related to puberty and fertility in TS. The authors present the protocol of *The Danish Turner Cryopreservation (DANTE) Study* and propose criteria for classifying patients for fertility preservation.

The average lifespan of individuals with TS is reduced, primarily due to a higher prevalence of congenital heart defects, arterial hypertension, aortic dissection, and metabolic disturbances including impaired glucose-insulin economy. Mitsch et al. conducted a review article on hyperglycemia in TS, exploring the factors contributing to elevated glucose levels among affected women. An early underlying deficiency in insulin secretion appears to be the leading determinant of heightened glucose levels in TS patients. Various potential causes were examined, including therapy with GH, HRT and oxandrolone; obesity, age, family history, hypogonadism, 45,X monosomy, autoimmunity, and disruption in insulin/glucagon/secretin secretion. However, consensus on the primary cause of impaired carbohydrate metabolism in this group remains elusive. Further examination of hyperglycemia and other metabolic syndrome components during GH therapy can be found in the work by Błaszczyk et al. These authors emphasize that insulin resistance and disturbances in carbohydrate metabolism are most pronounced during GH therapy in girls with TS. However, GH therapy does not seem to affect factors such as obesity, abdominal obesity, triglyceride levels, HDL concentrations, or hypertension. As metabolic disturbances can occur in women with TS throughout their lifespan, regular monitoring following transition to adult healthcare is required.

Ensuring optimal health in patients with TS necessitates adherence to the latest guidelines. Lam et al.'s study assesses this, drawing on data from 68 patients. Recommendations for documentation of height, weight, BMI, cardiac, and renal imaging exhibited the highest implementation rates. Conversely, recommendations for bone mineral density assessment, skin examination, otological review, ophthalmological assessment, and dental consultations were implemented less frequently. Moreover, liver function biomarkers were frequently overlooked.

Recent findings from two studies underscore the variability of symptoms based on genotype. Witkowska-Krawczak et al. illustrate how different karyotypes may be associated with different healthcare needs. For instance, individuals with complete 45,X monosomy display more prominent phenotypic characteristics, a higher prevalence of congenital circulatory system abnormalities, requires HRT more frequently and show lower spontaneous menstruation compared to those with mosaicism. The study also identified a higher incidence of autoimmunity linked to the X isochromosome. Suntharalingham et al. conducted a comprehensive investigation, utilizing whole exome sequencing in 134 adult women with TS compared with 23 46,XX controls, 101 46, XX women with primary ovarian insufficiency, and 11 46,XY controls. There were no significant changes observed at the gene or variant level on the X chromosome in women with 45,X monosomy with a specific autoimmune condition compared to those without it, nor were any changes found more frequently in women without a certain condition compared to those with it. However, the authors were able to confirm a correlation between autosomal TIMP3 variation and congenital cardiac anomalies.

In conclusion, the management of TS demands comprehensive and tailored healthcare approaches, including structured transition to adult care, which address the diverse needs of patients throughout their lifespan. From optimizing growth and hormone therapies to managing metabolic and cardiovascular risks, ongoing research and clinical efforts are essential for improving outcomes in TS care.

Author contributions

MW: Writing – original draft. MD: Writing – review & editing. AG: Writing – review & editing.

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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EDITED BY
Aneta Monika Gawlik,
Medical University of Silesia, Poland

REVIEWED BY
Roopa Kanakatti Shankar,
Children's National Hospital, United States
Xu Zhai,
China Academy of Chinese Medical Sciences, China

*CORRESPONDENCE
Catherina T. Pinnaro
catherina-pinnaro@uiowa.edu

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Hyperglycemia in Turner syndrome: Impact, mechanisms, and areas for future research

Cameron Mitsch[1], Eirene Alexandrou[2], Andrew W. Norris[2,3] and Catherina T. Pinnaro[2,3]*

[1]Department of Health and Human Physiology, The University of Iowa, Iowa City, IA, United States, [2]Stead Family Department of Pediatrics, University of Iowa, Iowa City, IA, United States, [3]Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa City, IA, United States

Turner syndrome (TS) is a common chromosomal disorder resulting from complete or partial absence of the second sex chromosome. Hyperglycemia, ranging from impaired glucose tolerance (IGT) to diabetes mellitus (DM), is common in TS. DM in individuals with TS is associated with an 11-fold excess in mortality. The reasons for the high prevalence of hyperglycemia in TS are not well understood even though this aspect of TS was initially reported almost 60 years ago. Karyotype, as a proxy for X chromosome (X_{chr}) gene dosage, has been associated with DM risk in TS – however, no specific X_{chr} genes or loci have been implicated in the TS hyperglycemia phenotype. The molecular genetic study of TS-related phenotypes is hampered by inability to design analyses based on familial segregation, as TS is a non-heritable genetic disorder. Mechanistic studies are confounded by a lack of adequate TS animal models, small and heterogenous study populations, and the use of medications that alter carbohydrate metabolism in the management of TS. This review summarizes and assesses existing data related to the physiological and genetic mechanisms hypothesized to underlie hyperglycemia in TS, concluding that insulin deficiency is an early defect intrinsic to TS that results in hyperglycemia. Diagnostic criteria and therapeutic options for treatment of hyperglycemia in TS are presented, while emphasizing the pitfalls and complexities of studying glucose metabolism and diagnosing hyperglycemia in the TS population.

KEYWORDS

Turner syndrome - TS, diabetes mellitus, impaired glucose tolerance, growth hormone, estrogen, X chromosome (human)

1 Introduction

Turner syndrome (TS) is a common, non-heritable genetic disorder affecting ~1 in 2000 females and is caused by complete or partial absence of the second sex chromosome (1). Most individuals with TS have short stature and primary ovarian insufficiency, which require timely treatment with recombinant growth hormone (GH), estrogen, and other adjuncts over prolonged periods to facilitate linear growth and induce and maintain puberty. Other

associated features of TS may include lymphedema, specific cognitive traits or deficits, congenital heart disease, autoimmunity, osteoporosis, renal malformations, and diabetes mellitus (DM) (2). This review will focus on the TS hyperglycemia phenotype – an indolent and gradually progressive decline in oral glucose tolerance that often culminates in frank DM (3). Despite the prevalence of hyperglycemia in TS, the pathogenesis has not been clarified, and as a result, no specific prevention or treatment exists. This review will cover the epidemiology and impact of hyperglycemia on those with TS, the hypothesized genetic and physiologic mechanisms associated with hyperglycemia in TS, and diagnostic and therapeutic approaches, while emphasizing the challenges of studying glucose metabolism in the TS population.

2 Search methods

A comprehensive literature search was conducted using PubMed (https://pubmed.ncbi.nlm.nih.gov) to retrieve all relevant primary research articles related to glucose metabolism in TS published in English through May 16, 2022, producing 184 articles. The search was carried out using the following keywords, either alone or in combination using the Boolean method: [("Turner Syndrome" OR "monosomy X") AND (diabetes mellitus OR "impaired glucose tolerance" OR hyperglycemia)]. We additionally performed searches to identify articles related to confounders of the study of hyperglycemia in TS, including obesity in TS and the impact of TS treatments on glucose metabolism, specifically recombinant growth hormone (GH), estrogen, and oxandrolone. We identified 190 unique articles after assessing for duplicates. The full text of these articles was reviewed and narrowed to 90 articles meeting eligibility criteria. We subsequently performed a manual search of the reference lists of all 90 articles and identified 5 additional eligible articles. The cumulative review consisted of 95 articles. A flow chart of our literature review methods is depicted in Figure 1.

3 Epidemiology/scope

Individuals with TS have a higher risk of mortality at all ages (4–6), and DM is associated with a further 11-fold raised mortality risk in TS (4). The high incidence of hyperglycemia in TS was noted over 50 years ago (7), but the true epidemiologic prevalence of hyperglycemia in TS remains difficult to estimate, partly because of selection bias in small, cross-sectional studies. Additionally, there is a lack of standardization in assessing glucose tolerance in TS patients, with studies reporting results using 50-, 75-, and 100-gram oral glucose tolerance tests (OGTT). Compounding the problem, these studies use varied diagnostic criteria, including those provided by the American Diabetes Association/National Diabetes Database (8, 9), Joslin (10), the United States Public Health Service (11), the World Health Organization (12), the Canadian Diabetes Association (13), and non-standard criteria (14–17). The prevalence of hyperglycemia in TS-specific studies using OGTT (assessed as described) is up to 83%, with higher rates reported in studies that enrolled older participants (3, 14, 18). However, asymptomatic hyperglycemia is not limited to adults and has been observed in up to 40% of children with TS aged 5 to 12 years old (16).

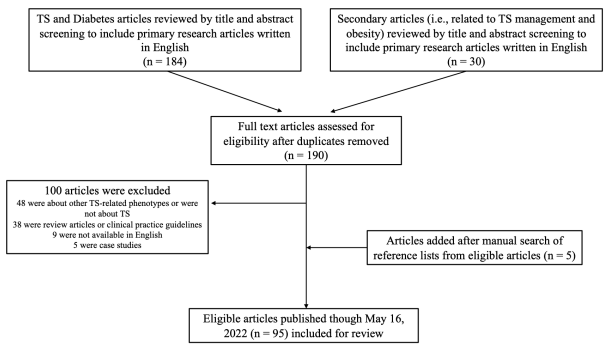

FIGURE 1
Flow chart of literature search.

Several treatment modalities used in the management of TS have the potential to alter glucose metabolism, especially recombinant GH, estradiol, and oxandrolone (2). Supraphysiologic levels of GH, as demonstrated in patients with acromegaly, lead to increased liver gluconeogenesis, as well as hepatic and peripheral insulin resistance (19). Likewise, insulin resistance is known to worsen in puberty for karyotypically-normal individuals, in healthy young women treated with oral contraceptive therapies, and in women with hyperandrogenemia (20, 21). However, both treatment-naïve and hormonally treated children and adults with TS show altered glucose metabolism (22), and the high prevalence of hyperglycemia in TS was noted prior to the introduction of hormonal therapies as components of TS management. Thus, hyperglycemia in TS cannot solely be deemed iatrogenic.

4 Effects of TS management on glucose homeostasis

4.1 Recombinant growth hormone

Recombinant GH at supraphysiologic doses (0.045-0.050 mg/kg/day, up to 0.068 mg/kg/day) is recommended in TS, typically starting around 4-6 years old and ideally, before age 13 years (2). Growth promoting treatments are utilized until growth plate fusion. Several studies have examined the influences of recombinant GH on glucose metabolism during this extended treatment period and upon its discontinuation. Overall, studies have demonstrated a worsening of insulin resistance without increased prevalence of IGT or DM in TS patients treated with recombinant GH (19, 22–24). A dose-related increase in insulin resistance was noted in patients treated with higher doses of recombinant GH (0.045 mg/kg/day *vs* 0.0675 mg/kg/day and 0.09 mg/kg/day), as evidenced by increased area under the curve (AUC) for insulin during OGTT, insulinogenic index, and urinary C-peptide (24). There was no apparent difference in glucose metabolism in once daily versus twice daily divided doses of recombinant GH. Additionally, fasting insulin showed a sustained increase throughout GH therapy, in contrast to glucose-stimulated insulin, which showed no progressive worsening beyond four years of treatment (19, 22). Finally, insulin resistance induced by recombinant GH appeared to be

reversible, as insulin levels declined upon discontinuation of treatment, although remained above pre-treatment levels (19, 22, 23). Studies involving non-TS controls also saw increased insulin levels in 46, XX females; therefore, the elevated post-treatment insulin levels in TS individuals were felt to be positively correlated with post-pubertal status and increasing age (22, 23). Thus, while recombinant GH treatment is responsible for reversible increases in insulin resistance, it does not explain the lifetime increased risk of hyperglycemia in TS.

4.2 Sex hormone replacement

Studies examining the effect of hormone replacement therapy (HRT) on glucose metabolism in TS demonstrate improvements in insulin sensitivity. Limited published studies have evaluated their effect on glucose tolerance. An investigation of the impact of six months of HRT with either oral or transdermal 17-beta-estradiol plus norethisterone in adult TS patients compared to healthy controls demonstrated that HRT did not worsen insulin resistance, but the AUC glucose on OGTT significantly increased (20). These differences were comparable between the transdermal versus oral estradiol groups, demonstrating no perceived benefit of one therapy over the other with regards to carbohydrate metabolism. A subsequent comparison of transdermal versus oral estradiol replacement on metabolic parameters in women with TS similarly did not demonstrate a perceived metabolic benefit of one therapy over the other although this study only measured fasting glucose (25). Additionally, HRT ameliorated the recombinant GH-induced increase in insulin resistance in women with TS (26). Another study also demonstrated that the GH-induced increase in insulin resistance was improved by the addition of estradiol; however, estradiol had no effect on glucose tolerance (27). Thus, HRT in TS seems to improve insulin sensitivity, but the effect on glucose tolerance (either alone or in conjunction with recombinant GH or alone) is not clear but is likely minor.

4.3 Oxandrolone

Oxandrolone is a non-aromatizable androgen utilized for adjunctive growth promotion in TS (28). Data regarding oxandrolone's effect on glucose metabolism are mixed. A randomized, placebo-controlled study comparing recombinant GH in combination with placebo or oxandrolone (either at 0.03 or 0.06 mg/kg/day), demonstrated no significant impact on insulin sensitivity (29). There were no differences between (recombinant GH + oxandrolone) versus (GH + placebo) groups with regards to developing IGT or decreased insulin sensitivity; however, fasting glucose levels and hemoglobin A1c (HbA1c) values decreased more in those treated with oxandrolone compared with placebo. In contrast, some studies have demonstrated a worsening of glucose tolerance with oxandrolone (21, 30). Specifically, AUC glucose and insulin levels during OGTT rose in patients treated with oxandrolone, either alone or in combination with recombinant GH (21). Despite these changes, HbA1c levels and fasting glucoses in treated individuals remained normal. This study used oxandrolone doses higher (0.125 mg/kg/day) than current recommendations (<0.05 mg/kg/day) (2). Similar increases in AUC insulin levels were noted during OGTT in individuals with TS on GH/oxandrolone combination therapy in a subsequent study, which also demonstrated a reversibility of glucose intolerance in TS individuals upon discontinuation of these treatments (30).

In summary, the effects of oxandrolone and estrogen on glucose metabolism are less clear than the effect of recombinant GH, but all may impact glycemic response as well as insulin resistance. Accordingly, recombinant GH, estrogen, and oxandrolone should be noted and controlled for when designing and interpreting clinical studies evaluating glucose metabolism in individuals with TS.

5 Clinical and genetic factors involved in hyperglycemia in TS

5.1 Non-TS-specific

5.1.1 Age

The incidence and prevalence of type 2 DM in the general population increases with age (31). Both cross-sectional (32) and prospective, longitudinal studies (3, 33) show that, not surprisingly, the risk of hyperglycemia also increases with age in TS. This phenomenon persists independent of current or prior recombinant GH use (33), and current (33, 34) or prior (34) estrogen-replacement therapy. Age-related risk of hyperglycemia was also noted in a study of children and adolescents who had never received hormone replacement of any kind (16).

5.1.2 Obesity

Obesity is also a known risk factor for metabolic syndrome and type 2 DM in the general population, and obesity is described as a common comorbidity of TS. However, there is no standard definition of obesity in TS. BMI correlates with several biochemical markers of obesity in TS, including C-reactive protein and interleukin-6 (35) and is the most assessed proxy of adiposity in clinical studies. However, when individuals with TS were matched to 46, XX women based on BMI, the women with TS demonstrated excess visceral and internal abdominal adipose tissue and intrahepatocellular lipids on magnetic resonance imaging (36). Two follow-up studies showed that alternative methods more specific to visceral fat may better represent metabolic risk in TS (37, 38). Body weights of those with TS are often greater than those of 46, XX girls and women of the same height (39), even though individuals with TS tend to have less subcutaneous extremity fat (39, 40). Those with TS have increased total fat mass and visceral fat with decreased total lean body mass (41, 42). For these reasons, it is plausible that typical BMI percentiles underestimate metabolic risk in individuals with TS.

Even with the aforementioned caveats, BMI is the most widely reported measure of obesity in glucose metabolism studies in TS. Most studies support linkage of increasing BMI with hyperglycemia in TS, but the risk is not obligatorily coupled to obesity. In a cross-sectional study consisting of children and adolescents with TS and controls, there was no difference in weight excess (reported as BMI-

standard deviation score) in those with TS who had abnormal OGTTs compared to the 31 individuals (those with TS and controls) who had normal OGTTs (16). In contrast, a large longitudinal study comprised of 113 TS patients demonstrated that BMI was higher among those with TS who had IGT compared to those with normal glucose tolerance (NGT) (3). BMI percentiles were also positively correlated with metabolic comorbidities including HbA1c in a recent natural history study of TS (43). Additionally, increasing BMI was associated with the development of hyperglycemia among those with TS who were not considered obese by BMI (32), and a longitudinal study (of a different group of individuals with TS) demonstrated a positive correlation of BMI with fasting glucose and HbA1c (43). Thus, it appears that within TS cohorts, increasing BMI is associated with increased risk of hyperglycemia even at non-obese BMI.

5.1.3 Family history of diabetes mellitus

An early observation noted in the study of TS was a high prevalence of DM and autoimmunity in the parents of individuals with TS (7). A second small study supported this observation (44). It was thus hypothesized that parental hyperglycemia or autoimmunity may impair meiosis and increase the risk of sex chromosome abnormalities such as TS (45, 46). Despite the initial enthusiasm for this topic, many well-designed follow up studies–which included comparable control groups–failed to demonstrate an association of family history of DM with risk to have a child with TS (33, 34, 47). Consistent with this negative conclusion, a study of a large group of individuals with TS found that the prevalence of DM in first-degree relatives of individuals with TS was similar to that in the general population (48). However, the relationship between family history of DM and development of TS-associated hyperglycemia has not been thoroughly explored. Family history of type 2 DM is a strong risk factor for the development of type 2 DM in the general population and largely represents genetic risk (49), but the impact of family history on the development of DM in TS is limited as most studies do not collect family history information in the setting of glycemic phenotyping.

5.1.4 Hypogonadism

There is both epidemiologic and experimental evidence to suggest that post-pubertal sex steroids contribute to sex-related differences in diabetes susceptibility. Specifically, the protective role of endogenous estrogens dissipates as women undergo menopause, evidenced by deleterious effects on body composition and glucose homeostasis (50) that can potentially be mitigated by estrogen replacement (51, 52). This is especially relevant to TS, as absent or incomplete pubertal development is one of the cardinal features (53), and pubertal induction and maintenance is a mainstay of TS treatment (2). Therefore, estrogen deficiency is a biologically plausible mechanism of increased diabetes incidence in TS. However, the increased incidence of hyperglycemia persists in individuals with TS who are actively on sex steroid treatment (18, 20, 54) and in pre-pubertal children with TS (16, 54). Thus, it is unlikely that estrogen deficiency alone underlies the increased risk of DM in TS. The effect of sex hormone replacement on carbohydrate metabolism in TS is reviewed in section 4.2 above.

5.2 TS-specific

5.2.1 X chromosome genetics and risk of hyperglycemia in TS

In addition to estrogen, X chromosome (X_{chr}) dosage has been shown to contribute to the favorable metabolic profile in 46, XX women (55, 56). Since TS is associated both with estrogen deficiency and haploinsufficiency for the X_{chr}, the TS hyperglycemia phenotype could be related to X_{chr} genetic factors, estrogen deficiency secondary to gonadal insufficiency, or their interaction.

Multiple karyotypes, all which result in haploinsufficiency of X_{chr} genes, are associated with TS. These include 45X (complete absence of the second X_{chr}), deletions of the short arm (i.e., p arm) of the second X_{chr}, isochromosome Xq (i.e., the second X_{chr} is comprised of 2 q arms and no p arms, equating to deficiency of the p arms), ring X_{chr} (i.e., the second X_{chr} is a ring containing variable amounts of X_{chr} material) and other structural aberrations (i.e., unbalanced X_{chr}-autosome translocations) (57). Any of these TS-associated karyotypes can exist with mosaicism for a typical 46XX, 46XY, 47XXX, or the aforementioned TS cell lines and may still present with features of TS depending on the prevalence and distribution of the of the TS cell line(s) (58).

Interestingly, even when compared to 46, XX women with primary ovarian insufficiency, women with TS demonstrate significant impairments in oral glucose-stimulated insulin secretion, which implicates X_{chr} factors, independent of estrogen deficiency, in the TS hyperglycemia phenotype (59). Karyotype, as a proxy for X_{chr} gene dosage, was independently associated with DM risk in TS, with isochromosome Xq demonstrating the greatest risk increase (32). This suggests that haploinsufficiency for Xp genes coupled with supernumerary copies of Xq genes (as is seen in isochromosome Xq) may underly the pathogenesis of the TS hyperglycemia phenotype. However, two subsequent studies reported that metabolic comorbidities, including hyperglycemia, are more highly associated with a different TS karyotype (i.e., ring X chromosomes) (58, 60). A strength of both these studies is the large number of TS individuals that were involved (>1000 each), while limitations include their observational, retrospective nature and their reliance on HbA1c to diagnose DM. Thus, there is no consensus on X_{chr} location of potential TS hyperglycemia-related genes.

X_{chr} inheritance in TS is biased, with approximately three-fourths of 45X TS individuals inheriting a maternal X_{chr} (61). This has led to the hypothesis that there may be X_{chr} genes which are differentially expressed depending on parent-of-origin. One recent study found no impact of X_{chr} parent-of-origin on fasting blood glucose in TS (62), but the lack of association is not surprising given that impaired fasting glucose is rare among those with TS (43), thus limiting statistical power in TS studies focused on this outcome. Another recent study also found no association of X_{chr} parent-of-origin with DM, but there were actually no individuals with DM in this study (63). Other metabolic phenotypes, including hypercholesterolemia (64, 65), are associated with parent-of-origin in TS, which suggests that there are imprinted genes on the X_{chr} that may underlie some of the TS-associated phenotypes.

Haploinsufficiency for X_{chr} genes leads to global hypomethylation and altered autosomal gene expression (66, 67). Several studies have

demonstrated abnormal gene expression profiles (32, 68) and differentially methylated X_{chr} and autosomal genes in individuals with TS (68, 69) Functional annotations of TS datasets (RNA sequencing and DNA methylation analysis of fibroblasts) demonstrate enrichment in genes involved in carbohydrate metabolism and glucose import (68, 70). Two recent studies have employed bioinformatics approaches to identify differentially expressed genes in TS and used pathway analysis to determine key differentially regulated pathways relevant to diabetes (71, 72). Candidate signature genes of DM in TS include 8 upregulated autosomal genes, including *SLC29A2, THBS1, GPRC5B, CSHL1, ADAM22, IGHM, WIZ, IGHD*, and 1 downregulated autosomal gene, *COX11* (72). It is not known if these altered autosomal genetic signatures are involved in the pathogenesis of diabetes in TS individuals. Despite significant recent progress made in characterizing the TS genome and epigenome, no specific genes or loci have been implicated definitively in the TS hyperglycemia phenotype. Figure 2 summarizes the evolution of risk factors for TS-associated hyperglycemia.

6 Physiological mechanisms hypothesized to underlie hyperglycemia in TS

Dysregulated secretion of GH, autoimmune beta cell destruction, impaired insulin secretion, increased insulin resistance, hyperglucagonemia, and an impaired incretin axis have all been hypothesized to be the primary or major physiological mechanism(s) contributing to hyperglycemia in TS. Many studies have drawn conclusions based on small sample sizes (median sample size 21). Early studies used hormonal assays that were hampered by a large degree of variability, which further complicates interpretation. Additionally, many studies were confounded by the absence of control groups matched by age, sex and/or adiposity, which are all variables known to impact glucose metabolism. Hypogonadal status and short stature make matching especially difficult in TS, given that women with TS may have increased adiposity that is differentially distributed anatomically compared to non-TS individuals (41, 42) as reviewed in section 5.2. Age of TS patients and TS management therapies should be accounted for (see sections 4 and 5.1 above for additional details) as potential confounders. Particular attention should be paid to studies including individuals with TS who are at or near the age of puberty– there is a well-described physiological decline in insulin sensitivity accompanied by compensatory insulin secretion that occurs during puberty (73), and it is unclear whether individuals with TS undergo this physiological transition, especially if not treated with sex steroids to facilitate pubertal induction and/or maintenance. Thus, comparing individuals with TS to age-matched controls during puberty may result in a biased conclusions of lower insulin secretion and decreased insulin resistance in TS. Table 1 summarizes the TS-non-specific and TS-specific factors that may contribute to hyperglycemia risk in TS.

6.1 Dysregulated endogenous GH secretion

Early in the study of hyperglycemia of TS, excess circulating GH was documented in 4 individuals with TS (74). Thus, it was hypothesized that excess GH secretion was responsible for the hyperglycemia seen in TS, similar to acromegaly. It should be cautioned that the early study utilized the tibia line assay, which can give values which are much higher than those from radioimmune assays (14). Five additional studies followed up on the GH hypothesis (17, 33, 47, 75, 76). The studies involved between 14-30 individuals of varying ages with TS. Three studies measured fasting GH levels using various radioimmunoassay techniques (17, 47, 76), and in one study, the assay method is not described (75). No difference in fasting GH levels was noted between individuals with TS and normal glucose metabolism and those with TS and hyperglycemia (75), or between individuals with TS and non-sex-matched controls. The third study did not report fasting GH levels (76). The fourth study observed higher fasting GH levels in TS patients than controls, however this difference was attributed to the fact that over half of the TS group was undergoing estrogen replacement (47). There also were no differences in average 24-hour GH levels or area under the curve for GH, which was sampled through an indwelling needle every 30 minutes for 24h in 10 patients (17). The fifth study reported GH levels in a combined group of individuals with TS and complete gonadal dysgenesis with 46XX

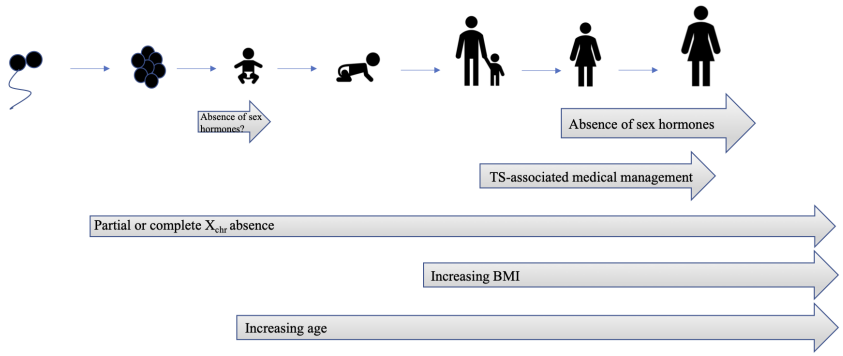

FIGURE 2
Evolution of risk factors for TS-associated hyperglycemia. There is an intrinsic risk of hyperglycemia due to syndrome-specific X_{chr} factors that is present at birth. Similar to type 2 DM in the general population, the risk for hyperglycemia increases with age and BMI. Temporary increases in insulin resistance are attributable to medical therapy, such as recombinant GH, and improve after discontinuation of therapy. Gonadal insufficiency and decreased production of sex hormones also contributes to risk of hyperglycemia, although is no.

karyotype, noting that those with DM had higher levels of plasma GH at the 15 and 30 minute time points; however, the frequency of abnormal GH responses in the diabetic group was not higher than in the non-diabetic group (33).

Two of these studies demonstrated normal rise in GH to hypoglycemic conditions (47, 76). Another pair of studies observed excessive increases in plasma GH levels during hyperglycemic conditions. This dysregulated GH response to hyperglycemia did not correlate with glucose tolerance (47, 75). Thus, while GH secretion to hypoglycemia in TS is intact, GH secretion may be dysregulated with respect to hyperglycemic conditions in individuals with TS. This may be exacerbated by HRT, but it does not seem to be a predominant mechanism underlying hyperglycemia in TS. A review of key GH studies in TS hyperglycemia can be found in Table 2.

6.2 Autoimmunity

A high prevalence of organ-specific autoantibodies was an early phenotypic association made in studies of TS (77, 78). Celiac disease, an autoimmune disorder that is strongly associated with the HLA-DQA1*0501 and DQB1*0201 alleles (79), was subsequently noted to be highly prevalent in TS (80), increasing the plausibility of a type 1 DM (T1D)-like mechanism underlying hyperglycemia in TS. Two early studies (81, 82) assessed human leukocyte antigen (HLA) associations with TS: one demonstrated no skewing of HLA frequencies among those with TS and their parents (82), while the second revealed that the frequencies of B38 HLA class 1 antigens were increased in the TS population (81). One additional study specifically looked for enrichment of HLA DR3 or DR4 haplotypes, two high risk T1D HLA haplotypes (83). These two haplotypes were not identified in those with TS, but they were not studied specifically in the context of TS individuals with a glycemic phenotype (84). An in-depth assessment of T1D genetic risk loci has not been performed in individuals with TS with regards to glycemia.

There is no consensus as to whether the frequency of T1D is increased in TS (85–88). However, most cases of DM in TS do not resemble classic autoimmune DM (58, 85). Longitudinal testing for islet autoantibodies has not been completed in the TS population, so it is not known if indolent autoimmunity contributes to the TS hyperglycemia phenotype. Cross-sectional measurements of islet cell autoantibody (ICA) have been performed but were not identified in any individuals with TS (3, 32, 54, 89, 90). Glutamic acid decarboxylase autoantibody levels (anti-GAD) were measured in 4 studies (32, 54, 91), with a positive assay in 2-10% of individuals with TS. A study of 113 women with TS (3) measured both ICA and anti-GAD antibodies and reported that 2/113 (1.8%) were autoantibody positive but did not differentiate which antibody(s). The time-course of insulin, glucose, and insulinogenic index in response to glycemic challenge did not differ between the antibody-positive and antibody-negative subjects with DM. A study following 134 patients with TS for a mean of 5.4 years similarly demonstrated a prevalence of T1D of 1.5% (92). In contrast to the previous data, in a German study of 24 individuals with TS and DM, 78% were positive for islet autoantibodies (85).

The supply of TS pancreas specimens available for histological study is very limited, and this is reflected in the paucity of research literature on pancreatic pathologies in TS. Reports of only two TS samples have been published, and there was no indication of insulitis or other typical histological findings of type 1 DM (47). However, neither sample was obtained from a TS individual with known DM. Given the natural history of hyperglycemia in TS, it is unlikely that an aggressive T1D-like autoimmune process is the cause of TS hyperglycemia. To evaluate this hypothesis rigorously, it will be necessary to do longitudinal studies that combine glycemic phenotyping and islet autoantibody assessments with genotyping for genetic variants linked to high risk for T1D. The next section (6.3) will discuss evidence suggesting that defects of insulin secretion do exist in TS, making such studies potentially more important to undertake. Key studies related to autoimmunity are summarized in Table 3.

TABLE 1 Summary of therapeutic, clinical, and genetic factors thought to be related to increased risk of hyperglycemia in TS.

Potential Risk Factor for DM in TS	Increases risk of diabetes in TS?	Supporting data
Recombinant GH	No	Responsible for reversible increases in insulin resistance but its use does not explain the lifetime increased risk of hyperglycemia in TS
Sex Hormone Replacement	No	Improves insulin resistance, effect on glucose tolerance is not clear
Oxandrolone	No	Unclear, often utilized in conjunction with recombinant GH and estrogen replacement
Age	Yes	Young individuals are still noted to have hyperglycemia at higher rates than expected, but risk does increase with age.
Obesity	Yes	No standard definition of obesity in TS, but increasing BMI is associated with increased risk of hyperglycemia in TS even at non-obese BMI.
Family history of diabetes mellitus	Unknown	Studies evaluating family history of DM in TS were not specifically evaluating that relationship to risk of DM in TS, rather they sought to see if parental DM was a risk factor for having a child with TS. These studies do not include enough data related to DM phenotype to conclude whether family history increases the risk of DM in TS.
Hypogonadism	Yes	HRT does not eliminate increased risk of hyperglycemia in TS, so unlikely to be sole contributing risk factor.
Complete or partial absence of the second sex chromosome	Yes	When compared with an age, sex, and BMI-matched cohort of women with primary ovarian failure (thus removing estrogen deficiency as a confounder), the TS group had higher rates of glucose abnormalities and demonstrated impaired insulin secretion.

TS, Turner syndrome; DM, diabetes mellitus; GH, growth hormone; HRT, hormone replacement therapy; BMI, body mass index.

TABLE 2 Summary of key studies evaluating growth hormone secretion in TS and its potential relationship to the TS-associated hyperglycemia phenotype.

Dysregulated GH secretion	
Costin et al. Carbohydrate intolerance in Gonadal Dysgenesis: Evidence for Insulin Resistance and Hyperglucagonemia. 1985.	Fasting and AUC GH levels were not different between individuals with TS and controls.
Lindsten et al. The Occurrence of Abnormal Insulin and Growth Hormone Responses to Sustained Hyperglycemia in a Disease with Sex Chromosome Aberrations, including a histological study of the pancreas in 2 such patients. 1967.	Fasting GH levels were higher in those with TS, and GH levels did not suppress to hyperglycemia. GH responses were normal to hypoglycemia in those with TS, but GH levels did not suppress to hyperglycemia. However, there was not an increased prevalence of IGT or DM in the group with abnormal GH responses.
Van Campenhout et al. Carbohydrate Tolerance in Gonadal Dysgenesis: 1979.	Glucose tolerance status on OGTT was associated with abnormal GH responses (i.e., lack of suppression) during OGTT, but those with DM had higher fasting GH levels.
Rasio et al. Diabetes Mellitus in Gonadal Dysgenesis. Studies of Insulin and Growth Hormone Secretion. 1976.	No differences in GH responses during OGTT when individuals with TS were divided by glucose tolerance status.

These studies suggest that endogenous GH secretion may be dysregulated in TS, but this does not seem to affect glucose metabolism. GH, growth hormone; TS, Turner syndrome; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; DM, diabetes mellitus.

6.3 Impaired insulin secretion versus insulin resistance

OGTT-based analyses indicate that insulin secretion is impaired in TS and that this impairment contributes to hyperglycemia. Seven studies utilizing OGTT conclude that insulin secretion is impaired in individuals with TS who have IGT (3, 14, 16, 20, 93–95); several of these studies used age- (16, 20, 94, 95), sex- (16, 20, 94, 95), and BMI-matched (94, 95) controls. Additionally, several other studies have demonstrated that there are defects in insulin secretion even in young, non-obese individuals with TS and NGT as compared to age and sex-matched controls. In one large study (n = 25 women with TS), early defects in insulin secretion were accompanied by heightened insulin sensitivity, suggesting that perhaps glucose intolerance progresses over time in TS as insulin secretory capacity falls and insulin resistance becomes apparent (59).

Abnormalities in insulin secretion are less apparent when assessed *via* intravenous glucose tolerance test (IVGTT) compared to OGTT. Only two of the six IVGTT studies demonstrated any impairment in early insulin secretion (20, 59), with a modest correlation of declining insulin secretion with age (59). Thus, although defects in insulin secretion are sometimes detected by intravenous glucose administration (IVGTT), they are less pronounced than the defects seen by oral glucose administration, i.e., by OGTT.

Insulin secretion in response to non-glucose stimuli has been evaluated in even fewer studies, all with small sample sizes and sometimes lacking control groups. Early phase insulin response to tolbutamide is likely decreased based on results from two studies (75, 96). Insulin secretion in response to glucagon seems to be normal in TS. The peak insulin levels were similar in TS and controls, although with a lower AUC in those with TS (96). Similarly, insulin secretion in response to arginine (17) and to arginine plus GLP-1 also do not appear to be different between individuals with TS and controls (94). The discrepancy in beta-cell capacity in response to different secretagogues is consonant with the fact these stimuli involve different mechanisms of insulin secretion [74] and may provide areas for future investigation with regards to the mechanism of impaired insulin secretion in TS.

Studies evaluating insulin resistance in TS are less consistent than those evaluating insulin secretion, suggesting that insulin resistance is not the primary driver of hyperglycemia in TS. Poor reproducibility of these studies, in part, reflects the variability with respect to methodology, age of TS participants, matching strategies, and medications utilized in the TS group. Five total euglycemic clamp studies have been published in TS (17, 94, 97–99) – two concluded that insulin resistance was increased in TS but did not use adiposity-matched controls (97, 98). Thus, it is possible that excess adiposity is an unmeasured confounder. When individuals with TS are matched on BMI, comparisons of insulin resistance as determined *via* euglycemic clamp are equivocal: one study did not find differences in insulin sensitivity (13 women with TS) (94), whereas the other did find lower insulin sensitivity (4 adolescents with TS) (99). The

TABLE 3 Summary of key studies evaluating autoimmunity as a potential mechanism contributing to TS-associated hyperglycemia.

Autoimmunity	
Dacou-Voutetakis et al. Increased Frequency of Hla B17 Antigen in Girls with Turner syndrome and their Fathers. 1993.	43 girls with TS were compared to 433 controls, with no difference in the frequency of HLA-DR3 or DR4.
Ibarra-Gasparini. New Insights on Diabetes in Turner syndrome: Results from an Observational Study in Adulthood. 2017.	1.8% of TS subjects had positive islet antibodies (14% of those with DM had positive islet antibodies), but those with positive antibodies had similar glycemic phenotyping to those who were antibody negative.
Lindsten et al. The Occurrence of Abnormal Insulin and Growth Hormone Responses to Sustained Hyperglycemia in a Disease with Sex Chromosome Aberrations, including a histological study of the pancreas in 2 such patients. 1967.	2 histological samples were examined, and neither showed evidence of insulitis. One sample demonstrated hyperplastic islet tissue, and those beta cells had significantly smaller nuclei than control samples.

Existing cross-sectional studies and clinical trajectory suggest that a predominant autoimmune mechanism underlying TS-associated hyperglycemia unlikely, but definitive genetic and longitudinal studies are lacking to completely conclude. TS, Turner syndrome; HLA, human leukocyte antigen; DM, diabetes mellitus.

negative study was matched on fat mass % and lean body mass % as determined by dual-energy X-ray absorptiometry, although the waist-hip ratio was higher in the TS group compared to the controls.

Additional evidence suggesting that insulin resistance is not the primary driver of hyperglycemia is highlighted in three studies comparing IVGTT-derived measures of insulin sensitivity in individuals with TS to controls (20, 59, 94). In all three studies, the subjects were matched on adiposity (either BMI or fat mass % or lean body mass %), and none of the TS patients demonstrated increased insulin resistance. In fact, the largest study (49 individuals with TS) revealed that those with TS had improved insulin sensitivity compared to controls (59). Furthermore, a study utilizing insulin tolerance testing reported that insulin sensitivity was higher in TS (14). Another stream of evidence also suggests that insulin resistance is not driver of early metabolic defects in TS, in that fasting glucose and insulin levels in euglycemic individuals with TS are similar or even lower than in controls (19, 36, 47, 96, 97, 100). OGTT-derived measures of insulin sensitivity also consistently demonstrate comparable insulin sensitivity in normoglycemic, non-obese TS women and controls (32, 59, 95, 101). Finally, to confirm the central obesity seen by abdominal magnetic resonance imaging, TS individuals underwent biochemical testing. All of them had the biochemical hallmarks of central adiposity but not of hyperinsulinemia (36).

Thus, based on multiple lines of evidence, insulin secretion in response to oral glucose seems to be diminished and may decline with age in TS. The insulin secretory responses to IV stimuli, including glucose, arginine, glucagon, and GLP-1 are less clearcut and warrant additional study with larger sample sizes and better matching. Insulin resistance does not appear to contribute consistently to early hyperglycemia in TS. It is likely that insulin resistance becomes evident as central adiposity increases in TS, and that this exacerbates the pre-existing defect in insulin secretion. Key studies summarized in this section can be found in Tables 4 and 5.

6.4 Hyperglucagonemia/dysregulated glucagon

Glucagon is a key counterregulatory hormone to insulin and has been hypothesized to play a role in the diabetogenic phenotype (102). Three studies, all published prior to 1991, investigated glucagon behavior in individuals with TS (17, 99, 103). All of them noted either normal or no differences in fasting glucagon levels. The third study also noted significantly higher glucagon levels in the individuals with TS following OGTT as well as diminished levels during insulin-induced hypoglycemia. These results may indicate abnormal glucagon secretion in TS but should be interpreted with caution as the controls in this study were not sex- or adiposity-matched. Synthesizing the limited data available, glucagon secretion may be dysregulated in TS, but there is insufficient evidence to say that hyperglucagonemia plays a predominant role in the heightened risk for hyperglycemia in TS. These studies are summarized in Table 6.

6.5 Impaired incretin secretion

As discussed in section 6.3, the early studies of hyperglycemia in TS reported disparate responses to intravenous and oral glucose challenges (15, 75). This implicates incretin hormones as possible contributors to hyperglycemia in TS. The incretin hormones, including glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like 1 peptide (GLP-1), are secreted by enteroendocrine cells in response to ingested macronutrients. The incretins potentiate insulin secretion in a glucose-dependent manner (104). OGTT followed by oral amino acid tolerance tests conducted in 20 individuals with TS demonstrated that those individuals with IGT detected on OGTT had similar plasma glucose and insulin responses to oral amino acids compared to those with NGT (75). These results suggest that individuals with TS and abnormal glucose tolerance can have normal tolerance and normal beta-cell sensitivity to oral amino acids and thereby provides further indirect evidence that alterations in incretin secretion may underly hyperglycemia in TS. Only two studies have directly measured incretin hormones during OGTT and contrast with each other. A small, early study of 12 adolescents with TS showed abnormal GIP response during the OGTT in those with IGT, and this impairment correlated with delayed insulin secretion (93). The other published study directly evaluating incretin concentrations in TS demonstrated no significant differences in serum GIP and GLP-1 levels between 13 individuals with TS and age-, sex-, BMI-, and lean body mass matched controls during OGTT, although notably only 2 women with TS had hyperglycemia (94). No TS studies have directly quantified the incretin effect in TS using isoglycemic IV glucose infusion. This is an area that warrants future study, as GLP-1 agonists are gaining traction to treat hyperglycemia

TABLE 4 Summary of key studies evaluating insulin secretion as a potential mechanism contributing to TS-associated hyperglycemia in.

Impaired insulin secretion	
Bakalov et al. Impaired Insulin Secretion in the Turner Metabolic Syndrome. 2004.	HOMA-B, I-AUC$_{30}$, I-AUC$_{180}$, and FPIR derived from OGTT were all significantly reduced in subjects with TS compared to controls (who were age and BMI matched women with primary ovarian failure). Subgroup analysis of the TS subjects with NGT continued to demonstrate lower insulin responses.
Hjerrild et al. Delayed Beta-Cell Response and Glucose Intolerance in Young Women with Turner Syndrome. 2011.	I-AUC$_{120}$ and I:G derived from OGTT were significantly reduced in subjects with TS compared to controls. FPIR derived from IVGTT was also reduced in subjects with TS.
Sheanon et al. Increased Prevalence of Beta-Cell Dysfunction Despite Normal HbA1c in Youth and Young Adults with Turner Syndrome. 2021.	Oral c-peptide and glucose minimal model-derived indices of beta cell function were significantly reduced in subjects with TS compared to controls.

These studies demonstrate that insulin secretion is impaired to oral glucose and is present prior to the evidence of hyperglycemia. TS, Turner syndrome; HOMA-B, Homeostatic model assessment of beta cell function; I-AUC, area under the insulin curve; FPIR, first phase insulin response; OGTT, oral glucose tolerance test; TS, Turner syndrome; BMI, body mass index; NGT, normal glucose tolerance; I:G; insulin-to-glucose ratio; IVGTT, intravenous glucose tolerance test.

TABLE 5 Summary of key studies evaluating insulin resistance as a contributor to TS-associated hyperglycemia.

Insulin resistance	
Caprio et al. Insulin Resistance: An Early Metabolic Defect of Turner's Syndrome. 1991.	Individuals with TS required significantly less GIR to maintain euglycemia than controls.
Hjerrild et al. Delayed Beta-Cell Response and Glucose Intolerance in Young Women with Turner Syndrome. 2011.	Insulin-stimulated glucose uptake was not different between individuals with TS and controls.
O'Gorman et al. An Evaluation of Early Cardiometabolic Risk Factors in Children and Adolescents with Turner syndrome. 2013.	WBISI (calculated using Matsuda index) from OGTT was not different between subjects with TS and controls. BMI and waist circumference were not correlated with WBISI in the TS group.
Alvarez-Nava et al. Insulin Sensitivity and Pancreatic B-Cell Function in Ecuadorian Women with Turner Syndrome. 2020.	HOMA-IR, Matsuda Index, QUICKI were not different between subjects with TS and controls.

These studies demonstrate that insulin resistance does not contribute consistently to early glucose abnormalities in TS. TS, Turner syndrome; GIR, glucose infusion rate; WBISI, whole-body insulin sensitivity; OGTT, oral glucose tolerance test; BMI, body mass index; HOMA-IR, homeostatic model assessment of Insulin Resistance; QUICKI, Quantitative Insulin Sensitivity Check Index.

in other conditions with high DM risk (105). Key studies discussed here are summarized in Table 7.

7 Clinical considerations

7.1 Recommended screening tests and potential limitations

The Clinical practice guidelines for the care of girls and women with TS recommend annual screening for DM after the age of 10 with HbA1c, with or without fasting plasma glucose (2). However, studies including HbA1c in TS are limited and show discordance between HbA1c and OGTT in the diagnosis of IGT and DM according to American Diabetes Association criteria in adults (3, 18, 106). It has similarly been demonstrated that 23% of girls with TS had abnormal OGTTs despite normal HbA1c (54). Isolated fasting glucose intolerance is uncommon in TS (14, 32, 35, 95) and paradoxically, a reduction of fasting insulin and plasma glucose concentrations has been associated with a deterioration in glucose tolerance in TS (20). As such, serial OGTT may be a more sensitive means to screen for glucose abnormalities in TS. A 2011 study demonstrated that a significant percentage of adult patients with TS lacked medical follow up, and those without transition care or preceding specialist care had an increased risk of having undiagnosed cardiovascular risk factor (107). Thus, it is crucial that patients with TS understand the necessity of and differences in metabolic screening as they transition to adult care.

7.2 Treatment considerations

There are no TS-specific treatment guidelines for DM. However, optimization of DM treatment should be a priority given the

TABLE 6 Summary of key studies evaluating hyperglucagonemia as a potential mechanism contributing to TS-associated hyperglycemia.

Hyperglucagonemia	
Zinman et al. Endocrine, Cytogenetic, and Psychometric features of patients with X-isochromosome 46,X,i(Xq) Turner's syndrome: A Preliminary Study in Nine Patients. 1984.	Fasting glucagon levels were reported as normal when compared to previously-studied controls, and did not change during OGTT.
Costin et al. Carbohydrate Intolerance in Gonadal Dysgenesis: Evidence for Insulin Resistance and Hyperglucagonemia. 1985.	No difference in fasting glucagon levels between individuals with TS and controls, but glucagon was higher at all time points throughout an OGTT in those with TS.
Stoppoloni et al. Characteristics of Insulin Resistance in Turner syndrome. 1990.	No differences in fasting glucagon levels between individuals with TS and controls.

The 3 small studies show no differences in fasting glucagon levels between individuals with TS and controls, but limited data is available to conclude if stimulated glucagon secretion is dysregulated. TS, Turner syndrome; OGTT, oral glucose tolerance test.

TABLE 7 Summary of studies evaluating impairment in incretin secretion or action to underly the TS-associated hyperglycemia phenotype.

Impaired incretin secretion or action	
Polychronakos et al. Carbohydrate Intolerance in Children and Adolescents with Turner syndrome. 1980.	Paired OGTT and IVGTT in TS showed that subjects with TS tolerated IV glucose infusion better than oral glucose (i.e., the mean insulin, glucose and Kt values were not different between those with normal oral glucose tolerance and abnormal oral glucose tolerance.
Heinze et al. Reduced Secretion of Gastric Inhibitory Polypeptide in Turner Patients with Impaired Glucose Tolerance. 1991.	Adolescents with TS had abnormal GIP response during the OGTT in those with IGT, and this impairment correlated with delayed insulin secretion.
Hjerrild et al. Delayed Beta-Cell Response and Glucose Intolerance in Young Women with Turner Syndrome. 2011.	No significant differences in serum GIP and GLP-1 levels between 13 individuals with TS and age-, sex-, BMI-, and lean body mass matched controls during OGTT

OGTT, oral glucose tolerance test; IVGTT, intravenous glucose tolerance test; TS, Turner syndrome, Kt, total glucose disappearance rate; GIP, glucose-dependent insulinotropic peptide; IGT, impaired glucose tolerance; GLP-1, glucagon-like peptide 1; BMI, body mass index.

significant contribution of diabetes to morbidity and mortality in TS. Additionally, DM is associated with lower health-related quality of life in TS (108) making this an important area of research. There are no published studies reporting the types of DM treatments used in TS and how they vary by age and in different health care settings. There are also no studies reporting the efficacy of targeted lifestyle modifications or anti-diabetic agents to mitigate the progression from IGT to DM in TS – given the natural history of hyperglycemia in TS (3), this should be an area of priority. It could be that most individuals with TS and DM are started on metformin in line with American Diabetes Association guidelines for the pharmacologic treatment of type 2 DM (109). However, given that insulin secretory defects seem to exist early on, even before the development of glucose abnormalities, and fasting hyperglycemia is atypical in TS, this drug may be a less appropriate first-line hyperglycemia treatment for individuals with TS.

8 Conclusion

Hyperglycemia is common in TS and is probably caused, at least in part, by an intrinsic defect in insulin secretion. Additional physiological studies are needed to clarify the mechanisms underlying hyperglycemia in TS. Hopefully, these efforts will culminate in physiology-informed therapy to prevent and/or treat hyperglycemia in TS patients and further inform screening for hyperglycemia in TS. The substantial increase in mortality associated with DM in TS is unexplained and warrants further study as these statistics are based on retrospective review of death certificate data and thus relevant clinical data is lacking. Future studies need to be designed with enough statistical power to account for the heterogeneity within the TS population, and this may be aided by TS registries and research networks (110). Genetics studies should continue to be pursued, as the specific X_{chr} contributions to DM in TS remain elusive.

Author contributions

CTP conceptualized the article. CTP and CM performed the literature search. CM, CTP, and EA wrote the first draft of the article. CTP and AWN critically revised the work. All contributing authors have read and approved submission to Frontiers in Pediatric Endocrinology. 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.

Publisher's note

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Aneta Monika Gawlik,
Medical University of Silesia, Poland

REVIEWED BY
Malgorzata Wojcik,
Jagiellonian University Medical College, Poland
Monika Obara-Moszynska,
Poznan University of Medical Sciences, Poland

*CORRESPONDENCE
Michał Erazmus
michal.erazmus@wum.edu.pl
Anna Małgorzata Kucharska
ankucharska@wum.edu.pl

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Predicted health care profile after transition to adult care in Turner syndrome children—experience of single center

Ewa Witkowska-Krawczak, Michał Erazmus*, Anna Majcher, Beata Pyrżak and Anna Małgorzata Kucharska*

Department of Pediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland

Introduction: Turner Syndrome (TS) is caused by the complete or partial loss of one of the X chromosomes in all or some female cell lines. The variable genotypes are responsible for a large phenotypic diversity, nevertheless most studies emphasize a weak correlation between genotype and phenotype. The study aimed to assess the occurrence of defects and diseases depending on the karyotype in patients with TS and correlation with the predicted health care profile after the transition to adulthood.

Materials and methods: 45 patients of the Department of Endocrinology and Pediatrics of the Medical University of Warsaw in 1990–2002 were analyzed. Girls were divided into 2 subgroups: "A", which included 16 patients with the karyotype 45,X, and "B", which included 29 girls with mosaic karyotypes. Based on the literature data, characteristic phenotypic features and the typical defects or diseases accompanying TS were selected, and the frequency of their occurrence was compared in both subgroups. Accordingly to this data, the predicted medical care profile was determined.

Results: In our study, patients with complete monosomy of the X chromosome had more characteristic phenotypic features. They needed sex hormone replacement therapy more often and started to menstruate spontaneously much less frequently (only 18.18% in monosomy vs. 73.91% in mosaic patients, $p = 0.006$). In patients with monosomy, congenital defects of the circulatory system were found more often (46.67% vs. 30.77%). The diagnosis in patients with mosaic karyotype was more often delayed, therefore the optimal time of growth hormone therapy was shorter. In our study, the X isochromosome determined the higher prevalence of autoimmune thyroiditis (83.33% vs. 12.5%, $p = 0.049$). We didn't find a correlation between the type of karyotype and health care profile after the transition, most of the patients needed more than 2 specialists. Most often, they required: gynecologists, cardiologists, and orthopedics.

Conclusions: After the transition from pediatric to adulthood, patients with TS need multidisciplinary care, but not all need the same kind of assistance. The phenotype and comorbidities determine the profile of patients' health care, however it wasn't directly related to the type of karyotype in our study.

KEYWORDS

turner syndrome, short stature, karyotype, puberty, congenital heart disease, autoimmune thyroiditis, quality of life

Introduction

Turner Syndrome (TS) was described in 1938 by Henry Hubert Turner (1). It occurs with a frequency of approximately 1:2.500 live female births. It is estimated that about 100 girls with TS are born annually in Poland (2). The syndrome is caused by the complete or partial loss of one of the X chromosomes in all or some of the female cell lines. Girls with TS are most frequently characterized by short stature, dysmorphic features and gonadal dysgenesis. They suffer more often than the general population from congenital defects of the circulatory system, urinary tract, endocrine disorders, eye and hearing disorders, and autoimmune diseases. There is a large phenotypic diversity. Current knowledge on the mechanisms of phenotypic features in TS is still incomplete. Most studies emphasize a weak correlation between genotype and phenotype (3). However the progress of genetic and molecular diagnostics allows for the identification of an increasing number of genes within the X chromosome responsible for the occurrence of individual symptoms (4). The kind of disorders associated with TS determines the kind of specialistic medical care for the whole life.

The study aimed to assess the occurrence of defects and diseases depending on the karyotype in young patients with TS and its correlation with predicted future health care profile after the transition to adulthood.

Materials and methods

45 patients who were in the childhood under the care of the Department of Endocrinology and Pediatrics in 1990–2020, were qualified for the study. The inclusion criterion for the study was the diagnosis of TS, based on the cytogenetic examination of at least 20 peripheral blood cells, analyzing metaphases from the standard culture with the formula 550–400 bands. There was no exclusion criteria. Based on the collected medical records, a retrospective analysis was performed.

The study group was divided into 2 subgroups: "A", which included 16 patients with the karyotype 45, X, and "B", which included 29 girls with mosaic karyotypes, regardless of their type. The distribution of particular types of karyotypes is presented in **Figure 1**.

Based on the "Clinical practice guidelines for the care of girls and women with Turner syndrome" (5), the frequency of TS diagnosis in both subgroups was analyzed according to age:

(1) <6 years of age. Optimal for the early initiation of treatment with recombinant human growth hormone (rhGH),
(2) 6–12 years of age—delayed implementation of rhGH treatment, optimal pubertal monitoring,
(3) > 12 years of age—suboptimal medical care, too late initiation of treatment (5).

Characteristic phenotypic features and the typical defects or diseases most often accompanying TS were selected (**Table 1**, **Figure 2**), and the frequency of their occurrence was analyzed in subgroups dependently on karyotype.

Patients older than 13-year-old (upper age limit of puberty initiation) were selected and analyzed for possible onset of puberty, (Tanner score, serum estradiol and LH, FSH concentrations, ovarian size in ultrasound). Also, patients older

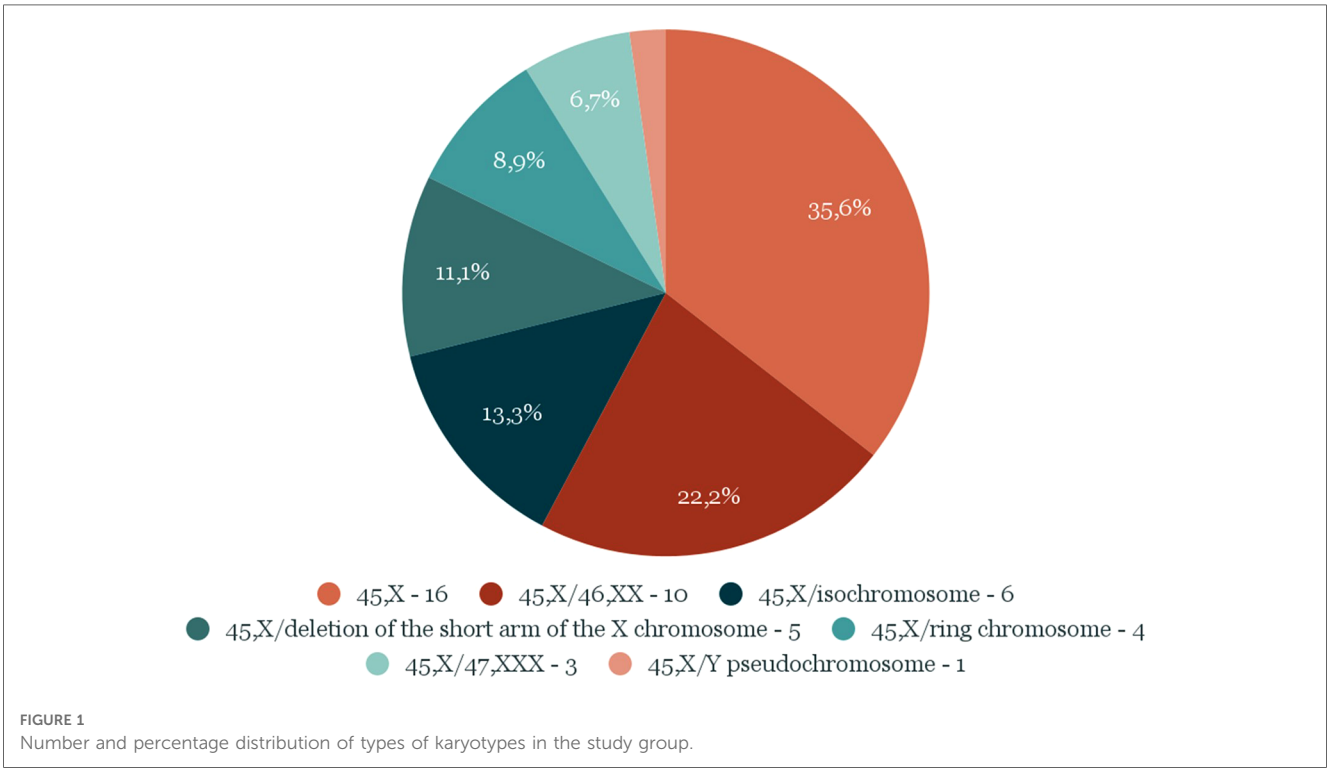

FIGURE 1
Number and percentage distribution of types of karyotypes in the study group.

TABLE 1 Distribution of the analyzed features among patients from the whole study group and "A" and "B" subgroups.

	Group "A"—45,X ($N = 16$)	Group "B"—mosaic chromosomes ($N = 29$)	Whole group ($N = 45$)	No data
Age of diagnosis				
<6 year of old	4/16 (25%)	4/29 (13,79%)	8/45 (17,78%)	
6–12 year of old	3/16 (18,75%)	16/29 (55,17%)	19/45 (42,22%)	
>12 year of old	9/16 (56,25%)	9/29 (31,03%)	18/45 (40%)	
Short stature	14/14 (100%)	24/26 (92,31%)	38/40 (95%)	5/45 ("A"—2/16; "B"—3/29)
Final growth				11/45 ("A"—4/16; "B"—7/29)
<3rd percentile	5/12 (41,67%)	13/22 (59,09%)	18/34 (52,94%)	
3–10th percentile	5/12 (41,67%)	8/22 (36,36%)	13/34 (38,24%)	
10–25th percentile	2/12 (16,67%)	1/22 (4,55%)	3/34 (8,82%)	
Phenotypic features				5/45 ("A"—2/16; "B"—3/29)
≤2	5/14 (35,71%)	15/26 (57,69%)	20/40 (50%)	
>2	9/14 (64,29%)	11/26 (42,31%)	20/40 (50%)	
Spontaneous onset of estrogen-dependent sexual development (patients >13 year of old)	2/11 (18,18%)*	17/23 (73,91%)*	19/34 (55,89%)	5/11 ("A"—5/16; "B"—6/29)
Spontaneous onset of menstruation (patients >15 year of old)	2/11 (18,18%)*	14/20 (70%)*	16/31 (51,61%)	14/45 ("A"—5/16; "B"—9/29)
Cardiovascular system defects	7/15 (46,67%)	8/26 (30,77%)	15/40 (37,5%)	4/45 ("A"—1/16; "B"—3/29)
Bicuspid aortic valve	4/15 (26,67%)	2/26 (7,69%)	6/40 (15%)	
Aortic regurgitation	3/15 (20%)	2/26 (7,69%)	5/40 (12,5%)	
Coarctation of the aorta	2/15 (13,33%)	2/26 (7,69%)	4/40 (10%)	
Patent foramen ovale	1/15 (6,66%)	2/26 (7,69%)	3/40 (7,5%)	
Aortic stenosis	0/15 (0%)	3/26 (11,54%)	3/40 (7,5%)	
Patent ductus arteriosus	1/15 (6,66%)	1/16 (3,85%)	2/40 (5%)	
Atrial septal defect	2/15 (13,33%)	0/26 (0%)	2/40 (5%)	
Autoimmune thyroiditis	3/16 (18,75%)	6/29 (20,69%)	9/45 (20%)	
Urinary system defects	2/15 (13,33%)	4/27 (14,81%)	6/42 (14,29%)	3/45 ("A"—1/16; "B"—2/29)
Hearing disorders	2/14 (14,29%)	10/23 (43,48%)	12/37 (32,43%)	8/45 ("A"—2/16; "B"—6/29)
Vision disorders	1/14 (7,14%)	6/22 (27,27%)	7/36 (19,44%)	9/45 ("A"—2/16; "B"—7/29)
Celiac disease	0/14 (0%)	2/25 (8%)	2/39 (5,13%)	6/45 ("A"—2/16; "B"—4/29)

*Statistically significant data (p-value <0.05).

than 15-year-old (upper age limit of menarche) were verified for spontaneous onset of menstruation.

All the above-described data were statistically processed using the chi-Square Calculator program.

On the basis of the above-developed data, a projected profile of specialist care after the age of 18 was created for each patient, depending on the health burdens.

Result

Age of diagnosis

The age of TS diagnosis in the study group ranged from the prenatal period to 17 years old. The average age of the karyotype testing was 10.16 years. Among patients with X chromosome monosomy, it was 10.56 years; among those with mosaic karyotypes, it was 9.93 years. We found no statistically significant difference between the mean age of diagnosis throughout the 3 decades of our observation.

In group "A" in 4/16 (25%) patients the diagnosis of TS was placed <6 years of age, in 3/16 (18.75%) patients between 6 and 12 years of age, in 9/16 (56.25%) the diagnosis was too late, >12 years of age. In group "B", the karyotype was determined in 4/29 (13.79%) patients <6 years of age, in 16/29 (55.17%) between 6 and 12 years of age, in 18/29 (40%) > 12 years of age (Table 1). There was no statistically significant difference in the age of diagnosis of the syndrome in the compared groups (p-value >0.05).

Growth

All girls with a karyotype 45, X had body height below 3 percentile for calendar age and received recombinant human growth hormone (rhGH) therapy. Among patients with mosaic karyotypes, height deficiency was diagnosed in 24/26 (92.31%), and all of them were treated with rhGH in the Program for Treatment of Short Children with TS. In 2/26 patients (7.69%), height was within the normal range for age (Table 1) and they did not require the rhGH treatment. Unfortunately, in the 5 oldest patients—2 from group "A" and 3 from group "B", retrospective data on body height have not been available.

In 34 patients—12 from group "A" and 22 from group "B", the final body height after the end of growth was also determined. Among patients with X chromosome monosomy, body height <3rd percentile was found in 5 (41.67%), 5 (41.67%) had height

in the 3rd–10th percentile channel, and 2 (16.67%) in 10th–25th percentile channel. Among patients with mosaic karyotype, 13 (59.09%) completed growth <3 percentile, 8 (36.36%) in the 3rd–10th percentile channel, 1 (4.55%) > 10 percentile (Table 1). The higher described differences were not statistically significant (p-value > 0.05).

Phenotypic features

Data of characteristic phenotypic features could be analyzed in 40 patients—14 from the "A" group and 26 from the "B" group. The list of the traits and their distribution in the entire study group are presented in Figure 2.

Depending on the number of phenotypic features, the following patients from both subgroups were compared:

(1) with a maximum 2 features—as an incomplete phenotypic picture of TS, which may occur incidentally and not be associated with the diagnosis, and

(2) having more than 2 characteristic features—as a phenotypic image clearly indicating the diagnosis of TS. In group "A" 2 or less of the features mentioned above were found in 5 (35,71%) patients, and in another 9 (64,29%), there were more phenotypic features. In group B, at least 2 traits were found in 15 (57,69%) girls, and the remaining 11 (42,31%) had more than 2 features (Table 1). No statistically significant difference was found between the type of karyotype and the number of phenotypic features (p-value > 0.05). It was also not seen that one of the features occurs significantly more often in combination with any of the types of karyotypes.

Puberty

Data concerning puberty was analyzed in 11 patients in group "A" and 23 in group "B" at the age of 13 years and older. As expected, pubarche was observed in 100% of them. Spontaneous onset of estrogen-dependent sexual development, assessed on the basis of mammary gland development, ovarian enlargement and increase in serum estrogen concentration, was found in 2 patients from the "A" group (18.18%) and 17 patients from the "B" group (73.91%), which was a statistically significant difference (p-value 0.0022*).

The number of patients at the age older than 15 years, in whom the spontaneous onset of puberty was analyzed, was 11 in group "A" and 20 in group "B". Menarche occurred in 2 girls with karyotype 45, X (18.18%) and 14 with mosaic karyotypes (70%)—this difference is also statistically significant (p-value 0.00574*).

All patients without spontaneous onset of puberty received estrogen replacement. Due to the preferences of the patients, 100% of them used the oral form of therapy. It was calculated that the average age of introduction therapy was 13.46-year-old.

Cardiovascular defects

Data on cardiovascular defects was gathered in 40 patients, including 15 in group "A" and 26 in group "B". The 3 most frequently detected defects were: bicuspid aortic valve (15% of patients), aortic regurgitation (12.5%), and aortic coarctation or subcoarctation (10%). Among patients

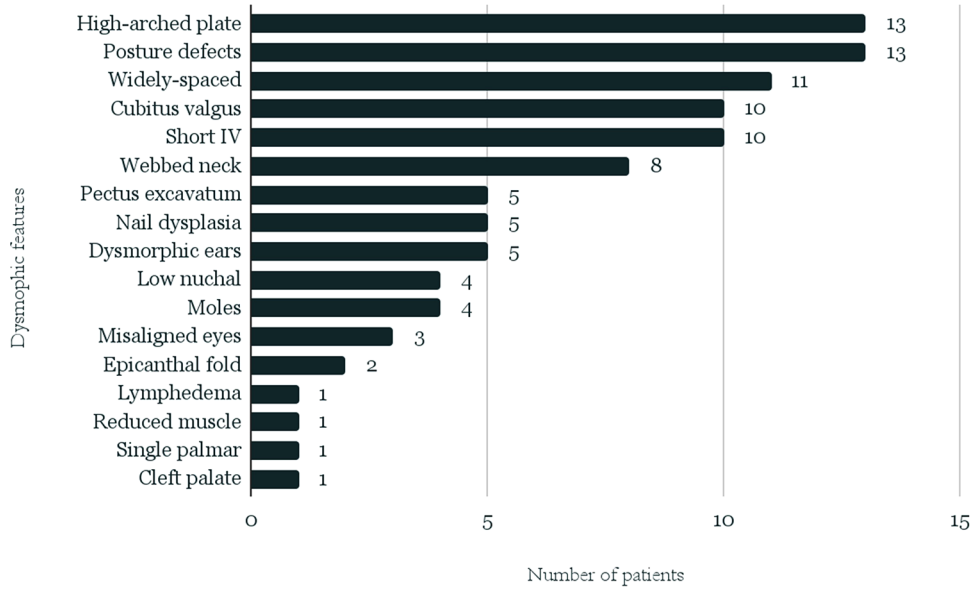

FIGURE 2
Distribution of the analyzed phenotypic traits among patients from the whole study group.

with monosomy of the X chromosome, cardiovascular defects were found in 7/15 (46.67%), while among those with mosaicism in 8/26 (30.77%)—a statistically insignificant difference (p-value > 0.05). The types of defects and the percentage of patients with them in each group are presented in Table 1. There was no significant predominance of any of the defects in any of the subgroups.

Autoimmune thyroiditis

Autoimmune thyroiditis was diagnosed in 3/16 (18.75%) girls from group "A" and 6/29 (20.69%) girls from group "B". Due to reports in the literature regarding the correlation of the occurrence of autoimmune thyroiditis with the isochromosome (15–18), this aspect was also checked. In the entire study group, autoimmune thyroiditis was found in 9/45 patients (20%), while in the group of girls with isochromosome in 5/6 (83.33%). A statistically significant difference was found between the number of patients with autoimmune thyroiditis in the isochromosome group (5/6) and among girls with all other karyotypes (4/39)—p-value 0.000031.

Other defects and accompanying diseases

Both subgroups of patients were also analyzed for the prevalence of urinary tract defects, vision and hearing disorders, celiac disease. The frequency of the above-described diseases in both studied subgroups is shown in Table 1. The patients were diagnosed with: horseshoe kidney, double or dilated calyx-pelvic system, upper calyx syndrome, chronic or recurrent exudative otitis media, hearing loss, myopia, astigmatism, monergism, and convergent strabismus. None of the diagnoses occurred significantly more often in patients with monosomy X chromosome than in patients with mosaic karyotypes.

Predicted specialist care after the age of 18

Based on the data described above and Clinical practice guidelines for the care of girls and women with Turner syndrome (5), a predictive profile of specialist care after the age of 18 was created for patients in the study group, which is presented in Table 2.

Discussion

Due to such a diverse genotypic and phenotypic picture of TS, numerous studies are being conducted to clarify the relationship between the type of genotype and the clinical picture of the disease, however there are not many studies trying to find key factors determining the type and quality of health care profile after the transition to adulthood.

During the pediatric period, the care of TS patients is usually well coordinated by the pediatric endocrinologists, because of rhGH treatment and puberty induction. However after the transition to adult care, medical management becomes decentralized and divided among several specialists. An efficient transition process and one center coordinating the entire treatment should improve the quality of life (QoL) of TS

TABLE 2 A predictive profile of specialist care in the study group after the transition to adult care.

Specialist	Number of patients	Health aspects	Recommendations (5)
Primary care	All patients	Special prevention	TSH, fT4, HbA1c, AST, ALT, GGT, ALP, clinical evaluation for scoliosis every year
Gynecologist	All patients	Prevention as in a healthy population	
		Persistent ovarian function	Observation for premature ovarian failure
			Consider oocyte cryopreservation
		Hypogonadism	Hormone Replacement Therapy
			Consider assisted reproductive therapy
Cardiologist	All patients	Special prevention	Transthoracic echocardiography (TTE) or cardiac magnetic resonance scan (CMR) every 10 years
		Pregnancy	TTE and CT/CMR 2 years before planned pregnancy
			TTE about 20 weeks of gestation
		Cardiovascular system defects—control and treatment	
Otolaryngologist	All patients	Special prevention	Audiometric evaluation every 5 years
		Hearing disorders—control and treatment	
Orthopaedist and rehabilitation specialist	15	Posture defects, valgus of the limbs—control and treatment	
Endocrinologist	9	Autoimmune thyroiditis—control and treatment	
Ophthalmologist	7	Vision disorders—control and treatment	
Dermatologist	4	Multiple moles—control	
Nephrologist	6	Urinary system diseases—control and treatment	
Gastroenterologist	2	Celiac disease—control and treatment	

patients. Many studies and recommendations emphasize the significantly lower QoL of adult patients with TS, so efforts should be made to improve this factor on many levels, starting primarily with the quality of medical care and effective transition (4). The QoL is dependent also on a proper education about the TS associated problems, additionally strong emotional support for these patients and constant psychological care, especially in adulthood should not be underestimated.

One of the most significant factors affecting the QoL of patients with TS is short stature, which is also the most frequent reason for the start of diagnostics. Indeed, short stature is the most common feature of girls with TS with classical monosomy as well as mosaic karyotypes (8). The earlier TS diagnosis allows for early rhGH treatment, however our study reveals that many patients with TS remain short adults. In our study group the final height position below 3rd percentile was present in 41,67% of patients with 45, X0 karyotype and 59,09% of patients with mosaic karyotypes.

Our study confirms the previously published observations, that the characteristic dysmorphic features of TS are more discreetly expressed in patients with a mosaic karyotype (6). This fact explains a general tendency towards delayed diagnosis in this group. In our study the average age of diagnosis of TS was 10,16 years, and there was no significant difference between the groups of patients with particular karyotypes (10,56 in patients with monosomy vs. 9,93 in patients with mosaicism, $p > 0{,}05$). A study population bias could explain this paradoxical observation, because mosaicism was presented by our patient almost 2 times more frequently than monosomy, discordantly with known literature data, where the prevalence of monosomy is estimated at 50%. Also, surprisingly, the mean age of diagnosis was much older than the average in many studies conducted in Western countries (19, 20). It indicates that the most frequent reason for the diagnostics in TS girls was short stature. It was the main characteristic feature that became more evident in school children.

Our study highlights the importance of karyotyping in all girls with short stature (21). The gold standard for karyotype evaluation is the examination of peripheral blood leukocytes. According to the guidelines, such an analysis should contain at least 20 cells because this methodology identifies at least 10% mosaic karyotypes with 95% sensitivity (9–11). It means that some patients with a mosaic karyotype can be overlooked with the standard diagnostic course, hence may never receive proper growth-promoting therapy or may never be diagnosed with comorbidities and then not receive appropriate specialist care, also after the transition to adulthood (12–14).

The results of our study revealed that the prevalence of comorbidities and systemic defects is similar in girls with a mosaic karyotype and classical monosomy, which was also reported by other authors (7, 8). It seems justified to highlight in the studies that the weak expression and sometimes even lack of characteristic dysmorphic features (so common in patients with a mosaic karyotype) cannot diminish vigilance and cause less active monitoring of comorbidities. It should be highlighted in the guidelines for pediatricians and physicians when the transition process is planned.

In our database, less than 55% of girls required hormonal induction of menstruation, while the up-to-date literature mentioned as much as 80%–90% (18). It probably was caused mainly due to the relatively high percentage of patients with mosaic karyotypes in our study group. In our study, there was an advanced average age of initiating estrogen replacement, followed by a delayed age of reaching the full sex hormone replacement. In the majority of patients, it was dependent on the desire to prolong the growth-promoting treatment delayed by the late TS diagnosis. It seems fully justified to ask whether the transition to adulthood care of TS patients should be postponed in such patients over the age of 18 years until they complete puberty. A separate problem noticed in our research is the preference of TS young girls for oral formulas of sex hormone replacement therapy. The main reason reported by girls and their parents was usually the fear of stigmatization in the peer environment when they use plasters. However, transdermal systems are recommended, therefore it seems important to encourage patients to use a more effective and safe method, both—during pediatric care and after the transition to adulthood (22).

A separate factor worsening QoL is gonadal dysgenesis and infertility. Oocyte cryopreservation is recommended for teenagers with TS from the age of 12, who have functioning ovaries. However, more and more studies emphasize the need to educate parents of younger girls with TS and the possible earlier decision about oocyte cryopreservation or ovarian tissue cryopreservation even from the age of 6, as premature ovarian failure generally occurs early in life in young women with TS, rapidly after the process of transition from pediatric care (5, 23, 24). Adult patients with TS will require a slightly different and more flexible approach during gynecological care. Medical staff and parents should start discuss this topic with adolescent girls while providing them with appropriate psychological support.

It should be remembered that in case of pregnancy, during obstetric care, patients with TS require careful cardiological care, frequent echocardiographic monitoring, and strict blood pressure control. Education on greater cardiovascular risk and the principles of a healthy lifestyle should be implemented from early childhood and then continued in internist care in adulthood.

The data available in the literature suggest that specific TS comorbidities are much more common among patients with some variants of mosaic karyotypes. It is well known i.e., that patients with TS suffer from autoimmune diseases more often than the rest of the TS population. In this group, autoimmune thyroiditis (AT) is more frequent, which is mentioned in many studies (25). This applies, for example, to the correlation of AT with the X isochromosome (15–18). Our analysis also confirmed this strong association—the X isochromosome determined the higher prevalence of AT (83.33% vs. 12.5% in monosomy and other karyotypes, $p = 0.049$).

It is essential to screen the presence of other autoimmune diseases in TS children, i.e., celiac disease. The clinical picture of celiac disease often takes atypical forms that can overlap TS symptoms, like menstrual disorders or infertility. The disease may develop over the years and could not be diagnosed until adulthood. Therefore, it is justified to emphasize the importance

of active screening. It is suggested to consider to check periodically the level of antibodies against tissue transglutaminase also after the transition in girls with TS, especially when suspicious symptoms or signs are present (5).

In childhood and adolescence, over 90% of TS patients require the treatment with rhGH, which is why the care of these girls is usually coordinated by an endocrinologist. In adulthood, however, the coordinating doctor may change. Depending on the local medical care organization, screening tests for diseases connected with TS can be provided by the general practitioner (GP). According to our country, the GP can monitor TSH, fT4 level and antithyroid antibodies, glucose level, blood pressure, BMI. When some abnormalities are detected, the patients could be referred to an adequate specialist. In our opinion, sometimes too much multidisciplinary care can lead to patient overload and reduced QoL, and therefore in some cases "less could be more".

In our study, we tried to find the key factors predicting the type and quality of health care profile after the transition to adulthood in patients with TS. We showed health problems affecting TS patients. Some require systematic control, while others require active screening, as they may appear in adult life. This highlights the importance of coordinated medical care after the process of transition. However, we didn't find a strong correlation between the type of karyotype and health care profile. Most of the patients needed more than 3 specialists, and mainly they required: gynecological, cardiological, orthopedics, and otolaryngological care.

Conclusions

An effective transition process can improve the quality of health care and QoL of TS patients. The majority of TS women need multidisciplinary medical care, which generally becomes more decentralized and divided among several specialists after the transition. The phenotype and comorbidities determine the profile of patients' health care, however it wasn't directly related to the type of karyotype. Accordingly to the most common health problem, main cooperation is needed between gynecologists, cardiologists, and orthopedics, as well as significant psychological support which is essential to prepare properly children and parents to the transition.

Data presented in our research show that patients with complete monosomy of the X chromosome have more characteristic phenotypic features of TS. They also start to menstruate spontaneously much less frequently and therefore need hormone replacement therapy more often. Congenital defects of the circulatory system are also found more often in monosomy. On the contrary, autoimmune thyroiditis was more frequent in patients with mosaic karyotypes. On the other hand, the diagnosis in patients with a mosaic karyotype is more often delayed; therefore, the optimal time of growth-promoting therapy is shortened, and these patients also risk less effective monitoring of comorbidities and a less efficient transition from pediatric care to adulthood. We did not find any other correlation between the type of karyotype and the presence of numerous other defects and diseases associated with TS. This implies the need for the same accurate monitoring and screening in all patients with TS, regardless of karyotype, because the quality of medical care in adult life also depends on it.

Author contributions

Planning—EW, ME, AK; Data base—EW, ME, AM; First draft—EW; Analysys of the methods, statistics—EW, AK, BP; Tables, figures—EW; Dicussion—ME, AK, BP; Conclusions—ME, AK, BP. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

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EDITED BY
Malgorzata Ewa Wiecek,
Medical University of Silesia, Poland

REVIEWED BY
Laura Melado,
ART Fertility Clinics LLC,
United Arab Emirates
Christina Bothou,
University Hospital Zurich, Switzerland

*CORRESPONDENCE
Casper P. Hagen
casper.hagen@regionh.dk

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AMH and other markers of ovarian function in patients with Turner syndrome – a single center experience of transition from pediatric to gynecological follow up

Casper P. Hagen[1,2]*, Margit Bistrup Fischer[1,2], Gylli Mola[1,2], Theis Bech Mikkelsen[1,2], Line Hartvig Cleemann[1,2], Claus Højbjerg Gravholt[3,4], Mette H. Viuff[3], Anders Juul[1,2,5], Anette Tønnes Pedersen[6] and Katharina Maria Main[1,2,5]

[1]Department of Growth and Reproduction, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark, [2]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), University of Copenhagen, Rigshospitalet, Denmark, [3]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark, [4]Department of Diabetes and Endocrine Diseases, Aarhus University Hospital, Aarhus, Denmark, [5]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark, [6]Department of Gynecology, The Fertility Clinic, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark

Turner syndrome (TS) is a chromosomal disorder that affects about 1 in 2500 female births and is characterized by the partial or complete absence of the second X chromosome. Depending on karyotype, TS is associated with primary ovarian insufficiency (POI). Approximately 50% of girls with a mosaic 45, X/46, XX karyotype may enter puberty spontaneously, but only 5-10% of women with TS achieve pregnancy without egg donation. In this review, we will evaluate the clinical use of markers of ovarian function in TS patients. Based on longitudinal studies of serum concentrations of reproductive hormones as well as ovarian morphology in healthy females and patients with TS, we will evaluate how they can be applied in a clinical setting. This is important when counseling patients and their families about future ovarian function essential for pubertal development and fertility. Furthermore, we will report on 20 years of experience of transition from pediatric to gynecological and adult endocrinological care in our center at Rigshospitalet, Copenhagen, Denmark.

KEYWORDS

ovarian function, fertility preservation, turner syndrome, anti mullerian hormone (AMH), FSH (Follicle Stimulating Hormone), inhibin B

Introduction

Various pathological conditions cause early loss of ovarian follicles resulting in absence or cessation of pubertal development and primary or secondary amenorrhea (premature ovarian insufficiency, POI). The most prevalent inherited condition of accelerated follicle loss is Turner syndrome (TS) affecting approximately 1:2500 liveborn females (1).

Due to complete or partial loss of one X-chromosome in all cells (e.g. 45,X) or part of the cells (mosaicisms, e.g. 45,X/46,XX), TS patients suffer from a variable degree of prenatal loss of follicles (2–5) (Figure 1).

When TS is diagnosed during childhood, patients and their families are often concerned about future reproductive potential. Will they develop similar to their teenage peers? Will they enter puberty spontaneously without hormone replacement therapy? Will they eventually achieve pregnancy? The increasing success rates of ovarian cryopreservation for future fertility in girls with cancer prior to gonadotoxic therapy have inspired similar protocols in patients with TS. In experimental settings, cryopreservation of ovarian tissue has been performed, and it is essential only to offer cryopreservation to patients with ovarian follicles (8, 9).

However, it is a challenge to assess ovarian activity in girls and it is even more difficult to predict future ovarian function. Apart from a transient neonatal gonadotropin surge, the hypothalamic-pituitary-gonadal (HPG) axis is quiescent until pubertal onset allowing only gonadotropin-independent growth of follicles reaching small antral stages. Therefore, in TS patients with streak ovaries, the usual lack of negative feedback and consequently hypergonadotropic hypogonadism is not evident prior to time of expected pubertal onset (8–15)(Figure 2).

Today, the best candidate as a marker of subtle ovarian activity is Anti-Müllerian Hormone (AMH) produced by granulosa cells in small growing follicles (11). Initially, the focus of attention on this peptide was the testicular production of AMH. Alfred Jost was the first to suggest that a substance produced from the developing gonad in the male fetus was responsible for the regression of the Müllerian ducts (ovarian ducts, uterus and the proximal one-third of the vagina) (12). This hormone is AMH, previously referred to as Müllerian Inhibiting Substance (MIS), produced by immature Sertoli cells in the male fetus (13, 14). AMH is a member of the TGF-beta family. It is encoded by the *AMH* gene (15) which is located on chromosome 19p13.3 (16). AMH exerts its effect through the single transmembrane receptor, AMH type 2 (AMHR2), leading to phosphorylation of Smad 1/5/8 that enter the nucleus and regulate transcriptional activity (17). In young patients with Differences of Sex Development (DSD), high serum concentration of AMH is a specific and sensitive marker of testicular tissue (immature Sertoli cells) in the gonad (18–21).

In females, circulating AMH originates exclusively from the ovaries (22). The function of AMH is not fully elucidated but knock-out mice models and human *in vitro* data indicate that AMH inhibits follicle growth as well as FSH induced aromatase activity (11, 23–25). Effects on recruitment from primordial follicles may be

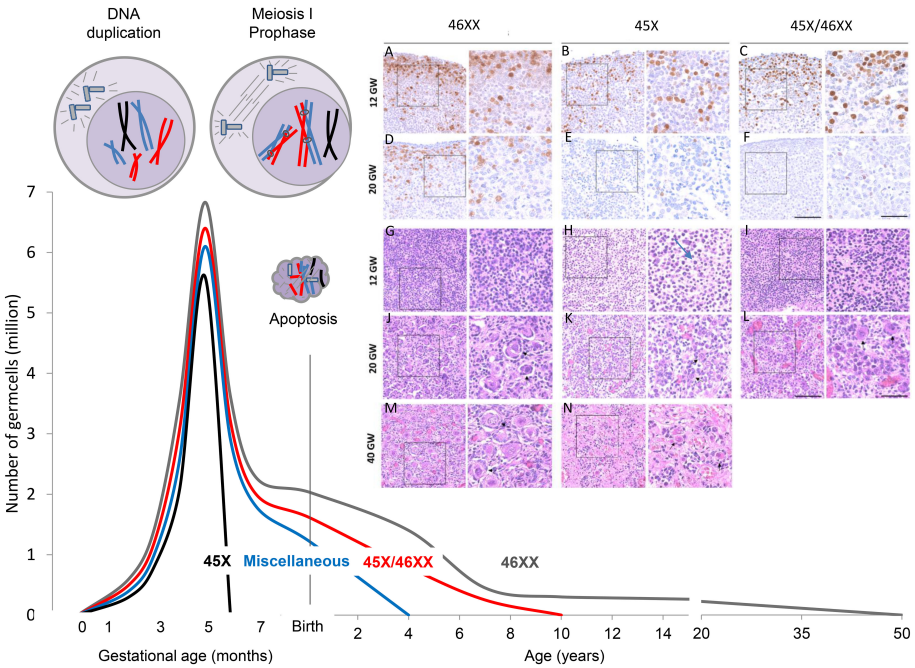

FIGURE 1
Accelerated loss of follicles depends on the TS karyotype. The mechanism is believed to be apoptosis caused by pairing failure of homologous chromosomes in meiosis I. This is schematically shown in the top left corner with only one duplicated X chromosome (black). Histology samples **A-N** modified from (5): In early fetal life, there are plenty of OCT4 positive oogonia present in 45, X ovaries **(B)**, but many of the germ cells are degenerated with contracted nuclei and a thin layer of cytoplasm (**H** arrow). Later in gestation, primordial and small growing follicles are present in the healthy ovary (J+M), whereas somatic cells and fibroblasts are abundant in the 45, X ovary (K+N). Schematic illustration of the number of germ cells in healthy females (46,XX, grey line) from early fetal life to time of menopause; data based on Baker et al. (6, 7).

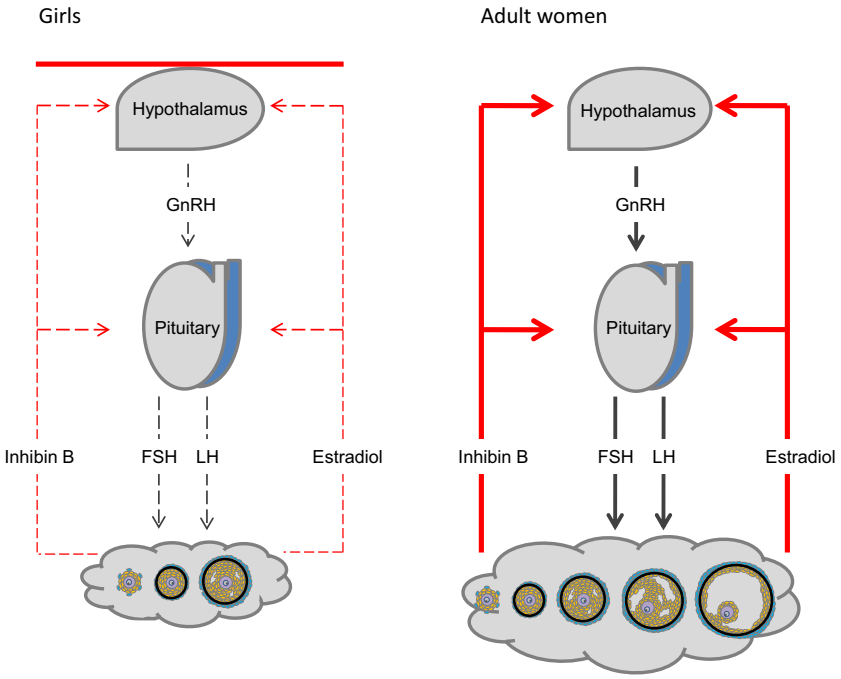

FIGURE 2
The hypothalamic-pituitary-gonadal (HPG) axis. Activity of the hypothalamus and pituitary is regulated by negative feedback of ovarian hormones (right). The HPG axis is centrally inhibited during mid-childhood (left). Follicles are primarily restricted to stages growing independently from FSH stimulation. Only occasionally FSH- induced follicle growth occurs.

species dependent. AMH promotes primordial follicle recruitment in cultured human ovaries (9) and *in vitro* and *in vivo* data from non-human primates support stimulating action of AMH on preantral follicle growth (26) (Figure 3). Thus, production and effects of AMH are follicle stage dependent and AMH seems to play an essential role as gate-keeper for FSH-induced follicle maturation, estradiol production, as well as regulator of the selection of the dominant follicle in the late follicular phase of the menstrual cycle. In humans, rare mutations of the gene encoding *AMH* result in premature ovarian insufficiency (27). Extragonadal effects of AMH have been proposed, and AMH may play a role in upregulation of GnRH dependent LH pulsatility (28). Circulating AMH levels are

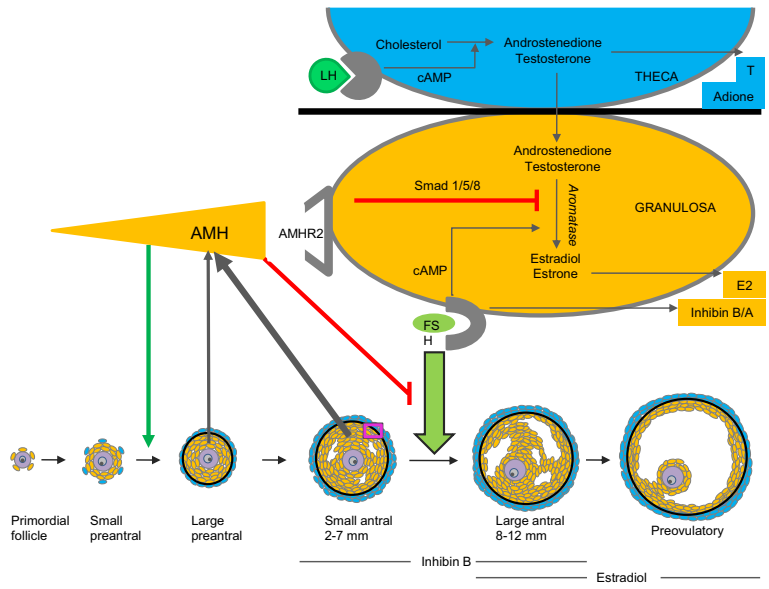

FIGURE 3
Model of AMH production and action. AMH is produced by granulosa cells of small growing follicles. It inhibits FSH-induced follicle growth as well as gonadotropin-induced aromatization of androgens to estrogens (9, 24–26).

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usually elevated in PCOS patients as well as in patients with granulosa cell tumors (29–31).

The unique source of AMH from follicles growing independently of FSH-stimulation poses several clinical advantages. Circulating AMH levels are more refractive to fluctuations of gonadotropin levels compared to hormones produced by larger follicles. Thus, circulating concentrations of AMH are relatively stable through the menstrual cycle (although cycle dependent fluctuations are more pronounced in women with higher AMH concentrations) (32–34). AMH is decreased app. 30% by oral contraceptive therapy (35) and 50% during pregnancy (36). In healthy adult women, serum levels correlate with the number of antral follicles (37). Due to a fine equilibrium between follicles in different stages (38, 39), AMH levels reflect the number of primordial follicles constituting the ovarian reserve (40) (Figure 4). In healthy adult women, circulating AMH is therefore predictive of the reproductive lifespan (41–45). Women with age specific low AMH tend to enter menopause earlier than women with higher AMH. However, considerable overlap exists, and the predictive value for AMH in a given woman concerning age at menopause is limited (46).

In this review, we will present data relevant when assessing AMH in girls and adolescents with TS. To interpret a given AMH measurement in a patient at risk of POI, it is essential to know details about AMH in healthy girls. Age specific reference ranges are mandatory. Additionally, cross sectional studies of AMH in relation to ovarian morphology are necessary to assess if AMH in girls reflects the number of small antral follicles – which may reflect the ovarian reserve of primordial follicles. Longitudinal studies of individual AMH levels are needed to evaluate the predictive value of AMH concerning future ovarian activity in healthy girls as well as in patients with TS. Further, we will briefly discuss the qualitative aspect of AMH concerning fecundability.

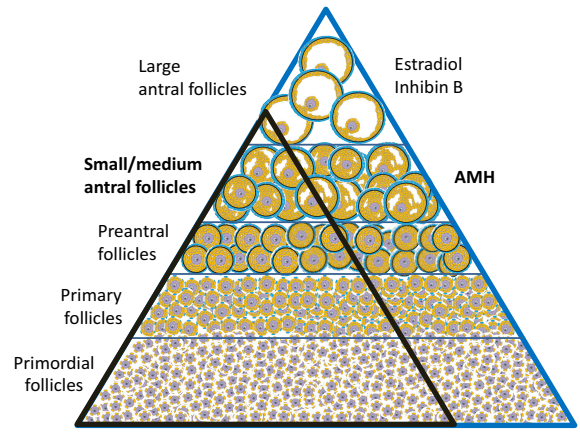

FIGURE 4
The number of follicles decline as they mature. Histological studies suggest that the number of follicles in different stages is in equilibrium; i.e. an individual with many primordial follicles have more preantral and antral follicles (blue triangle) compared with an individual with fewer primordial follicles (black triangle) (38, 39).

Karyotype as predictor of ovarian activity

In TS patients, the karyotype is strongly associated with ovarian status; i.e. the risk of POI is highest in monosomic patients compared to karyotypes with mosaicism including a healthy cell line (45,X/46,XX) or isochromosomes (6, 47–51). The mechanism causing accelerated loss of germ cells is believed to be apoptosis caused by pairing failure of homologous chromosomes in meiosis I. In early fetal life when the first oocytes enter the diplotene stage of meiotic prophase I, there are plenty of oogonia present in 45,X ovaries (Figure 1, histology section B, OCT4), but many of the germ cells are degenerated with contracted nuclei and a thin layer of cytoplasm (Figure 1, arrow in section H). Later in gestation, primordial and small growing follicles are present in the healthy ovary (Figure 1, J+M), whereas somatic cells and fibroblasts are abundant in the 45,X ovary (Figure 1, K+N). There are very few follicles.

In theory, the loss of follicles depends on the specific TS karyotype: Patients with 45,X are often born with streak gonads (Figure 1, black line) whereas TS patients with mosaicisms including a healthy cell line (45,X/46,XX) have approximately 50% chance of entering puberty spontaneously (Figure 1, red line) (6). All other TS genotypes caused by structural abnormalities of one X chromosome are referred to as miscellaneous having intermediate chance of preserved ovarian function (Figure 1, blue line).

The degree of mosaicism evaluated in 30 white blood cells may not be fully representative of the gonadal mosaicism (52). That is also the case when patients are diagnosed prenatally by non-invasive prenatal testing, amniocentesis, chorionic villus sampling or by fetal DNA in maternal blood sample. Furthermore, different tissue from the same patient – and even different cells from the same ovary may express variable degree of mosaicism (53, 54). Thus, the proportion of affected cells in peripheral blood is not always predictive of the remaining primordial follicles. This may explain cases of apparently monosomic patients with preserved ovarian function (55). There are even reports of 45,X patients with multiple unassisted pregnancies (56). Patients with miscellaneous karyotypes have an intermediate chance of maintaining ovarian activity, but from the limited number of patients with specific genotypes, it is not possible to clarify if certain loci are more prone for POI than others. Patients with TS including Y chromosome material are at risk of developing gonadoblastoma, and gonadectomy is recommended, although the degree of risk of gonadoblastoma still remains to be firmly established.

Thus, the karyotype based on DNA from white blood cells can be misleading concerning the degree of ovarian dysgenesis. The karyotype is a strong indicator of the degree of ovarian dysgenesis, but additional markers are needed to evaluate the ovarian function of girls and adolescents with TS.

Reproductive hormones

Detailed magnetic resonance imaging (MRI) and trans-abdominal ultrasound studies (TAUS) of ovarian follicle numbers

in healthy girls revealed that small antral follicles were present in all prepubertal girls (57). Large follicles were present after pubertal onset, and the number of large follicles increased as puberty progressed (Figure 5). This knowledge of ovarian morphology is important for interpreting circulating levels of reproductive hormones during childhood. Pubertal reactivation of the HPG axis and increasing levels of gonadotropins is essential for maturation of follicles into large antral stages responsible for steroid hormone production. Thus, inhibin B and estradiol (produced by granulosa cells) as well as testosterone and androstenedione (produced by theca cells) correlated strongly with the number of large follicles (57), independent of pubertal stages (Figures 6B–D).

These morphological findings and their association with hormone levels explain the clinical challenges the pediatrician faces when evaluating ovarian activity during the quiescence of the HPG axis in mid-childhood. Reproductive hormone levels in mid-childhood are therefore similar to healthy girls; i.e. low levels of LH and FSH from the pituitary as well as low or undetectable levels of inhibin B and estradiol produced by granulosa cells surrounding larger antral follicles (8, 9, 58). Centrally inhibited levels of FSH, albeit measurable, are rarely sufficient for follicle maturation beyond small antral stages (Figure 2). Thus, in our longitudinal study of reproductive hormone levels in TS patients through childhood, gonadotropins were not elevated in the majority of patients who did not enter puberty spontaneously

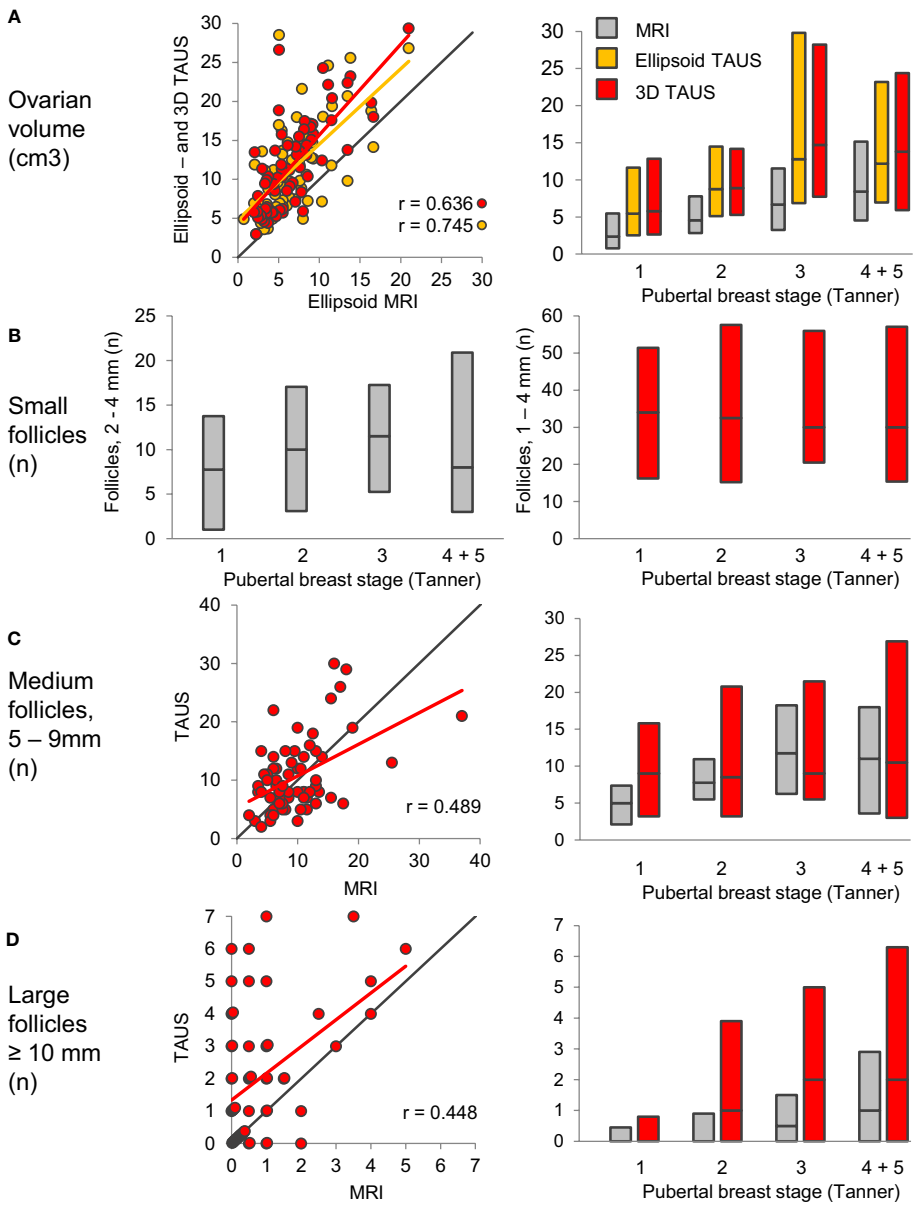

FIGURE 5
Ovarian volume **(A)** and follicle numbers **(B–D)** assessed by MRI (grey) and TAUS; Ellipsoid TAUS (orange) and 3D TAUS (red), according to pubertal breast stages. Bars indicate median, 10th and 90th percentiles. Black lines indicate lines of identity. Figure from (57).

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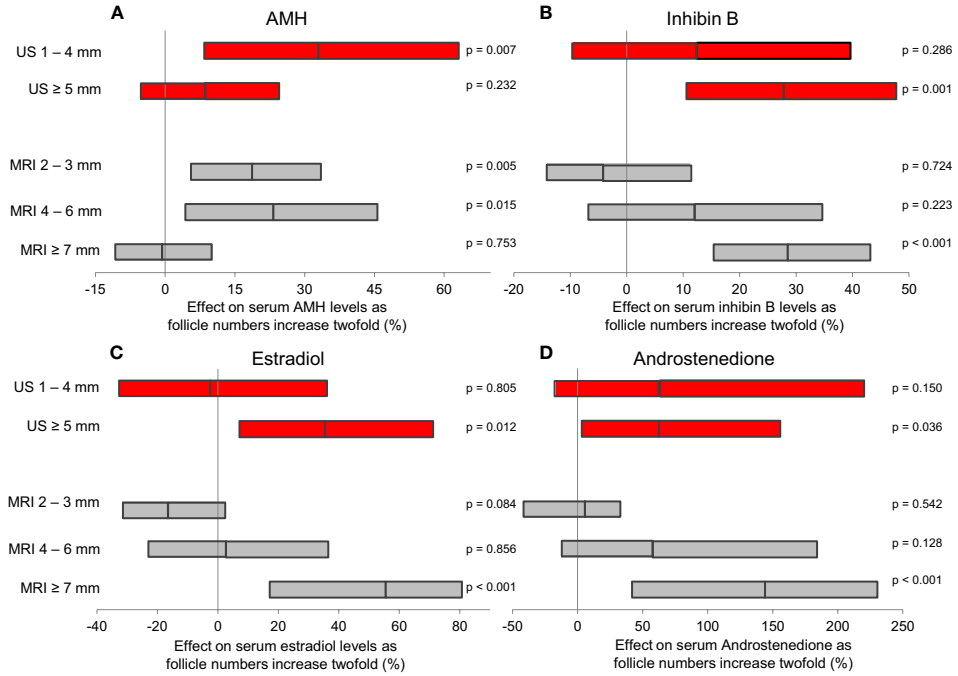

FIGURE 6
Results of multiple regression analyses evaluating which follicles contributed most to serum levels of ovarian hormones. The estimated effect of a two-fold increase (doubling) in follicle numbers is presented as geometric mean (95% CI). Both TAUS (red) and MRI (grey) revealed that AMH **(A)** is produced by smaller follicles than inhibin B **(B)**, estradiol **(C)** and androstenedione **(D)**. Figure from (57).

(FSH data seen in Figure 7) (47). However, there are indications that HPG activity during minipuberty does not end as abruptly in girls as in boys. Thus, FSH seems to be elevated in young prepubertal Turner syndrome patients up to 6 years of age (47, 58, 59). A single measurement of undetectable inhibin B was a prevalent finding in healthy girls and therefore not a very specific predictor of absent pubertal onset in TS patients. However, repeated blood samples increased the chance of revealing ovarian activity by detecting inhibin B produced by a randomly matured large follicle (47).

Introduction of ultra-sensitive liquid chromatography–mass spectrometry (LCMS/MS) indicates that estrone (E1) is measurable in the majority of healthy prepubertal girls (10). Further studies on circulating concentrations of estrone and estradiol (LCMS/MS) in girls with TS are needed to evaluate the predictive value of these biomarkers. Despite ultra-sensitive LCMS/MS methods enabling measurement of low levels of circulating androgens, these hormones are co-produced by the adrenals and therefore not specific for ovarian activity (60).

Even after spontaneous pubertal onset and/or menarche, it remains a clinical challenge to evaluate ovarian function. Irregular anovulatory cycles are prevalent in healthy girls up to 2-3 years after menarche (61). Furthermore, reproductive hormones may remain within the normal range before POI is clinically evident, despite significant depletion of the ovarian reserve (37, 62, 63).

Thus, during mid-childhood, the clinical use of gonadotropins and products from larger ovarian follicles (inhibin B, estradiol, testosterone and androstenedione) is hampered by central inhibition of the HPG axis.

However, in clinical follow up, repeated assessments prior to pubertal onset may reveal ovarian activity (detectable inhibin B levels) or hypergonadotropic hypogonadism (elevated FSH levels).

AMH in healthy girls

Interestingly, circulating AMH reflects the number of small and medium antral follicles in healthy peripubertal girls (57) (Figures 6A, 8). Thus, AMH is a unique marker of ovarian activity during mid-childhood quiescence of the HPG axis.

We established the first reference range of AMH in females measured with a sensitive assay. It was based on 926 healthy females from birth to 69 years of age (Figure 9). We observed a surge of AMH at time of the so-called "minipuberty" (the transient postnatal activation of the HPG axis) (64). This was confirmed in a recent detailed longitudinal study of healthy girls – even indicating a biphasic pattern of AMH and other reproductive hormones during the first year of life (65).

The transient stimulation of the ovaries during minipuberty results in increasing numbers of antral follicles producing AMH (66). AMH seems to increase from 4 to 8 years of age, but compared to other reproductive hormones, circulating levels of AMH are remarkably stable in childhood, puberty and adolescence (64, 67). However, inter-individually between girls, AMH levels vary 15-fold. These findings are in line with the dynamics of ovarian follicles, as the number of AMH-producing follicles (antral follicles < 6mm) varies between healthy peripubertal girls but the number of these small growing follicles do not increase after pubertal onset (57).

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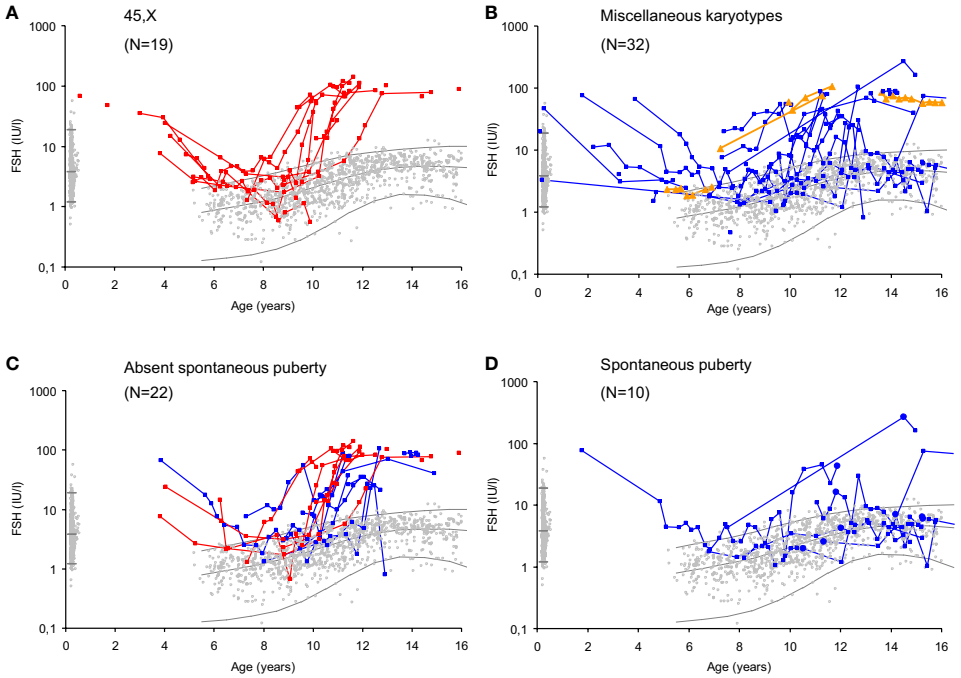

FIGURE 7
Serum FSH levels (IU/l) in girls with Turner syndrome (n=51) according to age, karyotype and spontaneous puberty onset compared to a reference range based on 2406 healthy Danish girls (grey dots). Lines represent geometric means and 95% prediction interval (± 2SD). Girls with 45,X monosomy (red, **(A)**; miscellaneous TS karyotypes before (blue) and after gonadectomy (orange) **(B)**; TS patients with absent spontaneous puberty **(C)** and spontaneous puberty **(D)**. Age at spontaneous pubertal onset is illustrated by closed circles. Figure from (47).

A recent long-term longitudinal study of healthy females followed from infancy to adolescence reveal remarkable stable levels of AMH through the entire childhood (68). If a girl had high AMH, she remained with high levels through infancy, childhood, puberty and adolescence, and vice versa if she had low levels, she maintained low levels. Thus, the predictive value of low concentration of circulating AMH in mid-childhood is both sensitive and specific of low AMH in adolescence. Due to individual tracking of activity from small growing follicles, AMH in mid-childhood - and even in infancy - was associated with the number of small follicles in the same girl at puberty and adolescence. A meta-analysis including data from several studies suggests that AMH increases in late adolescence (69). The study is based on data from different cohorts using different immunoassay which are difficult to convert to comparable levels (70, 71). Circulating AMH is present in different molecular forms (72) which may explain the discrepancy between AMH assays (73). There is a need of an international standard to enable comparison of AMH levels between study populations when measured at different laboratories.

Thus, in healthy girls, AMH is a unique reproductive hormone reflecting and predicting the number of small antral follicles. Individual circulating levels are stable through infancy, childhood, puberty, and adolescence.

Regulation of AMH

In healthy girls, circulating AMH levels are negatively associated with FSH levels prior to pubertal onset (74). Furthermore, detailed longitudinal data revealed a limited but significant increase of AMH prior to pubertal onset (+17%) followed by decreasing levels (-30%) two years after pubertal onset. These findings have been confirmed by two British cohorts of healthy peripubertal girls (75, 76) (Figure 10). Initially, we speculated that the post-pubertal decrease of AMH was caused by the pubertal increase of FSH, leading to increased maturation of follicles which would reduce the number of AMH producing follicles. However, our detailed study of ovarian morphology revealed that the number of AMH producing follicles (< 6mm) actually increased during early puberty (57). In the same study, independent of follicle numbers, estradiol levels were negatively correlated with AMH. Increasing estradiol during early puberty may therefore directly inhibit AMH production. Firm causal conclusions of the negative association between AMH and FSH as well as estradiol cannot be drawn from our human clinical data. However, direct inhibition of AMH expression by estradiol has been suggested by *in vitro* studies of granulosa cells from patients undergoing *in vitro* fertilization (77). Conversely, AMH reduces sensitivity and growth rate of follicles in response to FSH as well as inhibits aromatase expression in smaller follicles (11, 26, 78). Thus, AMH seems to inhibit estradiol production in small follicles, whereas estradiol may inhibit AMH production in large follicles. We have speculated that in prepubertal girls, AMH is essential to prevent FSH-induced growth as well as premature estradiol production from small growing follicles.

The data discussed above are from healthy girls with an intact HPG axis. Cellular studies suggest that FSH does not affect AMH

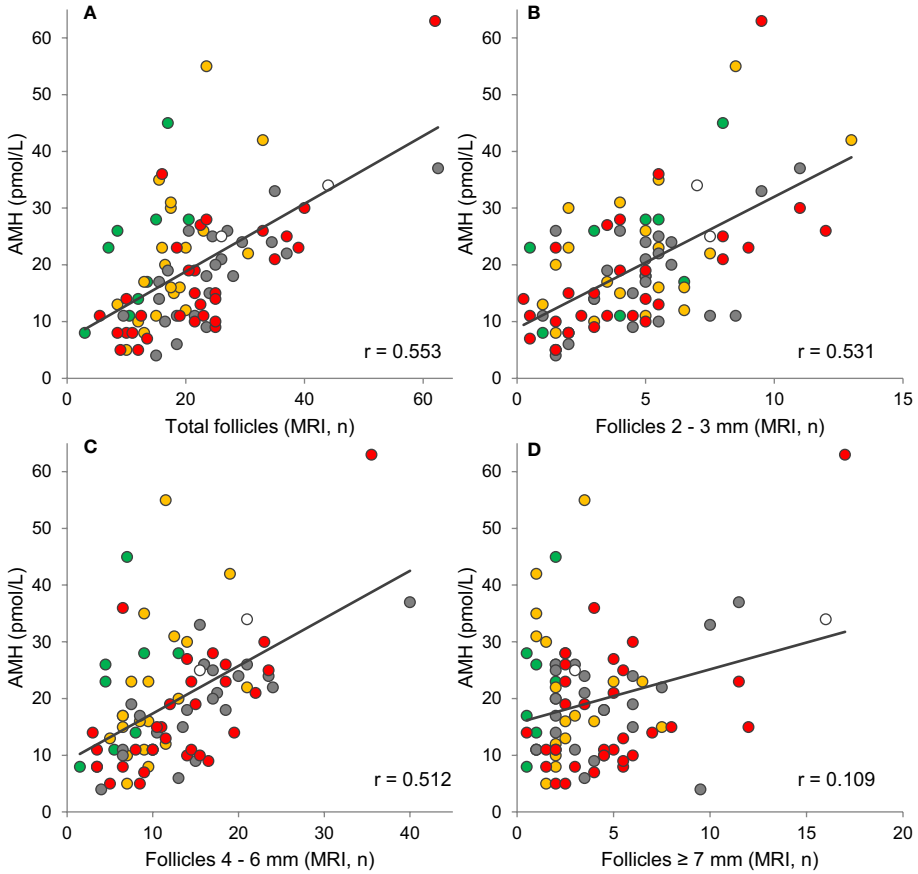

FIGURE 8
Correlations between AMH and **(A)** the total number of follicles (r=0.553, p<0.001), **(B)** small follicles (r=0.531, p<0.001), **(C)** medium follicles (r=0.512, p<0.001), and **(D)** large follicles (r=0.109, p=0.323) evaluated on MRI. Tanner stage B1, orange: B2, grey: B3, red: B4 + 5, white: Tanner stage unknown. Grey lines indicate tendency lines for correlations including all samples. Figure modified from (57).

production in granulosa cells from healthy women (79), however, these studies were performed on granulosa cells retrieved from ovarian stimulation which may affect the response. Further insight in regulation of AMH is gained from studies manipulating the HPG axis. From small cross-sectional studies of women on hormonal contraceptive treatment (HCT), AMH levels were considered independent of pituitary activity (36, 80). However, larger cross-sectional studies as well as recent longitudinal studies suggest that AMH levels are reduced app. 30% by HCT (35, 81, 82). Whether this is caused by direct inhibition of AMH expression by estradiol or

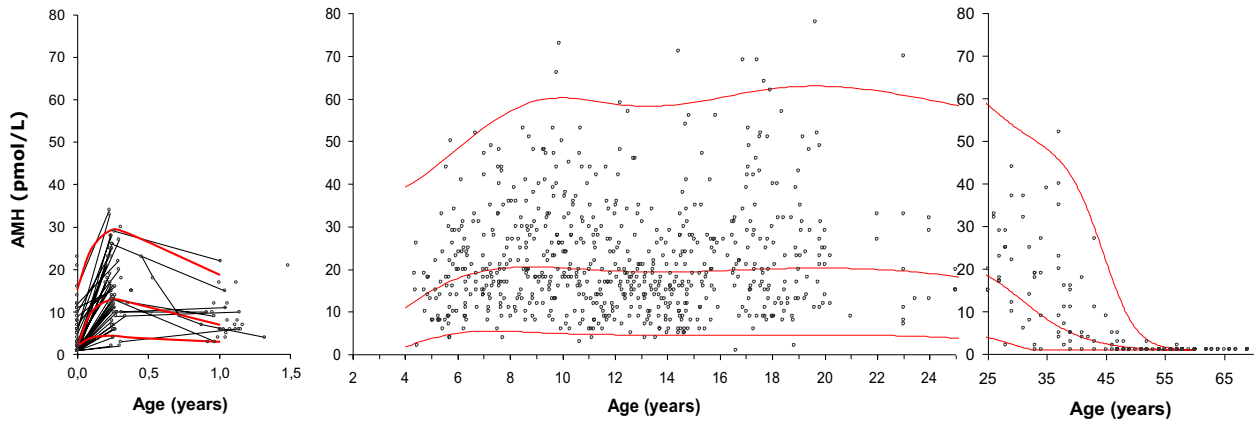

FIGURE 9
Serum AMH (pmol/L) in 926 healthy infants, girls, adolescents and adult women. Longitudinal values during infancy are connected with black lines. The red curves represent the median, the 2.5th percentile and the 97.5th percentile. Figure modified from (64).

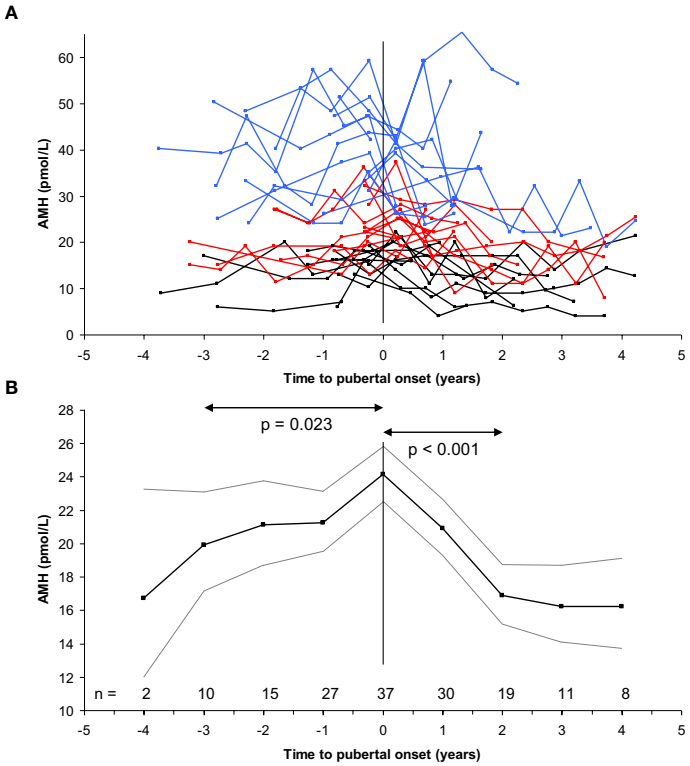

FIGURE 10
(A) Longitudinal AMH levels in 39 girls according to time to pubertal onset (breast development) (top). Blue, red and black lines indicate highest, medium and lowest tertile of AMH, respectively. **(B)** Variance component model (bottom) of the longitudinal data (black line: geometric mean, grey lines: +/- 1.96SD) revealed minor increase of AMH levels during the three years prior to pubertal onset (20 to 24 pmol/L, 17%, p=0.023), followed by decreasing levels two years after pubertal onset (24 to 17 pmol/L, 30%, p<0.001). Subsequently, AMH levels were stable. Figure based on data from (74).

the effect is due to reduced number of medium antral follicles caused by suppression of GnRH secretion by potent synthetic estrogens remains to be elucidated. In our study of AMH levels in girls with central precocious puberty before, during and after GnRH agonist treatment, AMH was reduced 50% in response to suppression of pituitary activity (83). Although ultrasound was not performed on these girls, previous studies suggest reduced number of medium sized antral follicles during GnRHa treatment (84). This would be a plausible explanation for our findings.

In conclusion, the negative correlation between AMH and FSH supports that a degree of negative feedback between pituitary gonadotropin secretion and ovaries is exercised even in prepubertal girls.

AMH as a predictor of fecundability in adult women

Whereas the value of AMH as a quantitative marker of follicles seems to be established, it remains contentious whether AMH is a marker of oocyte quality. Data from IVF settings strongly suggest circulating AMH as a marker of oocyte quality. AMH predicts the ovarian response (85, 86), and positive associations with the chance of conception (87) and livebirth (88, 89) have been reported. However, data from healthy women are less convincing. The first report of AMH as a marker of fertility in healthy women indicated that very low AMH predicted reduced fecundability in 100 women in their late reproductive life (30 – 42 years) (90). In another study of sub-fertile women who were unsuccessful in conceiving after 12 months of unprotected sexual intercourse (mean age 36 years), AMH levels in the 14 women achieving pregnancy during the following 6 months were not different from the 69 non-pregnant women (91). In a large prospective study of 186 healthy women (mean age 27 years) adjusted for male confounders, we found that high but not low AMH predicted reduced fecundability (Figure 11) (92). Our finding that high AMH was associated with reduced fecundability is most likely explained by a PCOS-like biochemical profile in the females with high AMH. The low AMH tertile included women witih AMH < 13 pmol/L which is well above the detection-limit of the assay (2 pmol/L) and the -2SD of the reference range in young adults (5 pmol/L). Thus, the size of the study population did not allow us to evaluate the effect of very low AMH. In support of our findings, a study of 1202 healthy women who had previously conceived did not find a reduced fecundability in women with low AMH (93). There is the possibility that sub-fertile PCOS patients may have been excluded in the study which may explain why high AMH was not associated with reduced fecundability in their cohort. In another study, AMH levels measured in the first trimester of pregnancy was not associated with fecundability (self-reported) in a retrospective

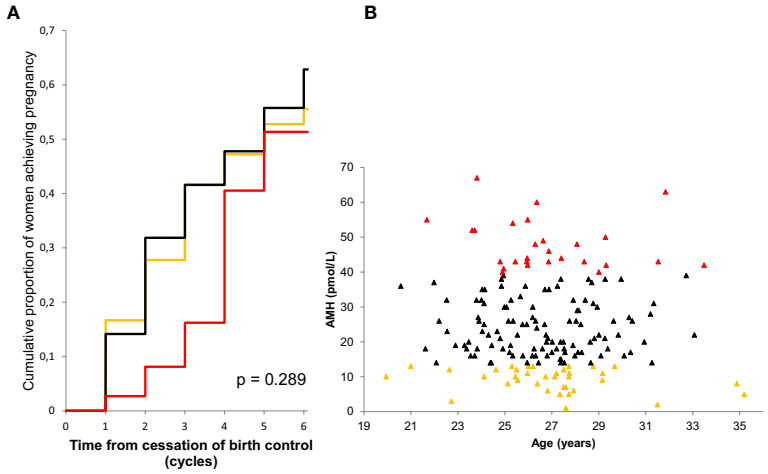

FIGURE 11
Kaplan-Meier curves showing the cumulative proportion of pregnancy by serum level of AMH **(A)**. Low AMH (quintile 1) orange line, medium AMH (quintiles 2-4) black line, high AMH (quintile 5) red line. P-value describes difference between curves (log-rank test). AMH as a function of age in 186 participating women **(B)**. Colors correspond to subgroups of AMH levels: low (orange), medium (black) and high (red). Figure from (92).

study of 87 healthy women conceiving naturally (mean age 31 years) (94). Other cohorts of different ethnicity support that low AMH is not associated with reduced fecundability (95).

Patients with TS have increased risk of autoimmune conditions, and untreated Hashimoto´s hypothyroidism may contribute to reduced fecundability in adult patients with preserved ovarian function.

In conclusion, AMH in adult women seems to be a quantitative rather than a qualitative marker of ovarian follicles. Further studies are necessary to elucidate if extremely low AMH affects time to pregnancy and to confirm whether low AMH predicts reduced fecundability in healthy women in late reproductive life.

AMH as marker of ovarian activity in ts patients

AMH has been associated with ovarian status in adolescent and adult patients with TS; i.e. low or undetectable AMH in patients with POI vs. AMH in the reference range in the majority of patients with ongoing ovarian function (64, 96–99). These cross-sectional data have been confirmed in a longitudinal follow-up study (48) (Figure 12). The longitudinal data from TS patients developing POI were sparse and we can therefore not firmly conclude on specific AMH values as predictors of absent pubertal onset or imminent POI. However, AMH was < 5 pmol/L (equals -2 SD) in all patients prior to clinical manifestation of POI. A cross sectional ROC analysis including data from all adolescent and adult patients revealed that AMH < 3 pmol/L seems to be a sensitive and specific marker of POI (both 95%) (48). These findings suggest an increased risk of imminent POI in TS patients with AMH < -2SD. For the clinician, the apparent predictive value of low AMH is useful when counselling adolescent TS patients with ongoing ovarian function about their risk of POI.

Taking into account that healthy girls maintain their relative AMH levels from infancy to adolescence (68) (Figure 13), it seems likely that undetectable AMH or AMH < -2SD is indicative of reduced ovarian activity in prepubertal TS patients. This was supported by our limited longitudinal data on young TS patients where all prepubertal girls with AMH < 4 pmol/L suffered from absent spontaneous pubertal onset (48). These findings are in line with a large European study where girls with TS having measurable AMH had a 19-fold increased chance of entering puberty spontaneously compared with patients with undetectable AMH (96). AMH is also undetectable or low in adult patients suffering from idiopathic premature ovarian insufficiency (100). FSH, LH, inhibin B, and estradiol may be unaffected until time of clinical manifestations of POI where the number of remaining follicles is severely reduced (37, 62, 63, 101). Our findings of multiple undetectable inhibin B measurements as a predictor of absent pubertal onset in young TS patients (47, 102) as well as decreasing inhibin B prior to POI in adolescent and adult patients (48). indicate that also inhibin B may be a valuable predictor of POI. However, single measurements of low or undetectable inhibin B should be interpreted with caution as this is a normal finding in healthy girls and adolescents (103).

Interestingly, adult Turner´s patients with ovarian function maintained their AMH levels during follow up, suggesting that they did not exhibit an accelerated depletion of their ovarian reserve compared to healthy controls (48). This is in line with UK biobank study where women who were not diagnosed with 45,X/46,XX had a similar number of children and did not enter menopause earlier than women with 46,XX (104). Of course there is a risk that the women in this study have a less severe phenotype compared with patients diagnosed with 45,X/46,XX. However, it suggests that patients with 45,X/46,XX have a chance for ongoing ovarian function and unaffected fertility comparable with healthy women. It also underlines the importance of continuous follow-up of such patients.

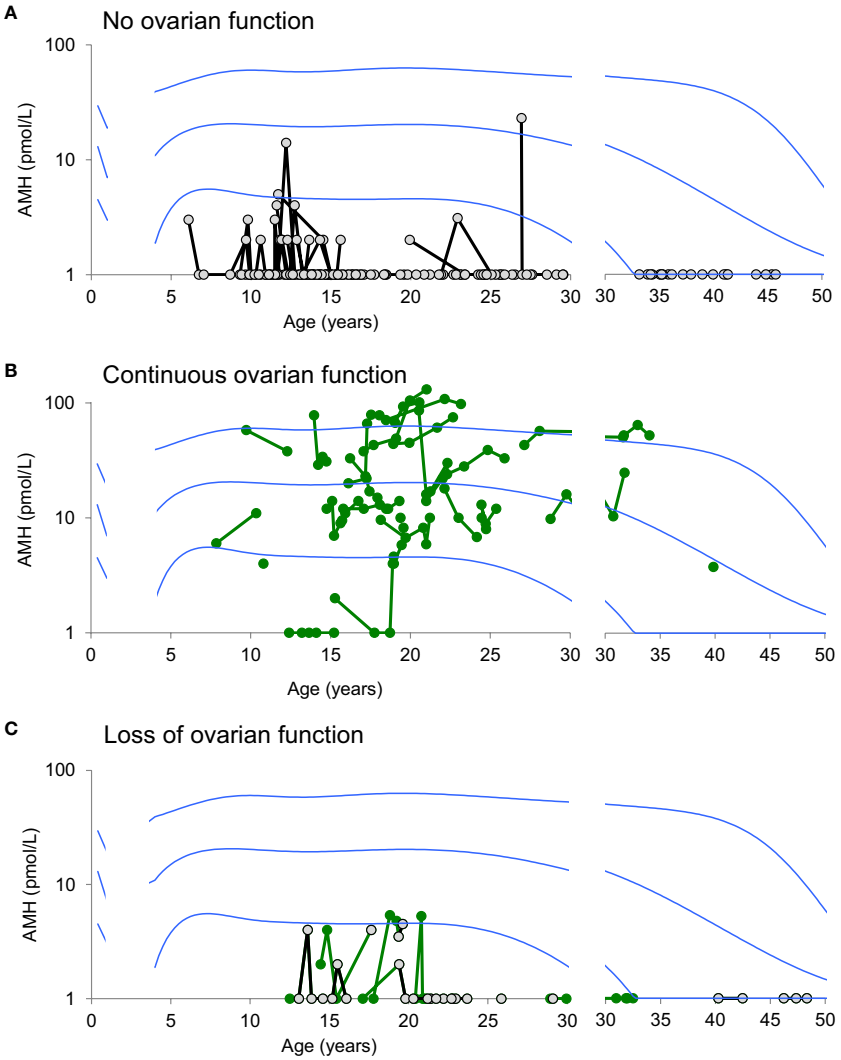

FIGURE 12
Serum AMH levels (pmol/L) in girls, adolescents, and women with TS according to age, reference range and their ovarian status: never ovarian function **(A)**, ongoing ovarian function **(B)**, and loss of ovarian function **(C)**. Green: patients with ongoing ovarian function; gray/black: patients with no ovarian function. Note the logarithmic Y-scale. Blue lines indicate the reference range (median, 2. and 97.5th percentiles). Figure from (48).

In conclusion, small studies of patients with TS suggest that AMH < -2SD is predictive of absent puberty and imminent POI, however larger studies are needed to qualify these findings further.

Ovarian cryopreservation in patients with TS

Hopefully, added understanding of the reproductive phenotype of patients with Turner's syndrome will lead to an improved evidence-based and individualized fertility counselling. Based on successful experience with ovarian cryopreservation and later auto transplantation in other patients at risk of POI (e.g. girls with cancer prior to gonadotoxic therapy, girls with thalassemia prior to bone marrow transplantation) (105–112), this procedure is now a treatment modality in clinical studies to young patients with TS in several centers. In Sweden, girls with TS have been offered cryopreservation since early 2000´s (50) and in the Netherlands, inclusion of girls with TS in a cryopreservation study has recently been finalized (113). In these studies, many patients had no follicles in the retrieved ovary. Although the karyotype, FSH, AMH, and inhibin B were all associated with the presence of follicles, the sensitivity and specificity of these markers were limited (50).

In this context, it is essential to evaluate ovarian activity. Surgery for ovarian cryopreservation should be avoided in patients without any ovarian follicles. Furthermore, surgery is not indicated in patients with ongoing ovarian function in adult life as they are likely to have a normal prognosis for pregnancy. Knowledge of markers and predictors of ovarian function in girls with TS is essential when counseling patients and their families in these matters. Importantly, studies have shown that life-birth rate after auto transplantation of frozen-thawed ovarian tissue is negatively correlated with increasing age and low AFC, which could indicate that low AMH at the time of cryopreservation

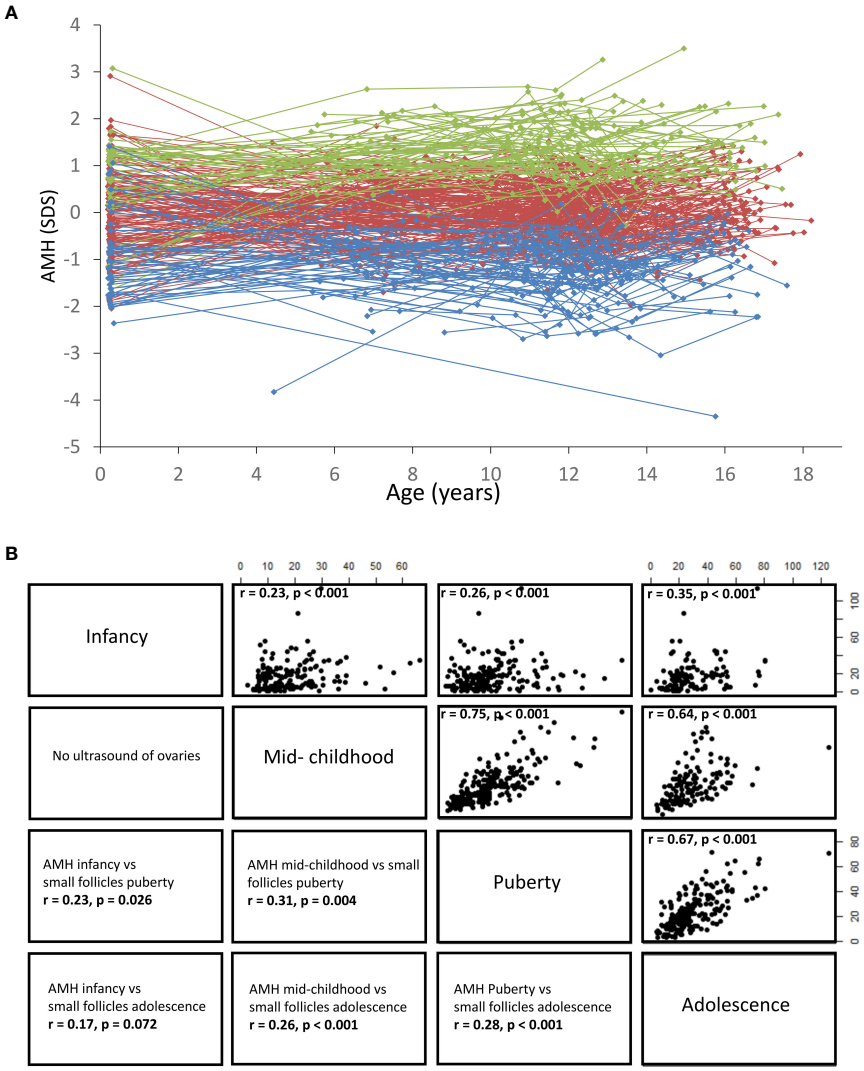

FIGURE 13
(A) Serum AMH concentrations shown as standard deviation scores according to age. Dots indicate individual values and longitudinal courses are connected by lines. All girls were divided into AMH quintiles (5 groups), based on the individual mean SD scores. Blue: 1st quintile, red: 2nd,3rd,4th quintile, green: 5th quintile. **(B)** Right side: Correlations (Spearman´s Rho, r value) between serum AMH concentrations (pmol/L) in infancy, mid-childhood, puberty and adolescence, all p < 0.001. Left side: Correlations (Spearman´s Rho, r value) between serum AMH concentrations and the number of small follicles (<4mm) assessed by transabdominal ultrasound. Figure based on data from (25).

plays a prognostic role (114, 115). To date, there are no reports on achieved pregnancies (or live births) in patients with TS after auto transplantation of ovarian tissue.

In prepubertal girls, harvesting of ovarian tissue usually includes laparoscopic retrieval of one of the ovaries inducing a small risk of bleeding and infection. If pregnancy cannot be achieved after auto transplantation, cryopreservation may induce false hope and later psychosocial harm (116). Apart from these ethical issues, the removal of one ovary may potentially cause even earlier loss of valuable ovarian function. Importantly, hidden nests of viable 46,XX oocytes with the potential of future fertilization may get lost.

Taking these considerations into account, we have designed a national protocol offering selected girls and adolescents with TS ovarian cryopreservation; The Danish Turner Cryopreservation (DANTE) Study (start of inclusion planned in 2023). Ideally, only patients with sufficient numbers of primordial follicles who in the future will experience POI before time of desired pregnancy will benefit from this intervention. In The DANTE Study, all Turner patients (2-18 years) are invited to participate, see Flow-diagram (Figure 14). The patient is initially screened for ovarian activity including Tanner staging by physical examination, assessment of circulating concentrations of reproductive hormones (e.g. AMH, FSH, LH, Inhibin B, estradiol), and transabdominal ultrasound of the ovaries to assess the number of antral follicles. If ovarian activity is very low (e.g. AMH < 2SD) or undetectable, the patient is not offered cryopreservation. Prepubertal girls will be followed longitudinally until POI can be confirmed at time of expected puberty.

In case of ovarian activity, the patient and her family receive information at a visit where both the pediatrician and a gynecologist participate. At this meeting we inform of expected fertility potential with and without ovarian cryopreservation based on current knowledge. Based on the initial screening, we will discuss the

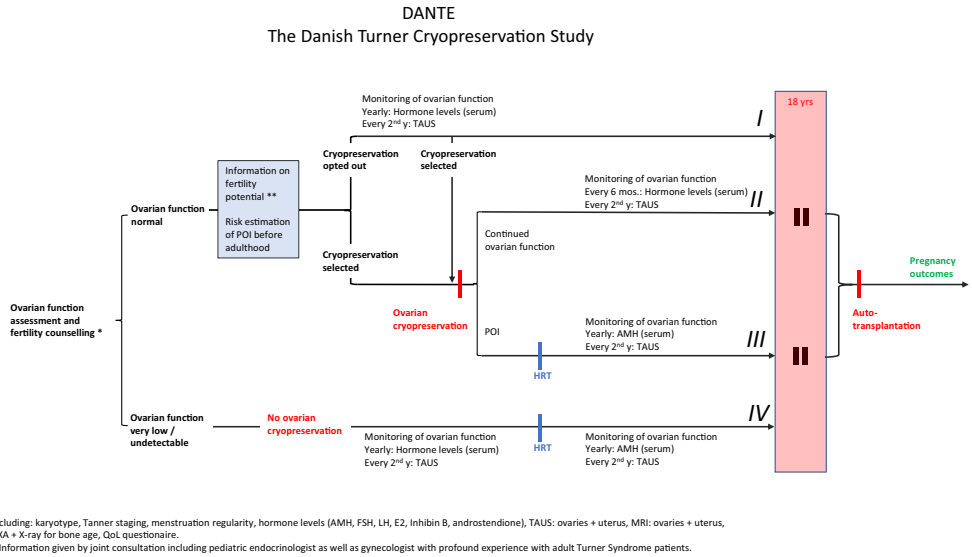

FIGURE 14
Study design of The Danish Turner Cryopreservation Study (DANTE). All Danish Turner Syndrome patients 2-18 years will be invited to participate. From an initial evaluation of ovarian function (Karyotype, Tanner staging, menstrual cycle regularity, circulating levels of reproductive hormones, transabdominal ultrasound and MRI of the ovaries) we will evaluate if ovarian function is normal or very low/absent. The patient will only be offered ovarian cryopreservation if parameters of ovarian function are within age-matched reference ranges. Based on thorough information concerning fertility potential including estimation of risk of POI before adulthood, the patient will decide for cryopreservation. The patient can opt out cryopreservation and monitor ovarian function closely (I) to select cryopreservation at a later stage if ovarian function declines (II and III). If initial evaluation of ovarian function reveals very low or no ovarian activity, the patients will not be offered cryopreservation (IV). POI (premature ovarian insufficiency): Prepubertal girls: AMH < -2SD and FSH > 2SD, transabdominal ultrasound: streak gonads or antral follicle count (AFC) < 10th percentile (57, 64). Post/ peripubertal: No spontaneous puberty, pubertal progression stopped or markedly delayed (117), or primary/secondary amenorrhea. Furthermore: AMH, E2 and inhibin B < -2SD, FSH > 2SD, streak ovaries and AFC < 10th percentile (57, 64).

chances of remaining ovarian function in adult life without intervention, if we expect to find enough follicles by cryopreservation, details about the procedure of future auto transplantation, the success rates in other groups of patients, and we will describe alternative methods of establishing a family (oocyte donation, adoption).

As an alternative strategy for fertility preservation in adolescents and young adults with TS, oocyte vitrification after ovarian stimulation could be considered (118–120). The first live birth after vitrification of oocytes in a woman with TS was recently reported (121).

Transition clinic

During the past 20 years, we have established joint clinics for adolescent patients in our tertiary center between pediatric endocrinologists and gynecologists as well as adult endocrinologists, as also recommended in the international guideline (122). We have seen nearly 600 patients in these joint transition clinics. Patients with TS are primarily transferred to the gynecological department after adolescence. If they suffer from hypothyroidism or other endocrine conditions, they are also transferred to the department of endocrinology. The pediatrician and the gynecologist/endocrinologist see the patients and their families at a joint consultation in familiar surroundings at the pediatric department one or more times before the age of 18 years. The content of the joint visit is highly individual. Usual topics include e.g. treatment of menstrual irregularities, information on hormone replacement therapy (HRT) including dose and treatment, contraception, sexually transmitted diseases, HPV vaccine, and fertility options. This is also an opportunity to evaluate transabdominal ultrasound of the internal genitalia with special focus on uterine growth by estradiol treatment. The patients are informed about what to expect after the transfer from pediatric to adult follow up. Many adolescents have reservations concerning gynecological examinations, and the transition clinic is an opportunity to stress that this is not a mandatory part of consultations at the gynecological department. We experience that the patients are better prepared and more confident to change to an adult setting, reducing the risk of drop out after referral. However, also the pediatricians and colleagues at the adult departments benefit mutually professionally and scientifically from these joint consultations facilitating sharing of knowledge in rare endocrine disorders, updates on guidelines from other disciplines, novel and emerging treatment options, new evidence, organization of departments, and inspiration to research projects bridging adolescents and young adult patients.

Summary

Girls with TS are at increased risk of premature ovarian insufficiency. Many of these patients are diagnosed in mid-childhood, but due to central inhibition of the HPG axis, it is difficult to evaluate ovarian activity in girls prior to pubertal onset.

Studies of ovarian morphology and reproductive hormones in healthy girls support that AMH is produced by granulosa cells surrounding small ovarian follicles. Even prior to pubertal onset, these follicles are continuously recruited from the pool of primordial follicles independently of gonadotropin-stimulation.

Circulating levels of AMH are predictive of the reproductive lifespan in healthy adult women. Our findings strongly indicate that the inter-individual variation of AMH in girls is indicative of the number of remaining primordial follicles – an important outcome in epidemiological research evaluating factors affecting prenatal establishment of the primordial follicle pool. Despite strong evidence of AMH as a quantitative marker of ovarian follicles, AMH does not predict the specific age at menopause for a given woman, nor is low AMH associated with reduced fecundability in young healthy women.

Marked inter-individual variation but little intra-individual variation of AMH in girls both reflects and predicts the number of small antral follicles. Thus, girls maintain their relative level of ovarian activity from follicles growing independently from FSH stimulation through infancy, childhood, puberty and into adolescence. Limited longitudinal data suggests AMH as a unique predictor of premature ovarian insufficiency in TS patients at risk of accelerated loss of follicles. AMH is therefore a key parameter when counseling patients and their families about future ovarian function. The karyotype of the patient as well as consecutive assessment of circulating levels of inhibin B and FSH may add to the predictive value of ovarian function of a given patient. This information is essential when considering whether the patient could benefit from ovarian cryopreservation.

Thus, the clinical use of AMH has been expanded from a marker of testicular tissue in rare DSD patients to a marker and predictor of ovarian activity used at a daily basis in pediatric endocrinology.

Author contributions

CH: deciding the topic for the review, drafting the manuscript. MF, GM, TM, LC, CG, MV, AJ, AP: revision of draft. KM: deciding the topic for the review, revision of draft. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

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EDITED BY
Rodolfo A. Rey,
Hospital de Niños Ricardo Gutiérrez, Argentina

REVIEWED BY
Agnieszka Zachurzok,
Medical University of Silesia, Poland
Anastasia Ibba,
Binaghi Hospital, Italy

*CORRESPONDENCE
Ewa Błaszczyk
ewa_p2@vp.pl

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Components of the metabolic syndrome in girls with Turner syndrome treated with growth hormone in a long term prospective study

Ewa Błaszczyk[1]*, Anna-Mariia Shulhai[2], Joanna Gieburowska[1], Kamil Barański[3] and Aneta Monika Gawlik[1]

[1]Department of Pediatrics and Pediatric Endocrinology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland, [2]Department of Pediatrics N°2, Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine, [3]Department of Epidemiology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

Background: Components of the metabolic syndrome are more common in patients with Turner syndrome (TS) than in the general population. Long-term growth hormone (GH) treatment also affects the parameters of carbohydrate metabolism. Therefore, all these factors should be monitored in girls with TS.

Objective: To assess the occurrence of metabolic syndrome components in TS girls before GH treatment and to monitor changes in metabolic parameters throughout GH therapy.

Patients and method: 89 TS patients were enrolled in the study. Clinical and laboratory data after the 1st (V1), 3rd (V3), 5th (V5) and 10th (V10) year of GH therapy was available respectively in 60, 76, 50 and 22 patients. The patients' biochemical phenotypes were determined by glucose 0', 120', insulin 0', 120', HOMA-IR, Ins/Glu ratio, HDL-cholesterol and triglycerides (TG) concentration.

Results: Obesity was found during V0 in 7.9% of patients,V1 - 5%, V3 - 3.9%, V5 - 2%, V10 – 0%. No patient met diagnostic criteria for diabetes. A significant increase in the basal plasma glucose 0' was found in the first five years of therapy (pV0-V1 < 0.001; pV0-V3 = 0.006; pV0-V5 < 0.001). V10 glucose 120' values were significantly lower than at the onset of GH treatment (pV0-V10 = 0.046). The serum insulin 0' and 120' concentrations as well as insulin resistance increased during treatment. No statistically significant differences in serum TG and HDL-cholesterol levels during GH therapy were found.

Conclusion: The development of insulin resistance and carbohydrate metabolism impairment have the greatest manifestations during GH therapy in girls with TS. Monitoring the basic parameters of carbohydrate-lipid metabolism in girls with TS seems particularly important.

KEYWORDS

turner syndrome, obesity, metabolic syndrome, growth hormone therapy, glucose homeostasis, insulin resistance/hyperinsulinemia, lipids

Introduction

The metabolic syndrome consists of many interrelated metabolic disorders including insulin resistance, glucose intolerance, dyslipidemia, visceral obesity and hypertension (1). These components of the metabolic syndrome have been described not only in adults but also in children (2, 3). The metabolic syndrome components occur more frequently in the population with Turner Syndrome (TS), and are detectable in childhood (4–6). Women with TS are more frequently obese with a central distribution of adiposity (7). A higher waist circumference and a thicker layer of subcutaneous adipose tissue are observed in girls with TS (8). Women with TS present a 2-times higher risk of type 2 diabetes and a 4.5-times higher risk of impaired glucose tolerance compared to the general population (9). Higher levels of triglycerides and total cholesterol are also found in adolescent patients with TS (10). Obesity leads to the development of cardiovascular diseases (CVD) and, in consequence, to increased mortality (11), but also another components of the metabolic syndrome, such as hypertension, dyslipidemia and type 2 diabetes, have been identified as significant determinants of CVD (12, 13). Heart defects (mainly aortic coarctation and bicuspid aortic valve) are more common in Turner syndrome (4, 14–16) and patients with TS are at a higher risk of hypertension, especially in the presence of heart defects, but also without concomitant congenital heart disease (4) (17). All these factors cause shorter life expectancy in the TS group (18): adult TS patients have a 4 to 5-fold increased rate of premature mortality connected with complications of congenital heart disease and premature coronary artery disease (19). In light of the above, all potential risks for the development of CVD in girls with TS should be monitored. Furthermore, growth hormone (GH) treatment, used in most TS patients, seems to play a role in metabolic disorders. Although heart imaging tests reported normal left ventricular morphology and function during GH treatment (20) and found no harmful effect of GH on aortic diameter in TS girls (21), some studies suggest GH treatment in supersubstituted doses in TS patients may induce a reduction of insulin sensitivity (22). Therefore, the influence of GH treatment on the risk of CVD should also be researched.

The aim of our study was to assess the occurrence of metabolic syndrome components in TS girls before and during GH treatment. Changes in metabolic parameters during subsequent years of GH therapy were monitored, especially the effect of GH treatment on insulin secretion and insulin sensitivity (7, 23). An additional aim was to examine the influence of puberty and the type of karyotype on the occurrence of components of the metabolic syndrome.

Subjects and methods

Our prospective study encompassed patients with TS, confirmed by karyotyping with routine G-banding (according to the recommendations of the American College of Medical Genetics), who started rGH therapy between 2003 and 2019 at the Department of Pediatrics and Pediatric Endocrinology with a dose of 47–66 μg/kg/day. Patients were qualified for the study at different ages, depending on the time of TS diagnosis and of qualification for GH treatment according to the Polish Drug Program. Some patients completed their GH therapy during the study, whilst others continue treatment beyond the end of data collection.

The criteria for inclusion into the study group were: TS, age from 3 to 18 years old, attending therapy at our center, and lack of coexisting diseases that could temporarily affect test results. Exclusion criteria were lack of consent of the legal guardian and/or the patient to participate in the study and/or taking medications that could temporarily affect the measured and laboratory parameters.

In the years 2003-2019, a total of 103 girls diagnosed with TS were qualified for GH therapy, of whom 89 were enrolled in the study. Each girl was examined and had laboratory tests before starting rGH treatment, and then clinical and biochemical parameters were monitored every 3-6 months according to the protocol until the end of GH therapy. At each visit, the patients were reminded of the importance of a healthy lifestyle. Data before the start of GH therapy (V0) was available for all participants. Clinical and laboratory data after the 1st(V1), 3rd (V3), 5th (V5) and 10th (V10) year of GH therapy was available respectively for 60, 76, 50 and 22 patients.

Clinical phenotype of study participants

The detailed anthropometrical analysis was based on weight and height measurements, along with body mass index (BMI) calculation, using the standard formula of weight (kg) divided by height (m) squared. Weight was measured with a Seca scale with a precision of 100 g, and height with a Harpenden stadiometer with a graduation of 0.1 cm. BMI was assessed by percentile curves. A BMI above the 97th percentile was classified as obesity, whilst a BMI between the 90th and 97th percentile as overweight based on the BMI chart developed by Institute of Mother and Child for healthy girls (https://imid.med.pl/pl/do-pobrania). Height was expressed as standardized values (height standard deviation score - hSDS) and was calculated using the following formula: hSDS = child's height – height for 50 pc/0 5 ∗ height 50 pc – height 3 pc. Based on the age, sex, BMI, and the appropriate reference standard, the BMI Z-score was calculated using the international (International Obesity Task Force; IOTF) body mass index (BMI) cut-offs (24).

The measurements of waist and hips circumference were obtained in an upright position, midway between the lowest rib margin and the iliac crest, and at the widest point of the hips, respectively, to the nearest 0.1 cm using an inelastic tape. The waist-to-height ratio (WHtR) and waist-hip ratio (WHR) were calculated by dividing both values. In the age group from 3 years to 11 years of age, waist circumference was determined using percentile charts according to the Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS (IDEFICS) study (3), in the older patients – according to the International Diabetes Federation (IDF) definition (25).

The Tanner staging was used for puberty assessment (26). Blood pressure was measured with an automated oscillometric device and some of the patients also had 24-hour blood pressure monitoring before the start of therapy – cut-offs for hypertension were established according to Blood Pressure Values Park's Pediatric Cardiology for Practitioners (https://doctorlib.info/cardiology/).

Body composition was determined in some patients using TANITA MC-980.

Biochemical phenotype of study participants

Morning fasting venous blood samples were collected to measure the concentrations of total cholesterol (TCh), HDL cholesterol (HDL-C) and triglycerides (TG). TCh, HDL-C and TG levels were analyzed enzymatically (Beckman Coulter, Brea, CA). An oral glucose load test of 1.75 g/kg was performed, with the determination of glucose and insulin levels at two-time points: 0' and 120'. An enzymatic test (hexokinase method) was used for the quantitative determination of glucose (Beckman Coulter). Insulin was determined using a chemiluminescence immunoassay on an IMMULITE 2000 analyzer. HOMA of insulin resistance (HOMA-IR) (fasting glucose [mmol/L] × fasting insulin [mIU/L]/22.5) were calculated as indices of IR. Fasting insulin [mIU/L]-to-glucose [mg/dL] ratio indices of the IR - the quotient of insulin concentration to fasting glucose >0.3 was also considered an IR marker (27). The concentration of TG, HDL-C, HOMA index and fasting glucose in children aged 3 to 11 years were qualified according to the IDEFICS study (3); in older girls, IDF percentile charts were used (25).

TSH (thyroid-stimulating hormone), free thyroxine (fT4) and IGF-1 (insulin-like growth factor 1) were also determined. Serum concentrations of fT4 and TSH were measured with a chemiluminescent immunometric assay (IMMULITE 2000 Free T4 and IMMULITE 2000 Third Generation TSH, respectively; Siemens) and IGF concentration was measured by solid-phase enzyme-labeled chemiluminescent immunometric assays (IMMULITE, DPC).

Statistical analysis

Data processing and statistical analyses were performed using «STATISTICA®v.13» (license № JPZ804I382130ARCN10J) software and Microsoft Excel (2013). The distribution of quantitative values was evaluated according to the Shapiro-Wilk test.

Considering the non-normal distribution of quantitative characteristics, their descriptive statistics were carried out in the median (Me), lower (Lq) and upper (Uq) quartiles. Comparative analysis of quantitative indicators in three or more groups was calculated using the Kruskal–Wallis H test, which was considered significant at $p<0.05$. Comparisons of groups were performed using Mann–Whitney U test with Bonferroni correction to assess the level of statistical significance.

The Pearson Chi-square test ($\chi^{2)}$ was used to analyze the frequency tables. An odds ratio (OR) and its 95% confidence interval (CI) were calculated to evaluate the impact of GH therapy duration on the development of metabolic syndrome criteria.

To evaluate the possible associations between the studied data, the Spearman correlation coefficients were determined. The significance of the differences between the values was considered significant at p ¾0.05.

Results

A total of 89 girls diagnosed with Turner syndrome were examined, of whom 46 (51.7%) had a 45,X karyotype and 43 (48.3%) had other karyotype subtypes (non-45,X). Based on patient history, 21 (23.6%) girls were born small for gestational age (SGA) and 68 (76.4%) were appropriate for gestational age (AGA). A history of parental obesity was reported in 28 (31.2%).

We analyzed the changes in anthropometric parameters, hormonal status, and carbohydrate and lipid metabolism parameters throughout the 10 years of GH therapy.

Analyzed metabolic syndrome components

The changes in anthropometric parameters and carbohydrate and lipid metabolism parameters are presented in Table 1. The body mass index increased during GH therapy and the BMI z-score was significantly higher than the BMI Z-score obtained during the first visit (BMI V0). After 5 years of GH therapy, BMI z-score (V5) was 2.8-fold higher compared to BMI V0, and after 10 years — it was 4.7-fold higher. An increased amount of high normal BMI (75-90 percentile) was found, especially after 5 and 10 years of GH therapy, from 20.4% in V0 to 27.7% in V10. According to BMI Z-score the number of overweight girls was as follows: V0 - 19.1%, V1 - 16.67%, V3 -18.42%, V5 -26%, and V10 -18.18%. The detailed data on the incidence of obesity and abdominal obesity are presented in Table 2.

A decrease in the WHtR index with a significant difference after 5 years (V5), and WHR with a significant difference after 10 years (V10) was observed.

After 1 year (V1) of GH in girls with TS, a significant increase in the basal plasma glucose 0' and glucose 120' was observed. With long-term GH therapy (10 years, V10) the glucose 0' was not statistically different compared to V0 data, whilst glucose 120' was significantly lower than at the onset of GH treatment (V0). The serum insulin 0' and 120' concentrations increased during treatment (Table 1). Glucose metabolism disturbances were found - impaired glucose tolerance (IGT) was recognized in V0 – in 3 patients (3.37%), V1 - 4 (6.67%), V3- 6 (7.89%), V5 - 4 (8%), V10- 2 (9.09%), the detailed data on the incidence of impaired fasting glucose (IFG) are presented in Table 2. No patient met diagnostic criteria for diabetes. The increase between V0 and V10 in prediabetes was insignificant (p=0.32).

HOMA-IR index and Insulin/Glucose ratio increased during GH therapy (Table 1).

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TABLE 1 Dynamics of the metabolic syndrome criteria changes depending on the duration of the GH therapy.

MS criteria	Duration of growth hormone therapy					Kruskal-Wallis Test (H) p-value	p-value Mann-Whitney U-test
	therapy onset V0 n=89	1 year / V1 n=60	3 years / V3 n=76	5 years / V5 n=50	10 years / V10 n=22		
Age, years	10.44 (6.06;12.57)	11.15 (6.73;12.67	12.54 (8.63; 14.35)	12.68 (9.15;15.28)	12.29 (10.39; 15.27)	H=23.69 p=0.000	$p_{V0\text{-}V1}$= 0.380 $p_{V0\text{-}V3}$=0.004* $p_{V0\text{-}V5}$=0.000* $p_{V0\text{-}V10}$=0.002*
BMI, kg/m^2	17.1 (15.5; 20.0)	16.8 (15.5; 20.0)	18.0 (16.2; 21.2)	18.6 (16.6; 21.1)	19.0 (17.0; 22.7)	H=13.08 p=0.023*	$p_{V0\text{-}V1}$=0.933 $p_{V0\text{-}V3}$=0.073 $p_{V0\text{-}V5}$=0.015* $p_{V0\text{-}V10}$=0.061
BMI z-score	0.13 (- 0.77; 1.11)	0.21 (- 0.67; 0.91)	0.34 (- 0.49; 1.05)	0.37 (- 0.28; 1.09)	0.62 (- 0.14; 1.01)	H=11.07 p=0.035*	$P_{V0\text{-}V1}$=0.320 $P_{V0\text{-}V3}$=0.078 $P_{V0\text{-}V5}$=0.043* $P_{V0\text{-}V10}$=0.023*
WC, cm	60.3 (56.8; 64.8)	59.8 (56.6; 69.0)	59.8 (55.9; 67.0)	62.8 (54.8; 66.3)	65.3 (59.50; 72.3)	H=2.507 p=0.775	$P_{V0\text{-}V1}$=0.820 $P_{V0\text{-}V3}$=0.840 $P_{V0\text{-}V5}$=0.653 $P_{V0\text{-}V10}$=0.093
WHtR	0.47 (0.44; 0.54)	0.48 (0.43; 0.52)	0.46 (0.43; 0.51)	0.45 (0.42; 0.48)	0.46 (0.42; 0.48)	H=9.63 p=0.048*	$p_{V0\text{-}V1}$=0.553 $p_{V0\text{-}V3}$=0.297 $p_{V0\text{-}V5}$=0.042* $p_{V0\text{-}V10}$=0.265
WHR	0.84 (0.82; 0.86)	0.88 (0.84; 0.91)	0.84 (0.80; 0.89)	0.83 (0.80; 0.87)	0.80 (0.77; 0.85)	H=10.55 p=0.032*	$p_{V0\text{-}V1}$=0.049* $p_{V0\text{-}V3}$=0.708 $p_{V0\text{-}V5}$=0.286 $p_{V0\text{-}V10}$=0.044*
hSDS	-2.98 (-3.77; -2.30)	-2.54 (-3.41; -2.04)	-2.34 (-3.33; -1.58)	-1.75 (-2.32; -1.38)	-1.66 (-2.39; -1.21)	H=30.75 P=0.000	$p_{V0\text{-}V1}$<0.001* $p_{V0\text{-}V3}$<0.001* $p_{V0\text{-}V5}$<0.001* $p_{V0\text{-}V10}$<0.001*
Glucose mg/dl	85.0 (78.0; 90.0)	91.0 (84.0; 98.0)	88.50(81.0; 95.8)	90.5 (84.5; 97.3)	85.5 (81.5; 95.5)	H=18.93 p=0.001*	$p_{V0\text{-}V1}$<0.001* $p_{V0\text{-}V3}$=0.006* $p_{V0\text{-}V5}$<0.001* $p_{V0\text{-}V10}$=0.105
Glucose 120', mg/dl	110.0 (89.0; 122.0)	119.4 (99.5; 139.8)	111.0 (100.0; 130.0)	107.9 (98.5; 126.0)	102.5 (93.3; 125.0)	H=10.15 p=0.043*	$p_{V0\text{-}V1}$=0.034* $p_{V0\text{-}V3}$=0.237 $p_{V0\text{-}V5}$=0.292 $p_{V0\text{-}V10}$=0.046*
Insulin 0', mIU/L	6.00 (3.0; 9.0)	14.5 (6.5; 78.5)	10.3 (7.0; 14.6)	13.0 (9.0; 17.1)	13.2 (9.8; 19.4)	H=13.04 p=0.023*	$p_{V0\text{-}V1}$=0.035* $p_{V0\text{-}V3}$=0.011* $p_{V0\text{-}V5}$=0.007* $p_{V0\text{-}V10}$=0.008*
Insulin 120', mIU/L	34.8 (14.0; 59.0)	37.0 (22.3; 74.8)	56.9 (35.0; 70.7)	59.0 (32.1; 84.2)	68.3 (46.1; 121.0)	H=17.809 p=0.001*	$p_{V0\text{-}V1}$=0.177 $p_{V0\text{-}V3}$=0.001* $p_{V0\text{-}V5}$=0.001* $p_{V0\text{-}V10}$=0.001*
HOMA-IR	0.94 (0.42; 1.44)	2.22 (1.36; 3.59)	2.03 (1.30; 3.07)	2.36 (1.61; 3.10)	2.44 (1.87; 3.08)	H=24.691 p=0.000*	$p_{V0\text{-}V1}$<0.001* $p_{V0\text{-}V3}$<0.001* $p_{V0\text{-}V5}$=0.001* $p_{V0\text{-}V10}$<0.001*
Ins/Glu	0.05 (0.02; 0.07)	0.12 (0.06; 0.15)	0.12 (0.08; 0.16)	0.11 (0.08; 0.17)	0.16 (0.11; 0.21)	H=45.01 p=0.000*	$p_{V0\text{-}V1}$=0.003* $p_{V0\text{-}V3}$<0.001* $p_{V0\text{-}V5}$<0.001* $p_{V0\text{-}V10}$<0.001*
HDL-C, mg/dl	58.7 (50.3; 68.8)	57.8 (52.5; 64.6)	55.9 (47.4; 63.9)	56.3 (52.0; 64.4)	64.7 (56.1; 71.3)	H=7.82 p=0.166	$p_{V0\text{-}V1}$=0.605 $p_{V0\text{-}V3}$=0.146

(Continued)

TABLE 1 Continued

MS criteria	Duration of growth hormone therapy					Kruskal-Wallis Test (H) p-value	p-value Mann-Whitney U-test
	therapy onset V0 n=89	1 year / V1 n=60	3 years / V3 n=76	5 years / V5 n=50	10 years / V10 n=22		
							$p_{V0\text{-}V5}$=0.443 $p_{V0\text{-}V10}$=0.174
TG, mg/dl	74.0 (59.3; 103.0)	89.1 (66.0; 104.0)	81.50(59.0; 109.8)	93.4 (66.0; 108.0)	84.5 (65.8; 110.0)	H=5.25 p=0.386	$p_{1V0\text{-}V1}$=0.222 $p_{V0\text{-}V3}$=0.247 $p_{V0\text{-}V5}$=0.066 $p_{V0\text{-}V10}$=0.158

BMI, body mass index; BMI z-score, body mass index z-score; WC, waist circumference; WHR, ratio of waist circumference to hip circumference; WHtR, ratio of waist circumference to height; hSDS, height standard deviation; HOMA-IR, index of insulin resistance; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.
*p<0.05 — statistically significant difference.

We did not observe a statistically significant difference in the serum triglycerides and HDL-C level during GH therapy (Table 1).

All the changes in metabolic parameters during GH therapy are presented in Figure 1.

Comparison of 45,X vs. non-45,X patients revealed higher basal glucose levels (p<0.05) and HOMA-IR (p < 0.0001) after one year of GH therapy in patients with monosomy. GH therapy longer than one year caused higher glycemia (p=0.0054) and HOMA-IR (p=0.0027) in patients with non-45,X karyotype. For the remaining metabolic parameters, no differences were found between 45,X and non-45,X patients during GH therapy (p>0.05).

Blood pressure was monitored throughout the 10 years of observation. Arterial hypertension was diagnosed in 9 (10.1%) girls before GH therapy, in 8 (13,3%) at V1, in 10 (13,1%) at V3, in 4 (8%) at V5 and in 2 (9%) at V10.

Spontaneous and induced puberty during observation was connected with an increased frequency of hyperglycemia and a higher HOMA index (p<0.05). No effect on the remaining metabolic parameters was found.

Additional analyzed parameters

During GH therapy HbA1c dynamic decrease was observed after 10 years of GH therapy (V10) (Table 3).

Although the TCh levels were statistically lower at V3, the long-term duration of therapy (V5 and V10) had no effect on TCh levels (Table 3).

IGF-1 levels increased 2.3, 2.6, 3.1 and 2.8 times at V1, V3, V5 and V10 respectively (Table 3). Long-term GH therapy did not cause changes in thyroid hormones fT4 and TSH (Table 3). Table 2 presents a comparative assessment of the frequency of metabolic syndrome criteria depending on the duration of GH therapy. The components of metabolic syndrome, such as hyperglycemia

TABLE 2 Comparative assessment of the metabolic syndrome criteria frequency depending on the duration of growth hormone therapy [n (%)].

Criteria	Obesity		Abdominal obesity		Impaired fasting glucose		Low HDL-C		Hipertriglicerydemia		Hypertension	
	n	%	n	%	n	%	n	%	n	%	n	%
V0	7	7.87	4	22.2	7	8.5	3	3.8	9	11.5	9	10.1
V1	3	5.0	3	15.0	16	45.7	2	3.9	9	16.3	8	13.3
V3	3	3.9	4	13.3	19	28.4	11	14.5	19	24.7	10	13.2
V5	1	2.0	2	9.1	10	21.7	1	2.1	8	17.1	4	8.0
V10	0	0	0	0	2	9.1	2	3.7	4	18.2	2	9.1
	Pearson's chi-squared $\chi2$ totally in the BMI group and intergroup comparisons p>0.05		Pearson's chi-squared $\chi2$ totally in the WC group intergroup comparisons p>0.05		Pearson's chi-squared $\chi2$ totally in the group of GLUC p<0,001; Pearson's chi-squared $\chi2$ in the V1 and V0 p=0.000; V3 and V0 p=0.002, V5 and V0 p=0.034; V10 and V0 p>0.05.		Pearson's chi-squared $\chi2$ totally in the group of HDL p<0,05; Pearson's chi-squared $\chi2$ in the compare V3 and V0 p=0.021. In other cases p>0.05.		Pearson's chi-squared $\chi2$ totally in the group of TG p>0,05; Pearson's chi-squared $\chi2$ in the compare V3 and V0 p=0.033. In other cases p>0.05.		Pearson's chi-squared $\chi2$ totally in the group and intergroup comparisons p>0.05	

n, number of patients; %, percent of patients, HDL-C, high-density lipoprotein cholesterol; Pearson's chi-squared test ($\chi2$). *p<0.05 — a statistically significant difference.
BMI Z-score for obesity was calculated using the international body mass index (BMI) cut-offs (IOTF). For the remaining parameters, IDF and IDEFICS criteria were used depending on the age of the patient.

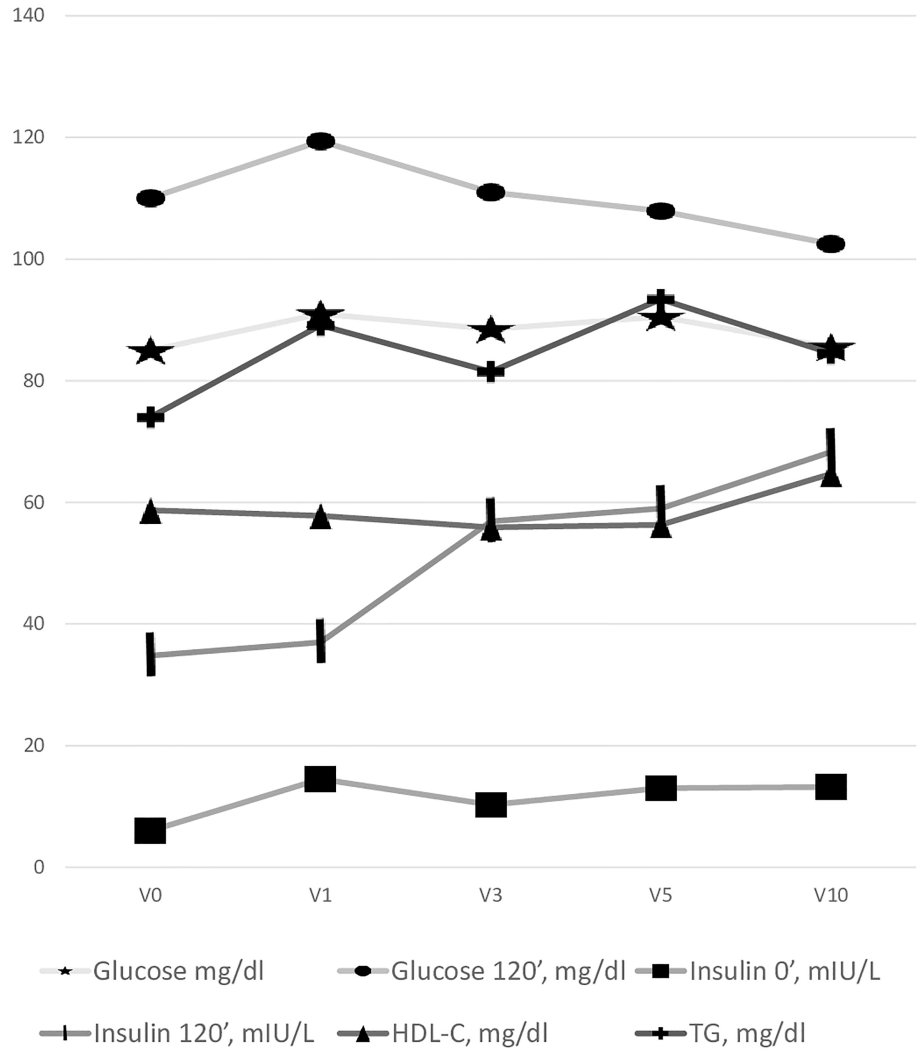

FIGURE 1
Changes in metabolic parameters during GH therapy.

(p<0.001), low HDL-C(p<0.05) and hypertriglyceridemia (p=0.033) were more frequently observed during the first three years of GH therapy. There were no statistically significant differences in the frequency of metabolic syndrome components between patients with IGF-1 SDS over +2 SDS and less than +2 SDS (p > 0.05).

The results of correlation analysis of the relationship between metabolic syndrome criteria, birth weight, and carbohydrate and lipid metabolism parameters are shown in Table 4.

The effect of GH therapy duration on the development of metabolic syndrome criteria in girls with TS and the relative odds ratio (ORs) are shown in Table 5.

Discussion

Many studies show that individual components of the metabolic syndrome are more common in girls with TS than in healthy girls. Our study showed that the percentage of overweight patients during observation was between 16.6-26%, whilst obesity was found in: 0 - 7.87% patients. A decrease in the WHtR index with a significant difference after 5 years and WHR with a significant difference after 10 years was observed. IGT was diagnosed in 3.37% of patients at the beginning of GH therapy and increased gradually throughout the treatment to 9.09%. No patient met diagnostic criteria for diabetes. A significant increase in the basal plasma glucose 0' was observed during the first five years of therapy. At 10-years of GH therapy, no differences were found in mean basal glucose compared to the onset of GH therapy. What is more, glucose 120' values were even significantly lower than at the onset of GH treatment. The serum insulin 0' and 120' concentrations increased during treatment. Both insulin resistance ratios increased during GH therapy. However, we did not observe a statistically significant difference in the serum triglycerides and HDL-C level during GH therapy. hSDS was correlated with TG concentration in the first three years of therapy.

Girls with TS tend to have greater waist circumference and subcutaneous adipose tissue than controls (8). The higher frequency of obesity is clearly visible in adult patients with TS (28, 29). Obesity

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TABLE 3 Dynamics of hormonal status and metabolic parameters in children with Turner syndrome depending on GH therapy duration [n=89; Me (Lq; Uq)].

Variables	Duration of growth hormone therapy					Kruskal-Wallis Test (H) p-value	p-value Mann-Whitney U-test
	therapy onset V0 n=89	1 year / V1 n=60	3 years / V3 n=76	5 years / V5 n=50	10 years / V10 n=22		
HbA1c, %	5.30 (5.00; 5.50)	5.25 (5.00; 5.50)	5.20 (5.00; 5.50)	5.30 (5.00; 5.50)	5.05 (5.00; 5.20)	H=26.12 p=0.000*	p_{V0-V1}=0.750 p_{V0-V3}=0.693 p_{V0-V5}=0.775 p_{V0-V10}=0.000*
TCh, mg/dL	175.0 (158.5; 201.0)	167.0 (152.0; 185.0)	161.0 (145.0; 176.0)	163.0 (145.0; 191.0)	166.50 (158.25; 186.50)	H=11,37 p=0.044*	p_{V0-V1}=0.138 p_{V0-V3}=0.001* p_{V0-V5}=0.060 p_{V0-V10}=0.187
fT4, ng/dL	1.39 (1.23; 1.60)	1.33 (1.10; 1.49)	1.36 (1.24; 1.51)	1.37 (1.21; 1.49)	1.40 (1.22; 1.55)	H=10.24 p=0.069	p_{V0-V1}=0.036* p_{V0-V3}=0.143 p_{V0-V5}=0.157 p_{V0-V10}=0.432
TSH, uIU/ml	2.88 (1.96; 3.49)	2.72 (2.00; 3.94)	2.67 (2.03; 3.45)	2.31 (1.86; 3.46)	2.41 (1.79; 3.49)	H=2.76 p=0.736	p_{V0-V1}=0.824 p_{V0-V3}=0.616 p_{V0-V5}=0.375 p_{V0-V10}=0.438
IGF-1, ng/ml	182.2 (84.3; 226.5)	416.0 (227.0; 550.0)	471.0 (320.5; 628.0)	558.5 (365.3; 684.5)	507.5 (332.5; 716.3)	H=77.01 p=0.000*	p_{V0-V1}<0,001* p_{V0-V3}<0,001* p_{V0-V5}<0,001* p_{V0-V10}<0,001*
IGF-1 SDS	-0.61(-1.05; -0.08)	1.64 (1.11; 2.19)	1.88 (1.34; 2.53)	2.17 (1.29; 3.14)	1.66 (0.73; 2.78)	H=86.09 p=0.001*	p_{V0-V1}=0.000* p_{V0-V3}=0.000* p_{V0-V5}=0.000* p_{V0-V10}=0.000*

HbA1c, glycated hemoglobin; TCh, total cholesterol; fT4, free T4; TSH, thyroid-stimulating hormone; IGF-1, insulin-like growth factor 1.
*p<0.05 — statistically significant difference.

in the TS group is known to increase the risk of hypertension (30) and leads to changes in lipoproteins profile (28), therefore routine screening of weight/BMI is recommended at every visit and at any age. Some studies have even reported a beneficial effect of rGH therapy on body composition (31, 32). In our study, an increase of BMI was observed, mainly due to the shift of the BMI value to the higher range of norms, while the number of overweight patients didn't change. The weight management is the most important health intervention at annual visits in adult TS patients (4).Although our study did not show a significant increase in waist circumference or waist-hip ratio during GH therapy, the measurements seem to be an important and easy to perform preventive test.

In relation to carbohydrate metabolism, Caprio et al. found reduced insulin sensitivity in TS girls in comparison with age-matched controls (5). It has also been shown that impaired glucose homeostasis in TS is not secondary to obesity or hypogonadism, but is due to haploinsufficiency for X-chromosome genes that impair beta-cell function and predispose to diabetes mellitus in TS (33). In our study we also found higher glycemia levels in girls with monosomy, unlike in other karyotypes (including mosaic karyotypes), before therapy. What is more, after one year of GH therapy, basal glucose levels increased significantly and HOMA index was determined in patients with 45,X karyotype. Interestingly, long-term GH therapy caused higher levels of glycemia and insulin resistance index in patients with non-45, X karyotype.

In our study we found a significant increase in the basal glucose concentration during the first years of GH therapy. Nevertheless, after 10 years of treatment there was no difference in basal glucose concentration compared to the onset of therapy. What is more, based on the glucose 120' values, it was significantly lower than at the onset of GH treatment. The serum insulin level during GH therapy increased with the increasing duration of treatment and it was accompanied by insulin resistance, which was confirmed by a rise in the HOMA-IR and Insulin/Glucose ratio. The decrease of insulin sensitivity can be a consequence of GH therapy or the effect of puberty induction (31, 34). Our results confirmed this observation as in our patients puberty was connected with a higher HOMA index.

Radetti et al. indicated that GH treatment in TS girls does not significantly increase the prevalence of impaired glucose tolerance or type 2 diabetes mellitus but decreases insulin sensitivity (35). Bannink, who investigated the effects of GH on carbohydrate homeostasis several years after discontinuing GH therapy, concluded that insulin sensitivity remained lower, whilst beta-cell function and fasting insulin levels remained higher than before treatment (36). However, most research on the impact of GH on

TABLE 4 Correlation between antropometrical parameters with metabolic syndrome criteria (Spearman correlation).

Variables		Birth Weight, g	BMI z-score	hSDS	WHtR first visit	WHR first visit	Bodyfat, %	MM, kg	TBW, kg	FFM, kg	glucose 0, mg/dl	insulin, 0 UI/l	HOMA-IR	TCh, mg/dl	HDL-C, md/dl	TG, md/dl
Birth Weight, g	r	1														
	P															
BMI z-score	r	0.280	1													
	p	0.010*														
hSDS	r	321	0.354	1												
	p	,003*	0.001*													
WHtR first visit	r	-0.231	0.804	-0.061	1											
	p	0.357	0.000*	0.809												
WHR first visit	r	0.082	0.468	0.007	0.577	1										
	p	0.748	0.050*	0.978	0.012*											
Body fat, %	r	0.172	0.780	0.136	0.844	0.511	1									
	p	0.456	0.000*	0.558	0.000*	0.034*										
MM, kg	r	0.387	0.397	0.405	-0.155	-0.400	0.292	1								
	p	0.043*	0.075	0.068	0.612	0.176	0.199									
TBW, kg	r	-0.005	0.431	0.318	0.234	-0.573	0.402	0.781	1							
	p	0.983	0.045*	0.184	0.465	0.046*	0.088	0.000*								
FFM, kg	r	0.232	0.298	0.316	-0.186	-0.499	0.157	1.000	0.783	1						
	p	0.338	0.216	0.187	0.563	0.049*	0.520	0.000*	0.000*							
Glucose 0, mg/dl	r	-0.008	0.076	-0.138	-0.396	-0.468	-0.087	-0.086	-0.165	-0.119	1					
	p	0.947	0.520	0.239	0.115	0.048*	0.716	0.720	0.514	0.639						
Insulin 0, UI/I	r	0.010	0.323	0.156	0.182	-0.144	0.522	0.597	0.572	0.339	0.014	1				
	p	0.932	0.006*	0.242	0.471	0.569	0.018*	0.005*	0.013*	0.169	0.917					
HOMA-IR	r	0.329	0.359	0.270	-0.313	-0.244	0.007	0.397	0.110	0.182	-0.022	0.351	1			
	p	0.010*	0.003*	0.036*	0.221	0.345	0.975	0.042*	0.665	0.469	0.864	0.028*				
TCh, mg/dl	r	-0.211	0.215	-0.001	0.478	0.299	0.390	0.083	0.364	0.113	0.032	0.298	-0.094	1		
	p	0.091	0.086	0.996	0.044*	0.299	0.169	0.778	0.222	0.714	0.806	0.034*	0.516			

(Continued)

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TABLE 4 Continued

Variables		Birth Weight, g	BMI z-score	hSDS	WHtR first visit	WHR first visit	Bodyfat, %	MM, kg	TBW, kg	FFM, kg	glucose 0, mg/dl	insulin, 0 UI/l	HOMA-IR	TCh, mg/dl	HDL-C, md/dl	TG, md/dl
HDL-C, md/dl	r	-0.143	-0.040	-0.118	-0.445	-0.116	-0.068	-0.123	-0.240	-0.209	0.220	0.042	0.248	0.037	1	
	p	0.258	0.753	0.354	0.111	0.693	0.818	0.675	0.429	0.493	0.091	0.770	0.083	0.773		
TG, md/dl	r	-0.021	0.271	0.573	0.264	0.080	0.008	0.114	0.479	0.231	0.141	0.047	0.098	0.428	-0.280	1
	p	0.872	0.036*	0.031*	0.363	0.786	0.978	0.698	0.038*	0.448	0.282	0.742	0.500	0.000*	0.025*	

hSdS – height standard deviation; BMI, body mass index; BMI z-score, body mass index z-score; WC, waist circumference; WHR, ratio of waist circumference to hip circumference, WHtR, ratio of waist circumference to height;; HOMA-IR, index of insulin resistance; TCh, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

carbohydrates proves that insulin secretion disorders return to their pretreatment values after discontinuation of GH treatment (35, 37, 38). In one study, even reduced abdominal adiposity and significantly better glucose tolerance in GH-treated vs -untreated girls with TS was found (31).

The pathophysiology of glucose homeostasis in TS is still not fully understood (39). A beta cell dysfunction and glucose homeostasis disorders in TS may be a consequence of haploinsufficiency of X chromosome genes (33). What is more, the development of genetic analysis suggests haploinsufficiency is not only one genetic mechanism for glucose disorders and epigenetic changes also should be taken into consideration (40). In TS reduced sensitivity of cells to insulin is observed with higher probability of hyperinsulinemia and glucose intolerance (5) as well as earlier development of type 2 diabetes (41). However, it cannot be forgotten that one of the adverse effects of treatment with GH can be carbohydrate intolerance or even diabetes (42). The more that doses recommended in TS are suprasubstitutional. GH therapy is started with a recommended dose of 45 to 50 µg/kg/day increasing to 68 µg/kg/day (4). It would be difficult to gather a group of patients with TS who would not be treated with GH to have a control group and unequivocally answer the question about the impact of GH influence on carbohydrate metabolism in TS. However, taking into account the literature data, it seems that carbohydrate disorders are included in TS, and GH is an additional risk factor for their development. Thus monitoring the parameters of carbohydrate metabolism seems to be justified at every stage of life in patients with TS. Gravholt et al. recommend lifelong annual measurement of HbA1c with or without fasting plasma glucose starting at the age of 10 years (4).

Similar to carbohydrate-impaired tolerance, lipid disorders are also more common in TS. Hypercholesterolemia was reported in 37–50% of women with TS, which is higher than in the general population (4, 28, 43). Pirgon et al. indicated that TS girls have a higher concentration of total cholesterol and triglycerides, and the concentration of LDL cholesterol correlates with the thickness of the intima-media complex, being a risk factor for atherosclerosis in girls with TS (28). In our data, we did not observe any statistically significant differences in the serum triglycerides and HDL-C levels during GH therapy or in long term observation of TCh concentrations. However, in one study endothelial function was better in GH-treated compared with GH-untreated TS girls, so GH may protect endothelial function in TS having a protective effect for cardiovascular system (44). Bannink et al. found that total cholesterol, LDL and HDL increased further after GH treatment discontinuation compared to 6 months after GH, resulting in higher TCh, but also higher HDL levels. The atherogenic index remained constant, though lower than in controls, hence GH therapy in girls with TS seems to have beneficial effects on serum lipids, visible even a few years after discontinuation of GH therapy (36). Accordingly, Gravholt et al. recommend annual lipid monitoring from the age of 18 years in the presence of at least one cardiovascular risk factor in TS patients (hypertension, overweight, tobacco, diabetes, and physical inactivity) (4).

Clinical guidelines suggest monitoring of IGF-1 levels and adapting GH dose in case of high IGF-1 levels (4). Although one

TABLE 5 The effect of GH therapy duration on the metabolic syndrome criteria development in children with Turner syndrome, OR (95% CI).

Criteria	BMI		WC		Glucose		HDL-C		TG		BP	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
V0	–	-	–	–	–	–	–	-	-	–	-	–
V1	0.38	0.07-1.91	0.68	0.11-3.24	9.02	3.25-25.04	1.02	0.16-6.33	1.50	0.55-4.06	1.36	0.49-3.77
V3	0.95	0.30-2.97	0.53	0.12- 2.48	4.24	1.65-10.84	4.23	1.31-15.82	2.46	1.03-5.86	1.34	0.51-3.50
V5	0.46	0.09- 2.32	0.35	0.05- 2.18	2.97	1.04-8.45	0.54	0.05-5.38	1.57	0.56-4.40	0.77	0.22-2.65
V10	0.53	0.06-4.55	0.42	0.16-2.07	1.07	0.20-5.56	0.92	0.21-3.69	1.70	0.47-6.17	0.89	0.18-4.44

OR, odds ratio; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHR, ratio of waist circumference to hip circumference; WHtR, ratio of waist circumference to height; HOMA-IR, index of insulin resistance; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

study conducted in GH-treated children showed a safety profile of GH, the incidence of type 2 diabetes was increased in relation to the general population, especially in patients with a risk factors of diabetes such as patients with TS (45). In our study we found no statistically significant differences in the frequency of metabolic syndrome components between patients with IGF-1 over +2 SDS and less than +2 SDS. So, presence of higher IGF-1 in our patient did not influence increase numbers of metabolic syndrome components.

As hypertension is common in TS, blood pressure should be measured at each visit. 24-h ambulatory monitoring is also helpful in detection of nocturnal or stress-related hypertensive episodes. What is important, treatment with GH had no evident effect on blood pressure (46, 47).

Components of the metabolic syndrome are present in the population of children and adolescents with TS, but they are also increasingly observed in the general population of children. However, so far there has been no consensus on the diagnosis of metabolic disorders in children (48). Several definitions of pediatric metabolic syndrome are known. Cook et al. adopted the adult metabolic syndrome definitions to create criteria that were applied to the 12-19 age group (49). However, the study group including teenagers makes it difficult to expand Cook's definition to younger children. The IDF definition includes younger children since metabolic syndrome can be diagnosed in children as young as 10 years old (25). The definition of Viner also covers younger children (aged 2-18); however, only obese children were included in that study (50). IDEFICS created definitions of metabolic syndrome using percentile charts for all parameters. The IDEFICS percentile charts include children from 3 to11 years (3), hence they can be useful in the youngest age group. Based on the above information, in our study we used the IDEFICS criteria as the most appropriate for the age group 3-11 years. Due to the lack of IDEFICS percentile charts for the older age group, we used the criteria proposed by the IDF for older girls.

The strengths of our study include: a large number of patients with a rare disease, enrolled in a prospective study at one center, according to one scheme. A limitation of our study is the different age of inclusion for GH therapy.

In conclusion, monitoring the basic parameters of carbohydrate-lipid metabolism in TS seems crucial in preventing the development of cardiovascular diseases at all stages of life, from childhood through adolescence to adulthood. Our study showed that of all metabolic syndrome criteria in girls with TS, the development of insulin resistance and carbohydrate metabolism impairement have the greatest manifestations during GH therapy in girls with TS. In our clinic, anthropometric parameters are monitored at each visit, and the parameters of carbohydrate and lipid metabolism are determined at regular intervals. In the presence of abnormal results, patients and parents are encouraged to follow a healthy lifestyle and consult a dietitian. Other research from our center shows that medical follow-up in the transition phase is still inadequate (51), so improvement in transitional health care and caring for adult TS patients is warranted through better awareness raising of patients and their parents from the onset of diagnosis, and through promoting healthy behaviors as early as in adolescence.

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 The Ethics Committee of the Medical University of Silesia (resolution number NN-013-96/I/03 and KNW/0022/KB1/162/15/16). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

EB and AG designed the study, prepared the database, wrote the manuscript, monitor patients. A-MS analyzed the patient database

and wrote the manuscript. JG monitor the patients and collected samples for biochemical analysis. KB helped during statistical analysis. 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.

Publisher's note

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EDITED BY
Aneta Monika Gawlik,
Medical University of Silesia, Poland

REVIEWED BY
Giorgio Radetti,
Ospedale di Bolzano, Italy
Giulia Rodari,
IRCCS Ca 'Granda Foundation Maggiore Policlinico Hospital, Italy

*CORRESPONDENCE
Kerstin Albertsson-Wikland
kerstin.albertsson.wikland@gu.se

†These authors have contributed equally to this work and share first authorship

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Normalization of puberty and adult height in girls with Turner syndrome: results of the Swedish Growth Hormone trials initiating transition into adulthood

Berit Kriström[1†], Carina Ankarberg-Lindgren[2†], Marie-Louise Barrenäs[3], Karl Olof Nilsson[4] and Kerstin Albertsson-Wikland[3*]

[1]Department of Clinical Science, Pediatrics, Umeå University, Umeå, Sweden, [2]Department of Pediatrics, Institute of Clinical Sciences, Göteborg Pediatric Growth Research Center, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [3]Department of Physiology/Endocrinology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [4]Department of Clinical Sciences, University Hospital Malmö, Lund University, Malmö, Sweden

Objective: To study the impact of GH dose and age at GH start in girls with Turner syndrome (TS), aiming for normal height and age at pubertal onset (PO) and at adult height (AH). However, age at diagnosis will limit treatment possibilities.

Methods: National multicenter investigator-initiated studies (TNR 87-052-01 and TNR 88-072) in girls with TS, age 3–16 years at GH start during year 1987–1998, with AH in 2003–2011. Of the 144 prepubertal girls with TS, 132 girls were followed to AH (intention to treat), while 43 girls reduced dose or stopped treatment prematurely, making n=89 for Per Protocol population. Age at GH start was 3–9 years (young; n=79) or 9–16 years (old; n=53). Treatment given were recombinant human (rh)GH (Genotropin® Kabi Peptide Hormones, Sweden) 33 or 67 µg/kg/day, oral ethinyl-estradiol (2/3) or transdermal 17β-estradiol (1/3), and, after age 11 years, mostly oxandrolone. Gain in $height_{SDS}$, AH_{SDS}, and age at PO and at AH were evaluated.

Results: At GH start, $height_{SDS}$ was −2.8 (versus non-TS girls) for all subgroups and mean age for young was 5.7 years and that of old was 11.6 years. There was a clear dose–response in both young and old TS girls; the mean difference was (95%CI) 0.66 (−0.91 to −0.26) and 0.57 (−1.0 to −0.13), respectively. The prepubertal $gain_{SDS}$ (1.3–2.1) was partly lost during puberty (−0.4 to −2.1). Age/$height_{SDS}$ at PO ranged from 13 years/−0.42 for $GH_{67young}$ to 15.2 years/−1.47 for GH_{33old}. At AH, GH_{67old} group became tallest (17.2 years; 159.9 cm; −1.27 SDS; total $gain_{SDS}$, 1.55) compared to $GH_{67young}$ group being least delayed (16.1 years; 157.1 cm; −1.73 SDS; total, 1.08). The shortest was the $GH_{33young}$ group (17.3 years; 153.7 cm: −2.28 SDS; total $gain_{SDS}$, 0.53), and the most delayed was the GH_{33old} group, (18.5 years; 156.5 cm; −1.82 SDS; total $gain_{SDS}$, 0.98).

Conclusion: For both young and old TS girls, there was a GH-dose growth response, and for the young, there was less delayed age at PO and at AH. All four groups reached an AH within normal range, despite partly losing the prepubertal gain during puberty. Depending on age at diagnosis, low age at start with higher GH dose resulted in greater prepubertal height gain, permitting estrogen to start earlier at normal age and attaining normal AH at normal age, favoring physiological treatment and possibly also bone health, hearing, uterine growth and fertility, psychosocial wellbeing during adolescence, and the transition to adulthood.

KEYWORDS

adult height, estrogen, growth hormone, height gain, prepubertal growth, pubertal growth, timing of puberty, Turner syndrome

1 Introduction

The main characteristics of Turner syndrome (TS), a sex-chromosomal pathology syndrome, are short stature and gonadal failure. The etiology of short stature is multifactorial and may depend partly on haplo-insufficiency of the *SHOX* gene (1, 2). Growth for girls with TS is reduced in all phases of growth, being fetal–infancy, childhood, and puberty, compared to non-TS girls growth models (3, 4) and references (5, 6). As a result, TS is associated with an adult height (AH) approximately 20 cm below that predicted based on mid-parental height (MPH). Research from adult women with TS has shown that having an AH within the normal range and undergoing puberty at a normal time relative to their peers are of great importance for quality of life (QoL) (7, 8). For this reason, growth-promoting and puberty-inducing therapies have been used in girls with TS for many years.

Androgens were used as growth promoter even before recombinant human (rh) growth hormone (GH) was approved in 1986 (9). In Sweden by 1986, girls with TS aged 9 years or older were included in national investigator-initiated multicenter trials of rhGH (33 μg/kg/day) and estrogen replacement therapy (ERT) (10). These studies were in 1987 expanded to include three investigator-initiated trials of rhGH treatment also including girls from 3 years of age (4). From 1991, the rhGH dose was increased in line with the upper dose used in subsequent GH-trials for optimizing pubertal growth in GH deficiency (GHD) and for other possible indications (67 μg/kg/day) (9, 11–13). Such a temporal study design allowed the assessment of dose–response (14). It should also be possible to evaluate both growth response and GH responsiveness during the first year of treatment (15) and with individualized growth prediction models (16, 17) by using the one for TS (18).

What have we learned about growth in girls with TS since the first trials were initiated more than 35 years ago? Growth-promoting treatment with rhGH at different doses and with or without ERT and androgens in girls with TS has shown varying results both in short-term studies (14, 19, 20) and long-term studies to AH (21) undertaken in different countries (10, 22–31) and from worldwide outcome databases as pioneering KIGS (32–36).

Today, there is consensus that, if age at diagnosis permits, puberty should be induced at a "normal age" in girls with TS (37). Due to the known physiological effects of estrogen on most tissues and organ systems, puberty should ideally be induced using a dosage regimen that mimics the increasing serum estradiol levels observed in normal female puberty (38, 39), and girls should subsequently be maintained on a dose that results in serum levels appropriate for young adult women, which is twice that recommended for postmenopausal women (40–42), considering uterine size with fertility aspects (43, 44), future bone (45), and cardiovascular health (46, 47).

The goal for girls with TS in the 1980s, as it is now, was to normalize height during childhood so that puberty could be induced within the normal age range, allowing for normal tempo of the progress of subsequent pubertal growth and maturation and the attainment of a normal AH within the expected normal age range. To achieve this, it is necessary to balance projected height with age at puberty induction, using incremental doses of ERT. This is further complicated by delays in the age at diagnosis of TS, which can limit the time available for growth-promoting treatment. The key question addressed in the present analysis was what difference would there be when starting treatment in the young girl compared with that in an older girl using rhGH, oxandrolone, and estrogen? This is now investigated in the present study, using long-term data from the above-mentioned investigator-initiated multicenter trials conducted in Sweden between 1987 and 2011, which followed 132 girls with TS to AH, partly presented at the fifth TS meeting (14) and at ESPE (48).

2 Material and methods

2.1 Ethics

The trials (TRN 87-052-01 and TRN 88-072) were approved by the Ethical Committees of Sweden at the university hospitals in Lund (221/87), Göteborg, Linköping, Umeå, Uppsala (all 76-88), and the Karolinska Institute (88–40). National approval for the last

part of the study was received from Lund (400/91). Informed consent was obtained from the parents and from the girls if they were old enough to understand.

2.2 Study subjects

2.2.1 Inclusion criteria

The study included girls with TS (karyotype from at least 25–30 lymphocytes) aged between 3.0 and 15.9 years and with height standard deviation scores (SDSs) below −1 compared with the reference population of healthy Swedish girls born approximately the same years (6). All Turner karyotypes were accepted, except for those associated with a Y-chromosome cell line. Girls with normalized thyroid function and moderately well-treated epilepsy were accepted.

2.2.2 Exclusion criteria

Girls were excluded if they (1) had severe diseases including coeliac disease; (2) had been previously treated with GH, sex hormones, or corticosteroids; (3) had a Turner karyotype containing a Y-chromosome cell line; or (4) they were unable to attend.

2.2.3 Intention to treat population

Between 1987 and 1998, 144 girls with TS who were naive to GH treatment were enrolled in Swedish multicenter studies in accordance with the criteria above; they received GH at a dose of 33 or 67 μg/kg/day (see study design), either alone or alongside treatment with oxandrolone and estrogen, depending on their age. Girls starting GH treatment from 1987 to 1991 received the 33 μg/kg/day dose, while girls starting treatment from 1991 onwards received 67 μg/kg/day. At GH treatment start, 88 girls were 3–9 years old, and 56 were over 9 years of age; 67 girls received 33 μg/kg/day, and 65 received 67 μg/kg/day doses. Overall, 132 of the 144 girls were followed to AH and constituted the intention-to-treat (ITT) population (Figure 1). Girls were assigned to subgroups based on age and GH dose. After enrollment, there were 12 girls not followed to AH, two were excluded owing to missing data and five owing to the initiation of GnRH-analogue treatment in TS girls with spontaneous puberty. Five developed other diseases after enrollment: two with coeliac, two with epilepsy, and one with high blood sugar, however, not diabetes mellitus.

2.2.4 Per protocol population

The per protocol (PP) population constituted 89 girls (Figure 1). Protocol violations occurred in 43 girls; these included significant GH dose reduction or premature GH treatment cessation when the girl was satisfied with her height (n=35 with height velocity more than 2.5 cm/year) or before AH was attained (n=8 with another 2.2–7.9 cm until AH) (Figure 1).

2.2.5 Pretreatment characteristics

The pretreatment characteristics of the four ITT study subgroups are shown for the ITT population in Table 1 for the PP population and in Supplementary Tables S1 and S2. Karyotype 45,X was found in 73 girls, mosaicism in 5 girls, and structural abnormalities in 53 girls.

2.2.6 Laboratory analyses for diagnostic and safety purposes

GH–IGF axis. Before the start of treatment, a 24hGH profile was obtained with 30 min sampling, and serum IGF-1, IGFBP1, and IGFBP3 were determined. IGF-1 and IGFBP3 were determined again after 10, 30, and 40 days, and thereafter at the yearly visit (51). GHBP was also analyzed (52).

TSH–thyroxine axis. TRH, TSH, free thyroxin fT4, and fT3 were determined before GH treatment start and every 6 months thereafter: TSH, fT4, and fT3 (53)

Gonadal axis. FSH, LH, DHEAS, androstenedione, estradiol, and SHBG were analyzed before GH treatment start and yearly

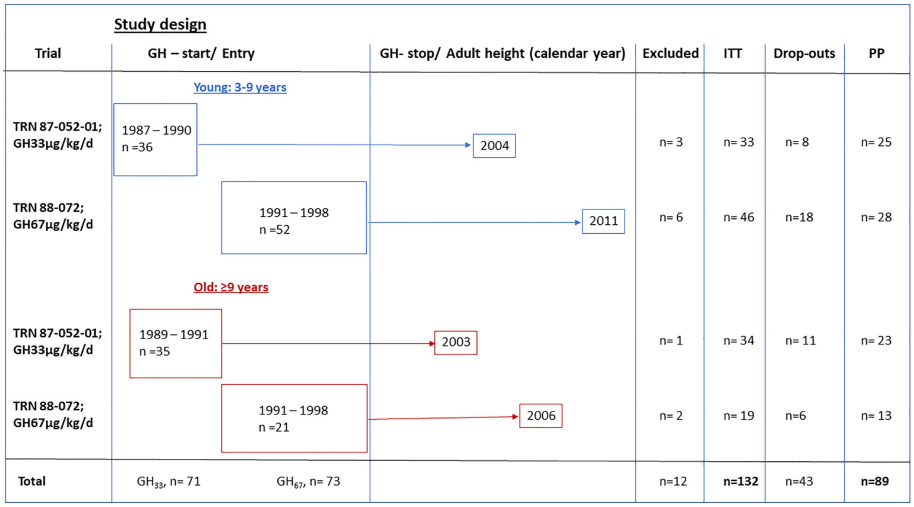

FIGURE 1
Study design for the GH trials of the temporal designed study. Calendar year at enrollment and GHstart and GHstop and adult height indicated, as excluded from intention to treat (ITT) and dropouts from per protocol (PP) population.

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TABLE 1A Pre-treatment characteristics for the young groups 3—9 years at GH start in the ITT population versus normal population (6, 49, 50).

Variables	Dose 33 µg (n=33)	Dose 67 µg (n=46)	p-value	Difference between groups Mean (95% CI)	Effect size
Karyotype 45X	25 (80.6%)	25 (54.3%)			
Mosaic	1 (3.2%)	2 (4.3%)			
Other	5 (16.1%)	19 (41.3%)	0.051		
Karyotype missing	2	0			
At birth					
GA (weeks)	38.6 (1.8) 39 (34; 42) n=33	38.5 (2.0) 39 (33; 42) n=46	0.86	0.095 (−0.784; 0.981)	0.049
Length (SDS)	−2.53 (1.41) −2.68 (−5; 0.9) n=33	−1.86 (1.17) −1.78 (−4.34; 0.23) n=46	0.023	−0.670 (−1.242; −0.098)	0.525
Weight (SDS)	−1.61 (1.41) −1.39 (−5.11; 0.37) n=33	−1.12 (1.22) −1.1 (−3.92; 1.7) n=46	0.11	−0.489 (−1.080; 0.104)	0.375
Mother height (SDS)	−0.23 (0.94) −0.18 (-2.73; 1.3) n=33	0.01 (1.21) -0.10 (-2.09; 2.63) n=46	0.35	−0.232 (−0.734; 0.265)	0.210
Father height (SDS)	−0.21 (0.90) −0.26 (-1.79; 1.70) n=33	−0.14 (1.10) 0.10 (-3.33; 1.74) n=46	0.77	−0.069 (−0.529; 0.395)	0.067
MidParental Height (SDS)	−0.27 (0.90) −0.44 (-2.05; 1.86) n=33	−0.08 (1.10) -0.19 (-3.36; 2.07) n=46	0.42	−0.187 (−0.650; 0.276)	0.183
DiffMPH (SDS)	−2.26 (1.71) −2.17 (−5.79; 1.48) n=33	−1.78 (1.32) −1.83 (−4; 0.93) n=46	0.15	−0.483 (−1.162; 0.191)	0.323
Pre-treatment height velocity, before GHstart (cm/year)	5.49 (1.73) 5.4 (3.14; 12.3) n=33	5.54 (1.45) 5.47 (2.51; 9.48) n=45	0.89	−0.053 (−0.774; 0.657)	0.034

For categorical variables n (%) is presented.
For continuous variables, mean (SD)/median (Min; Max)/n= is presented.
For comparison between groups, chi-square exact test was used for non-ordered categorical variables, and the Fisher's non-parametric permutation test was used for continuous variables. The confidence interval for the mean difference between groups is based on Fisher's non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
GA, gestational age; GH, growth hormone; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SD score between the height of the girl and the heights of her parents.

thereafter. LHRH was analyzed yearly from 5 years of age onwards; FSH forms were explored (54).

Glucose metabolism. Intravenous glucose tolerance test, b-glucose, HbA1c, and urine test for protein and glucose were conducted at treatment start and yearly thereafter. HbA1c was analyzed every 6 months, and urine was tested for protein and glucose every 3 months.

Coeliac disease. Gliadin antibody test was performed (55).

Blood status. Hb, LPK, Na, K, urea, and ALP were analyzed every 6 months.

GH antibody analysis. GH antibody analysis was performed.

2.3 Study design

A total of 144 girls were included consecutively and were assigned to four subgroups based on age at diagnosis (3–9 vs. >9–15.9 years) and GH dose (33 vs. 67 µg/kg/day) at treatment start. Before any analysis, data from 12 girls were excluded, 5 due to LHRH agonist treatment because of short stature at age of onset of spontaneous puberty. At GH treatment start, the ITT population included 79 girls aged 3–9 years (young group) and 53 girls aged over 9 years (old group). The 67 girls who started GH treatment before mid-1991 received 33 µg/kg/day dose, and the 65 who started treatment after this point received 67 µg/kg/day dose (Figures 1, 2). The 132 girls were assigned to groups as follows:

- $GH_{33young}$ (n=33): girls were enrolled 1987–1990 (Turner III) and started treatment with 33 µg/kg/day GH aged 3–9 years; the last girl reached AH in 2004.
- GH_{33old} (n=34): girls were enrolled 1989–1991 (Turner IV) and started treatment with 33 µg/kg/day GH aged >9 years; last girl reached AH in 2003.

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TABLE 1B Pre-treatment characteristics for the old groups ≥9 years at GH start in the ITT population, SDS versus Swedish references (6, 49, 50).

Variables	Dose 33 µg (n=34)	Dose 67 µg (n=19)	p-value	Difference between groups Mean (95% CI)	Effect Size
Karyotype 45X	13 (38.2%)	10 (52.6%)			
Mosaic	2 (5.9%)	0 (0.0%)			
Other	19 (55.9%)	9 (47.4%)	0.42		
Missing	0	0			
At birth					
GA (weeks)	39.3 (1.4) 39 (36; 42) n=33	38.2 (1.8) 38 (34; 41) n=17	0.033	1.10 (0.11; 2.00)	0.699
Length (SDS)	−1.98 (1.40) −1.81 (−5.49; 0.97) n=34	−1.81 (1.24) −1.67 (−4.48; 0.31) n=18	0.68	−0.167 (−0.953; 0.606)	0.124
Weight (SDS)	−1.49 (1.08) −1.24 (−4.73; 0.42) n=34	−1.13 (1.08) −0.98 (−3.07; 1.41) n=19	0.25	−0.359 (−0.983; 0.252)	0.332
Mother height (SDS)	−0.27 (0.70) −0.18 (−1.46; 1.09) n=33	−0.04 (0.88) −0.18 (−1.78; 1.73) n=19	0.31	−0.227 (−0.672; 0.223)	0.295
Father height (SDS)	0.03 (0.83) 0.05 (−1.71; 1.82) n=31	0.17 (0.87) 0.14 (−1.79; 1.59) n=19	0.60	−0.135 (−0.624; 0.362)	0.160
MPH (SDS)	−0.16 (0.73) −0.09 (−1.50; 1.23) n=31	0.08 (0.82) 0.00 (−1.55; 1.78) n=19	0.29	−0.241 (−0.691; 0.210)	0.315
DiffMPH (SDS)	−1.89 (1.43) −1.71 (−5.89; 0.96) n=31	−1.98 (1.37) −1.87 (−4.4; 0.69) n=18	0.83	0.091 (−0.750; 0.921)	0.064
Pretreatment height velocity, before GHstart (cm/year)	3.51 (1.05) 3.54 (1.14; 5.32) n=34	3.79 (0.63) 3.9 (2.82; 4.75) n=16	0.33	−0.278 (−0.846; 0.277)	0.297

For categorical variables, n (%) is presented.
For continuous variables, mean (SD)/median (Min; Max)/n= is presented.
For comparison between groups chi-square exact test was used for non-ordered categorical variables, and the Fisher's non-parametric permutation test was used for continuous variables.
The confidence interval for the mean difference between groups is based on Fisher's non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
GA, gestational age; GH, growth hormone; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SD score between the height of the girl and the heights of her parents.

$GH_{67young}$ (n= 46): girls were enrolled 1991–1998 (Turner V) and started treatment with 67 µg/kg/day GH at 3–9 years; last girl reached AH in 2011.

GH_{67old} (n=19): girls were enrolled 1991–1998 (Turner V) and started treatment with 67 µg/kg/day GH aged >9 years; last girl reached AH in 2006.

2.4 Hormonal treatment

2.4.1 GH treatment

RhGH [Genotropin®, Kabi Peptide Hormones (Turner III–IV), Kabi Pharmacia Corp. (Turner V), Stockholm, Sweden] 33 or 67 µg/kg was injected deep subcutaneously every evening (56). Girls on the 67 µg/kg/day dose were started on 33 µg/kg/day; the dose was increased stepwise over a period of 1–6 months to avoid water retention and edema. GH dose was adjusted to body weight every 3 months. GH treatment was stopped when growth velocity fell below 2 cm/year or when the girl was satisfied with her height (n=43).

2.4.2 Oxandrolone treatment

Oxandrolone (Anavar®, Searle Ltd., Chicago, USA) 0.05 mg/kg/day was allowed according to the protocol from 11 years of age (bone age ≥9 years) if growth velocity and/or height criteria were not satisfied despite good compliance according to the investigator's clinical judgment. When initiated during childhood, oxandrolone treatment was used until AH in 54% of the young girls, and among the older girls, 94% had started oxandrolone 1–2 years after GH start, i.e., before the start of estrogen replacement therapy (puberty onset).

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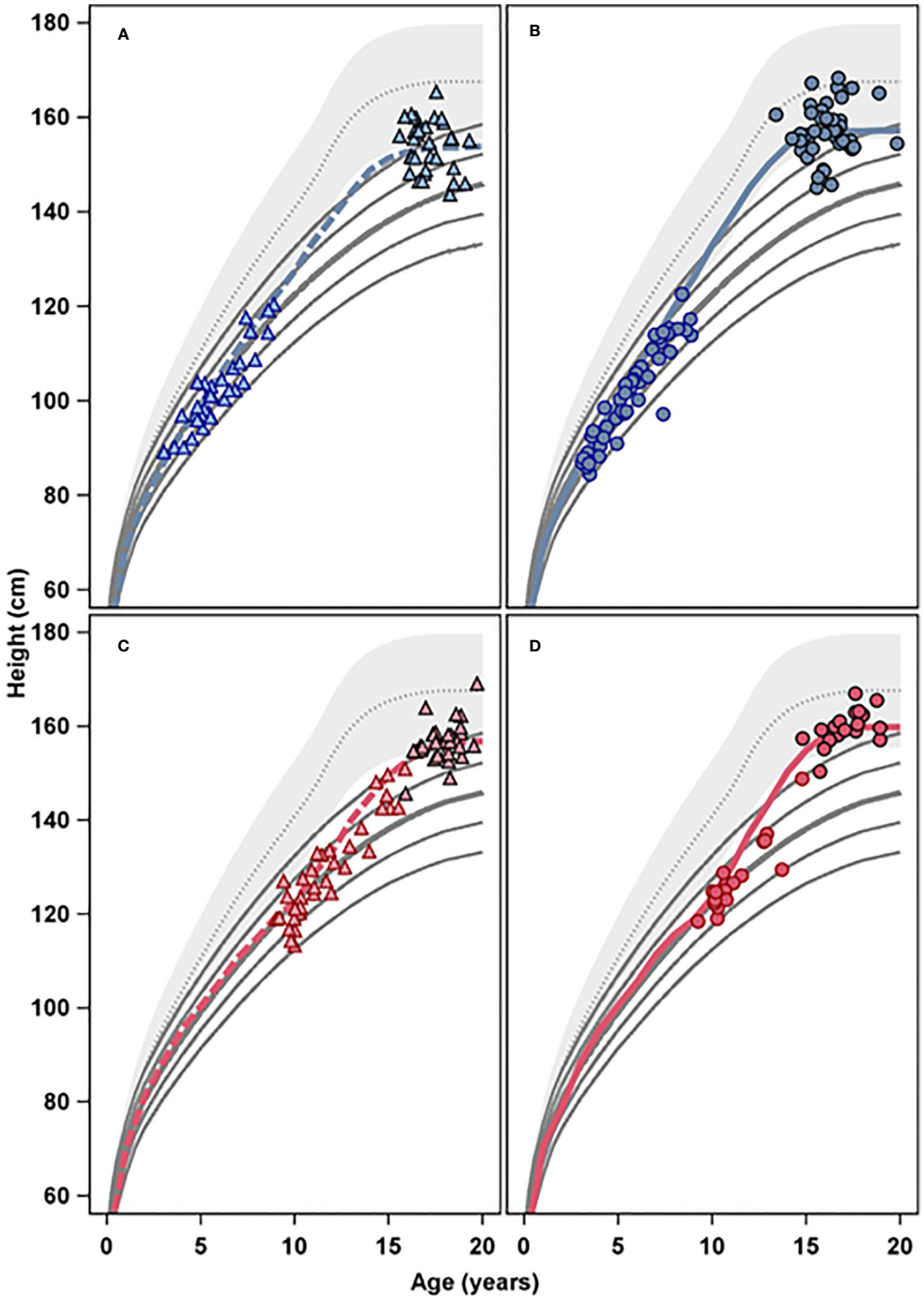

FIGURE 2
The four panels present height (cm) versus age (years) for the four different age and treatment groups, GH $_{33\ young}$ **(A)**, GH $_{67\ young}$ **(B)**, GH $_{33\ old}$ **(C)**, and GH $_{67\ old}$ **(D)**; open symbols represent GH start and filled symbols represents adult height. The colored lines represent mean growth for the four treatment groups, blue for the young groups, and red for the old groups, respectively, in relation to height reference from the healthy girls (gray area) (6) and from girls with Turner Syndrome (solid lines) (5).

2.4.3 Estrogen replacement therapy

2.4.3.1 Oral ethinyl-estradiol

During the 1980s and early 1990s, puberty was initiated using oral ethinyl-estradiol (EE2) (Etivex®, Leo Pharma Corp., Malmö, Sweden). Age at treatment initiation was at the investigator's discretion; the intention was to start treatment with a dose of 25 ng/kg/day at an appropriate annual visit when the patient was close to 13 years of age. For the first year, the dose was increased by 25 ng/kg/day every 3 months; thereafter, there was a yearly dose increment of 100 ng/kg/day. The older girls with gonadal failure, group GH_{33old} and GH_{67old}, started EE2 at 13–14 years of age with the same starting dose. For girls >14 years, wishing for a more rapid pubertal development, a starting EE2 dose of 100 ng/kg/day was allowed with yearly dose increments of 100 ng/kg/day. Gestagen (Medroxi-progesterone, Gestapuran®, Leo Pharma Corp., Malmö, Sweden) was added when EE2 dose reached 300 ng/kg/day.

2.4.3.2 Transdermal 17β-estradiol

In 1997, ERT was changed to transdermal 17β-estradiol for most girls. At that time, starting dose was a 5-µg patch (Estraderm® Serono, Schweiz), and from 2001, this became a 6.25–12.5-µg patch (1/4–1/2 part of the matrix patch Evorel® (=Systen®) 25 µg/24 h; Janssen-Cilag Pharmaceutica N.V, Beerse, Belgium), corresponding to 0.13 ± 0.03 µg/kg bodyweight with application of the piece of patch initially only at nighttime (38). Girls were maintained on the initial dose for 9 months, with the aim of inducing breast development. Thereafter, the dose was increased by 6.25 µg every 6 months until what was then considered to be the adult regimen was reached (a continuous 25-µg patch, changed twice/week). Gestagen was added approximately 2 years after the start of ERT.

2.5 Monitoring

Height (mean out of three measures using a Harpenden stadiometer), sitting height, and weight were measured at baseline and thereafter every 3 months. AH was considered to have been achieved at the time when growth velocity was below 1 cm/year. Pubertal maturation was assessed according to breast development Tanner stage 1–5.

3 Methods

3.1 Growth evaluation

3.1.1 Using references of healthy girls

Birth length and weight were converted to SD scores (SDS) relative to a reference population of ~800,000 healthy newborns born in Sweden from 1990 to 1999 (49). All measurements were corrected for gestational age. Height at start of GH treatment and the last recorded height before puberty (prepubertal height) were converted to SDS using the childhood component (3) applied to the Swedish reference population born in 1974 (6), to calculate gain in $height_{SDS}$ during the prepubertal and pubertal period.

The difference from current $height_{SDS}$ to mid-parental height (MPH) SDS is referred to as $diff_{SDS}$. $Diff_{SDS}$ was calculated at different time points including GH treatment start, the start of puberty, and at AH. MPH_{SDS} was calculated as: (father's $height_{SDS}$ + mother's $height_{SDS}$)/1.61 (50).

AH was measured in centimeters and converted into SDS with heights transferred to "age 18 years for AH" for the reference population, irrespective of actual age at AH (6).

GH efficacy, i.e. height gain, during the childhood growth period (prepuberty), is given in SDS according to the childhood function from the ICP model (3) applied in the used reference (6). Gain in centimeters from GH start until onset of puberty (last recorded prepubertal height) is not included, as it only reflects time from treatment start. Height gain during puberty is calculated in centimeters and by calculating AH_{SDS} minus $height_{SDS}$ at last prepubertal visit. Total gain in $height_{SDS}$ was calculated using AH_{SDS} minus $height_{SDS}$ at GH start.

Duration of puberty was calculated based on the difference between age at the last prepubertal visit or at the visit when ERT was started and the age when AH was attained.

3.1.2 Using reference for girls with Turner syndrome

All lengths and heights were also converted to SDS relative to a reference for girls with TS obtained from data on spontaneous untreated growth in girls with TS in Sweden, Denmark, and the Netherlands (5), and versus the Childhood component of the Turner ICP-growth model, used here for calculating height gain during puberty (4).

3.2 Statistical analyses

Continuous variables were described using the mean, SD, median and range, and categorical variables using n and %.

For comparison between the two groups, Fisher's exact test was used for dichotomous variables, A chi-squared test was used for non-ordered categorical variables, a Mantel–Haenszel chi-squared test was used for ordered categorical variables, and Fisher's non-parametric permutation test for comparison of two means was used for continuous variables. The main results from the comparison between two groups regarding dichotomous and continuous variables were presented as mean difference with 95% confidence interval (CI). For continuous variables, effect size between the two groups was also given. Effect size was defined as mean difference/pooled SD.

A forward stepwise linear regression was used to select independent predictors for each outcome variable. Only those predictors with a univariate relationship with $p<0.1$ to each outcome variable were included as possible predictors. The explained variance (r^2) was calculated for each model, together with beta-coefficient with 95% CI for each predictor, i.e., independent variable.

All tests were two-tailed and conducted at the 5% significance level.

4 Results

4.1 GH-dose dependency of height outcomes

A clear GH-dose response effect was found in girls classified as young or as old at time of GH start, both in the ITT and the PP population (Figures 3, 4, 5). For the ITT population, the progress in $height_{SDS}$ from birth to AH, for three comparisons, is presented: for pre-treatment characteristics, young in Table 1A and old in Table 1B,

A: for comparisons between GH dose within each age group, young, in Tables 2A, 3A and old in Tables 2B, 3B;

B: for comparisons for all girls versus dose, see Supplementary Tables S3A–C, and

C: for comparisons for all girls versus age, Supplementary Tables 4A–C, all versus the Swedish reference for non-TS girls (6).

Corresponding information for the PP population is presented in Supplementary Tables S1, S2, S6, S7.

Auxological data according to a TS reference (5) is presented in Table 4, for the ITT population and in Supplementary Table S5 for the PP population.

Figure 2 show changes in $height_{SDS}$ versus both references.

4.1.1 Prepubertal gain in $height_{SDS}$

The young age group (3–9 years). At the start of treatment, there was no difference in mean $height_{SDS}$ (both −2.8) or mean age (5.8 versus 5.7 years, respectively) between the low- and high-dose groups. At 1 year of treatment (see Table 2A), and at 2 years of treatment, the GH_{33} group was significantly shorter than the GH_{67} group, mean (SD) of −2.04 (0.77) versus −1.48 (0.77), respectively, a difference in $height_{SDS}$ of 0.56 ($p < 0.01$) increased to 0.96 ($p<0.0001$) after 4 years on treatment and was maintained throughout the childhood growth period. Only two girls in the GH_{67} group started Oxandrolone during the first year of GH treatment, and no further girls started this treatment during the second year.

At start of puberty, the $GH_{33young}$ group had reached a $height_{SDS}$ of −1.10 compared with −0.423 for the $GH_{67young}$ group (p=0.0017); mean duration of prepubertal treatment was 8.9 and 7.3 years, respectively. Total prepubertal gain in $height_{SDS}$ from GH start to last prepubertal visit/start of puberty was 1.68 for the $GH_{33young}$ group and 2.36 for the $GH_{67young}$ group (p=0.0002); the mean difference in $height_{SDS}$ was −0.68 (95% CI, −1.008 to −0.363) (Table 2A, Figure 3).

The old age group (>9 years). At start of treatment, there was no difference in mean $height_{SDS}$ (both −2.8) or mean age (11.8 versus 11.2 years, respectively) between the low- and high-dose groups. At 1 year on GH treatment, $height_{SDS}$ was −2.34 for the GH_{33old} group and −1.76 for the GH_{67old} group (p=0.0076). During the first year of treatment, 13 (68%) girls in the GH_{67} group were started on Oxandrolone, while 9 (26%) from the GH_{33} group were started on Oxandrolone during the second year (Table 2B, Figure 3).

At start of puberty, $height_{SDS}$ was −1.47 for the GH_{33old} group versus −0.734 for the GH_{67old} group (p=0.0007). After 3.4 and 2.9 years of GH treatment, respectively, total prepubertal gain in $height_{SDS}$ was 1.33 for the GH_{33old} group versus 2.09 for the GH_{67old} group (p=0.0018); the mean difference between groups was 0.756 (95% CI, −1.219 to −0.282; Table 2B, Figure 3).

Comparisons of GH dose between the age groups. At +1 year on GH 33 μg/kg/day, there was no significant difference in mean dose between the young and old groups: GH_{young} and GH_{old} groups received 35.8 and 33.7 μg/kg/day, respectively (p=0.077); at the last prepubertal visit, doses were 34.8 and 34.0 μg/kg/day, respectively (p=0.31).

For the dose group 67 μg/kg/day, there were also no significant differences in terms of dose between the young and old groups at either time point (comparable values for +1 year were 53.9 and 56.5 μg/kg/day, respectively, and that at last prepubertal visit were 56.8 and 56.2 μg/kg/day, respectively(p=0.39)) (Tables 2A, B).

4.1.2 Pubertal growth

The young age group (3–9 years). A negative change in $height_{SDS}$ from start of puberty to AH was found: −1.18 for the GH_{33} versus −1.29 for the GH_{67} group (p=0.36). When expressed in centimeters, the corresponding values for pubertal growth were 3.25 and 7.67 cm, respectively ($p<0.0001$); the mean difference between groups was −4.42 (95% CI, −6.50 to −2.33) (Table 3A, Figure 3).

The old age group (>9 years). The change in $height_{SDS}$ during puberty for this group was also negative: by −0.351 for the GH_{33} group versus −0.536 for the GH_{67} group (p=0.29); mean difference was 0.15 (95% −0.147 to 0.535). When expressed in centimeters, height gain during puberty was 7.15 and 9.16 cm for the GH_{33} and the GH_{67} group (p=0.25), respectively (Table 3B, Figure 3).

Comparisons of GH dose between the age groups. The calculation of mean dose for the total treatment period was 34.9 vs. 34.6 μg/kg/day for the young and old GH_{33} groups, respectively. The corresponding values for the young and old GH_{67} groups were 55.1 vs. 57.7 μg/kg/day.

4.1.3 Total gain in $height_{SDS}$

The young age group (3–9 years). For the young group of girls, the total gain in $height_{SDS}$ was 0.49 for the GH_{33} group compared with 1.07 for the GH_{67} group (p=0.0006); mean difference was 0.57 (95% CI, −0.905 to −0.257). Total time on GH treatment was 10.2 versus 9.43 years, respectively (p=0.082) (Table 3A, Figure 3).

The old age group (>9 years). For the old group, the total gain in $height_{SDS}$ was 0.98 for the GH_{33} group versus 1.55 for the GH_{67} group (p=0.012); mean difference was 0.57 (95% CI, −1.031 to −0.125). Total time on GH treatment was 5.19 and 5.28 years, respectively (p=0.85) (Table 3B, Figure 3).

Comparisons between and over age groups. When the total gain in $height_{SDS}$ from GH start to AH in the PP population was compared for the two dose groups, independent of age at GH start, the GH_{33} groups experienced a significantly lower gain in $height_{SDS}$ (0.893) compared to the GH_{67} groups (1.23) (p=0.020); mean difference was 0.391 (95% CI, −0.714 to −0.064) (Supplementary Table S5).

4.1.4 Adult height

The young age group (3–9 years). Adult $height_{SDS}$ was −2.28 for the $GH_{33young}$ versus −1.71 for the $GH_{67young}$ group (p=0.0083); mean difference was 0.57 (95% CI, −0.990 to −0.148). When expressed in centimeters, AH was 153.7 versus 157.2 cm in the

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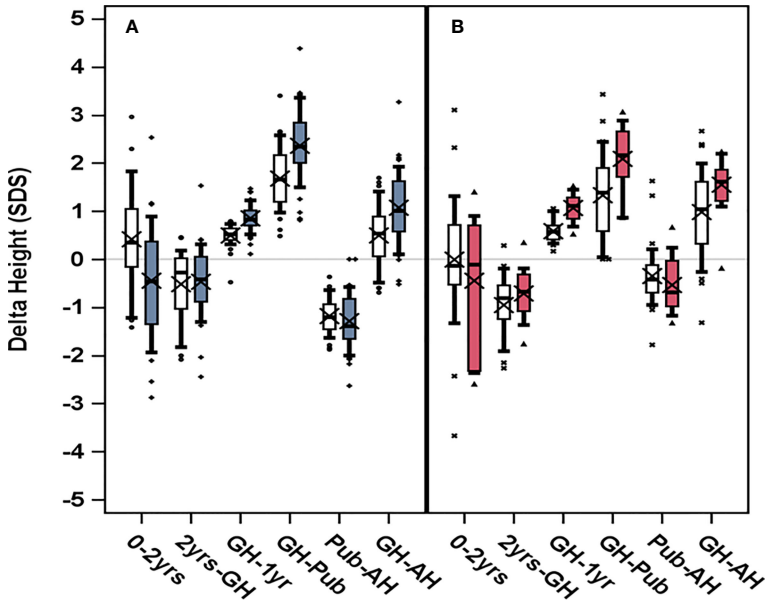

FIGURE 3
Change in height$_{SDS}$, delta height, trough different growth periods for girls who started GH treatment at **(A)** age 3–9 years to the left, blue, or **(B)** after age 9 years to the right, red. GH dose 33 µg/kg/day are depicted in light color boxes; dark color boxes (blue for young and red for old) represent GH dose 67 µg/kg/day. Boxplots show 5th, 25th, 50th, 75th, and 95th percentiles, and "X" represents the group mean value. Height$_{SDS}$ was calculated in relation to reference from non-TS girls (6), for infancy (0–2 years), for childhood (2 years GH) as pretreatment growth, for prepubertal GHstart to onset of puberty (GH-Pub) as prepubertal gain, for onset of puberty to adult height (Pub-AH) as pubertal gain, and for the total period on GH (GH-AH) as total gain.

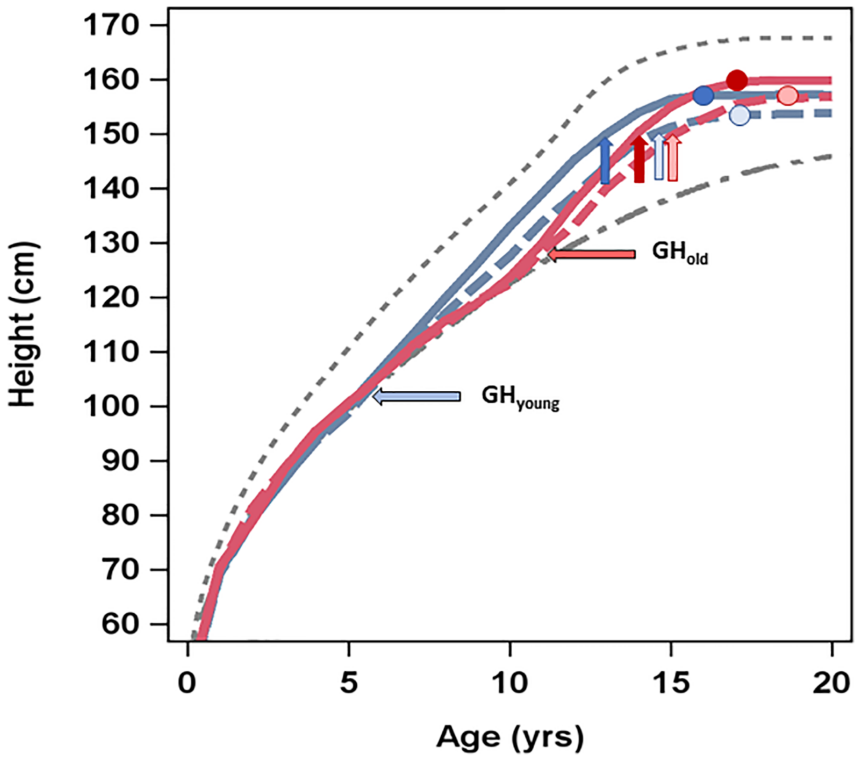

FIGURE 4
Average height (cm) over time by age (years) at GH start and GH dose. The upper (dotted) line presents average height over time in healthy girls (6), the lower (dashed) line shows the average height over time in untreated girls with Turner syndrome (5), and the four lines in between present the four treatment groups: GH$_{33young}$ (blue dotted line), GH$_{67young}$ (blue solid line), GH$_{33old}$ (red dotted line), and GH$_{67old}$ (red solid line). Horizontal arrows depict age (years) at GH start for the different groups. Vertical arrows depict age at puberty onset, and circles show height and age at attained adult height.

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TABLE 2A Auxology during GH treatment for the young 3—9 years ITT population SDS versus the Swedish references (6, 49, 50).

Variables	Dose 33 µg (n=33)	Dose 67 µg (n=46)	p-value	Difference between groups Mean (95% CI)	Effect size
At GH start					
Age (years)	5.84 (1.48) 5.52 (3.04; 8.9) n=33	5.66 (1.76) 5.55 (3.07; 8.89) n=46	0.65	0.174 (−0.585; 0.921)	0.106
Height$_{SDS}$	−2.78 (0.78) −2.84 (−3.97; −1.13) n=33	−2.78 (0.72) −2.71 (−5.39; −1.52) n=46	0.97	0.006 (−0.331; 0.344)	0.008
Diff MPH$_{SDS}$	−2.51 (0.97) −2.35 (−4.23; −0.56) n=33	−2.70 (0.88) −2.7 (−4.5; −1.08) n=46	0.36	0.193 (−0.225; 0.614)	0.209
+ 1 yr All prepubertal					
Mean GH dose$_{Year\ 1}$ (µg/kg/day)	35.8 (5.8) 35.4 (25.5; 61) n=33	53.9 (7.6) 55.8 (28; 66.4) n=46	<.0001	−18.1 (−21.2; −15.0)	2.61
Height$_{SDS}$	−2.28 (0.74) −2.26 (−3.57; −0.66) n=33	−1.93 (0.71) −1.9 (−4.35; −0.51) n=46	0.036	−0.350 (−0.675; −0.021)	0.486
At puberty start					
Age (years)	14.7 (1.1) 14.7 (12.5; 16.9) n=33	13.0 (1.4) 13.3 (9.1; 16.8) n=46	<.0001	1.65 (1.05; 2.25)	1.25
Height$_{SDS}$	−1.10 (0.90) −1.09 (−3.18; 0.27) n=33	−0.42 (0.96) −0.40 (-3.02; 1.26) n=46	0.0017	−0.676 (−1.095; −0.247)	0.724
Diff MPH$_{SDS}$	−0.83 (1.10) −0.60 (-3.39; 0.80) n=33	−0.34 (1.06) −0.37 (-2.41; 1.83) n=46	0.049	−0.489 (−0.971; −0.001)	0.455
Mean GH dose$_{Pre\ puberty}$ (µg/kg/day)	34.8 (3.2) 34.1 (30.3; 46.7) n=33	56.8 (5.7) 58.1 (37.7; 65.4) n=46	<.0001	−22.0 (−24.2; −19.9)	4.55
Gain in height$_{SDS}$ GH start - Puberty onset	1.68 (0.67) 1.66 (0.48; 3.4) n=33	2.36 (0.74) 2.34 (0.81; 4.38) n=46	0.0002	−0.682 (−1.008; −0.363)	0.960

For continuous variables Mean (SD)/Median (Min; Max)/n= is presented.
For comparison between groups the Fisher´s Non Parametric PermutationTest was used for continuous variables.
The confidence interval for the mean difference between groups is based on Fishers non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
GH, growth hormone; ns, not significant; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SD score between the height of the girl and the heights of her parents; vrs: versus.

low- and high-dose groups, respectively (p=0.0083), mean difference was 3.5 cm (95% CI, −6.02 to −0.90) (Table 3A, Figure 4).

The old age group (>9 years). Adult height$_{SDS}$ was −1.82 for the GH$_{33}$ versus −1.27 for the GH$_{67}$ group (p=0.015); mean difference was 0.546 (95% CI, −0.985 to −0.112). When expressed in centimeters, AH was 156.5 versus 159.9 cm in the low- and high-dose groups, respectively (p=0.015); mean difference was 3.4 cm (95% CI, −5.99 to −0.68) (Table 3B, Figure 4).

Comparisons between and over age groups. If considering only age at GH start and not dose in the PP population, the GH$_{young}$ group attained an AH$_{SDS}$ of −1.95, while the GH$_{old}$ group attained an AH$_{SDS}$ of −1.60 (p=0.075); mean difference was 0.341 (95% CI, −0.709 to 0.037) (Supplementary Table S6C).

4.1.5 Spontaneous or induced puberty

Data from the girls with spontaneous puberty. Of the 32 girls with spontaneous onset of puberty in the ITT population, 22 attained full pubertal development spontaneously. Data from these 22 girls were as follows: the groups GH33/67$_{young}$ and GH 33/67$_{old}$ reached AH$_{SDS}$ mean (SD) of −2.32(0.47)/−2.13(0.85) versus −2.00(0.89)/−1.84(0.54), respectively, with pubertal height gain (cm) 2.4/12.1 versus 9.7/17.6. Pubertal duration ranged from 1.8 to 4.2 years.

Data from the girls with induced puberty. Most girls needed puberty induction; divided into the groups GH33/67$_{young}$ and GH 33/67$_{old}$, AH$_{SDS}$ was mean (SD) of −2.28(0.92)/−1.59(0.95) versus −1.72(0.80)/−1.01(0.47), respectively.

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TABLE 2B Auxology during GH treatment for the old ≥9 year ITT population versus the Swedish references (6, 49, 50).

Variables	Dose 33 µg (n=34)	Dose 67 µg (n=19)	p-value	Difference between groups Mean (95% CI)	Effect size
At GH start					
Age (years)	11.8 (2.1) 11.1 (9; 15.9) n=34	11.2 (1.5) 10.7 (9.2; 14.8) n=19	0.32	0.540 (−0.523; 1.652)	0.286
Height SDS	−2.80 (0.77) −2.76 (−4.43; −1.36) n=34	−2.82 (0.54) −2.93 (−3.96; −1.44) n=19	0.90	0.024 (−0.376; 0.426)	0.034
Diff MPH_{SDS}	−2.57 (0.74) −2.49 (−3.95; −0.86) n=31	−2.90 (0.83) −2.86 (−4.01; −1.44) n=19	0.15	0.329 (−0.125; 0.788)	0.424
+ 1 yr All prepubertal					
Average GH $dose_{Year\ 1}$ (µg/kg/day)	33.7 (3.4) 33.7 (26.7; 40.7) n=34	56.5 (5.8) 56.1 (44; 71.4) n=19	<.0001	−22.7 (−25.2; −20.2)	5.21
$Height_{SDS}$	−2.34 (0.77) −2.35 (−3.87; −0.81) n=28	−1.76 (0.60) −1.77 (−2.83; −0.41) n=19	0.0076	−0.582 (−1.002; −0.160)	0.825
At puberty start					
Age (years)	15.2 (1.5) 15.3 (11.2; 18) n=34	14.1 (1.7) 14.5 (11.2; 16.6) n=19	0.026	1.07 (0.13; 1.98)	0.668
Height SDS	−1.47 (0.72) −1.48 (−3.43; 0.18) n=34	−0.73 (0.71) −0.58 (−2.02; 0.30) n=19	0.0007	−0.732 (−1.145; −0.320)	1.02
Diff MPH_{SDS}	−1.33 (0.84) −1.46 (−3.02; 0.67) n=31	−0.82 (0.81) −0.88 (−2.33; 0.68) n=19	0.040	−0.513 (−1.000; −0.026)	0.620
Average GH dose prepuberty (µg/kg/day)	34.0 (2.5) 33.4 (29.8; 41) n=32	58.2 (6.5) 59.1 (43.1; 68.9) n=19	<.0001	−24.2 (−26.8; −21.6)	5.49
Gain in $height_{SDS}$ GH start-puberty onset	1.33 (0.88) 1.38 (0; 3.43) n=34	2.09 (0.67) 2.16 (0.86; 3.06) n=19	0.0018	−0.756 (−1.219; −0.282)	0.928

For continuous variables Mean (SD)/median (Min; Max)/n= is presented.
For comparison between groups the Fisher's non-parametric permutation test was used for continuous variables.
The confidence interval for the mean difference between groups is based on Fisher's non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
GH, growth hormone; ns, not significant; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SD score between the height of the girl and the heights of her parents; vrs, versus.

4.2 Age outcomes

4.2.1 Age at puberty onset

4.2.1.1 Induced puberty

The young age group, 3–9 years. Age at start of puberty was significantly greater for the $GH_{33young}$ compared with the $GH_{67young}$ group (14.7 versus 13.0 years, respectively) (p<0.0001) and occurred at a $height_{SDS}$ of −1.10 versus −0.423, respectively (p=0.0017) (Table 2A). Age at pubertal onset for those started on ERT was significantly greater for the $GH_{33young}$ compared with the $GH_{67young}$ group (14.6 versus 13.5 years, respectively) (p<0.001).

The old age group, >9 years. Girls in the older age group receiving GH_{33} were older at the start of puberty than those receiving GH_{67} (15.2 vs. 14.1 years, respectively) (p=0.026). $Height_{SDS}$ at start of puberty was −1.47 in the low versus −0.734 in the high-dose group (p=0.0007) (Table 2B). However, age at induction of puberty was similar for low- and high-dose groups (15.5 versus 15.2 years, respectively).

Comparisons between and over age groups. When age at onset of puberty was compared for the four subgroups, puberty was induced at a significantly earlier age for those in the $GH_{67young}$ group versus the other groups (Figure 4). For those girls receiving oral EE2, age at onset of puberty ranged from mean (SD) 13.8 (1.1) to 15.5 (1.1) years, with lower age in the younger group. For those receiving transdermal estradiol patches, mean age at onset of puberty was 13.4 (1.1)–16.8 (n=1) years, with lowest age in the $GH_{67young}$ group.

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TABLE 3A Auxology at adult height for the young 3—9 years ITT population versus the Swedish references (6, 49, 50).

Variables	Dose 33 µg (n=33)	Dose 67 µg (n=46)	p-value	Difference between groups Mean (95% CI)	Effect Size
At adult height					
Age (years)	17.3 (1.2) 17 (15.6; 21.4) n=33	16.1 (1.2) 16 (13.4; 19.8) n=46	0.0002	1.13 (0.60; 1.66)	0.968
Adult height (cm)	153.7 (5.4) 155.1 (143.7; 165.4) n=33	157.2 (5.8) 156.8 (145.1; 168.3) n=46	0.0083	−3.47 (−6.02; −0.90)	0.617
Height$_{SDS}$	−2.28 (0.89) −2.05 (−3.93; −0.36) n=33	−1.71 (0.95) −1.77 (−3.7; 0.12) n=46	0.0083	−0.571 (−0.990; −0.148)	0.617
Diff MPH$_{SDS}$	−2.01 (0.99) −1.84 (−4.53; −0.43) n=33	−1.63 (0.93) −1.66 (−4.07; 0.49) n=46	0.083	−0.384 (−0.816; 0.050)	0.403
Gain in height$_{SDS}$ Puberty onset – adult height	−1.18 (0.38) −1.21 (−1.88; −0.37) n=33	−1.29 (0.56) −1.39 (−2.63; 0) n=46	0.36	0.105 (−0.122; 0.331)	0.213
Gain height (cm) Puberty onset—adult height	3.25 (2.63) 2.7 (−0.2; 10.1) n=33	7.67 (5.60) 6.2 (0; 23.3) n=46	<.0001	−4.42 (−6.50; −2.33)	0.958
Mean GH dose$_{Total}$ (µg/kg/day)	34.9 (3.5) 34 (30.3; 48.1) n=33	55.1 (6.2) 55.6 (37.3; 65.9) n=46	<.0001	−20.1 (−22.5; −17.8)	3.83
Time on GH (years)	10.2 (1.8) 10.3 (5.3; 13) n=33	9.43 (2.02) 9.72 (5.36; 13.87) n=46	0.082	0.776 (−0.098; 1.670)	0.400
Duration puberty (years)	2.57 (1.05) 2.4 (0.76; 5.28) n=33	3.09 (1.59) 3.08 (0; 9.26) n=46	0.11	−0.517 (−1.143; 0.113)	0.373
Gain in height$_{SDS}$ GH start – adult height	0.49 (0.68) 0.53 (-0.69; 1.69) n=33	1.07 (0.73) 1.00 (−0.52; 3.27) n=46	0.0006	−0.577 (−0.905; −0.257)	0.816

For continuous variables, mean (SD)/median (Min; Max)/n= is presented.
For comparison between groups the Fisher's non-parametric permutation test was used for continuous variables.
The confidence interval for the mean difference between groups is based on Fisher's non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
AH, adult height; GH, growth hormone; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SD score between the height of the girl and the heights of her parents.

4.2.1.2 Spontaneous puberty

Spontaneous onset of puberty was seen in 32 girls, 10 of whom later needed ERT ($GH_{young33}$: n=1; $GH_{young67}$: n=2; GH_{old33}: n=6; and GH_{old67}: n=1).

The young age group, 3–9 years. Age at onset of spontaneous puberty was significantly greater for the GH_{33} compared with the GH_{67} group [mean (SD) 16.1 (0.2) years (n=2) versus 11.7 (1.4) years (n=12)] (p<0.05).

The old age group, >9 years. For the older girls, those on GH_{33} also started puberty later, at a mean (SD) age of 14.6 (1.9) years (n=12), compared with those on GH_{67}, who started puberty at 11.8 (0.5) years (n=6) (p<0.01).

4.2.2 Age at adult height

The young age group: 3–9 years. The $GH_{33young}$ group reached AH significantly later, at age 17.3 years, compared with $GH_{67young}$ group, who reached AH at 16.1 years (p=0.0002), with a duration of puberty of 2.57 versus 3.09 years (p=0.11), respectively (Table 3A).

The old age group, >9 years. The older girls on GH_{33} reached AH at 18.5 years compared to 17.2 years for the GH_{67} group (p=0.0050), with a duration of puberty of 3.30 and 3.07 years (p=0.65), respectively (Table 3B).

4.2.2.1 Comparisons between and over age groups

When age at AH was compared between the two dose groups in the PP population, independent of age at GH start, the GH_{33} group was significantly older than the GH_{67} group when they attained AH (17.8 versus 16.6 years, respectively; p<0.0001) (Supplementary Table S6C). When the total gain in height$_{SDS}$ from GH start to AH was compared for the two dose groups, independent of age, the GH_{33}groups gained significantly less height$_{SDS}$ (0.839) compared with the GH_{67} groups (1.23), (p=0.020) (Supplementary Table S6C).

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TABLE 3B Auxology at adult height for the ≥9 year old ITT population versus the Swedish population (6, 49, 50).

Variables	Dose 33 µg (n=34)	Dose 67 µg (n=19)	p-value	Difference between groups Mean (95% CI)	Effect Size
At adult height					
Age (years)	18.5 (2.0) 18.2 (15.9; 25.9) n=34	17.2 (1.2) 17.6 (14.8; 18.9) n=19	0.0050	1.29 (0.36; 2.32)	0.741
Adult height (cm)	156.5 (5.0) 156.2 (144; 169.2) n=34	159.9 (3.8) 159.7 (150.4; 167) n=19	0.015	−3.32 (−5.99; −0.68)	0.717
HeightSDS$_{t0}$	−1.82 (0.83) −1.87 (−3.88; 0.27) n=34	−1.27 (0.62) −1.3 (−2.83; −0.1) n=19	0.015	−0.546 (−0.985; −0.112)	0.717
Diff MPH$_{SDS}$	−1.67 (0.94) −1.84 (−3.92; 0.03) n=31	−1.35 (0.78) −1.44 (−2.71; −0.18) n=19	0.23	−0.317 (−0.842; 0.193)	0.358
Gain in height SDS **Puberty onset—adult height**	−0.35 (0.63) −0.42 (−1.78; 1.63) n=34	−0.54 (0.54) −0.69 (−1.33; 0.66) n=19	0.29	0.186 (−0.147; 0.535)	0.311
Gain in height (cm) **Puberty onset—adult height**	7.15 (5.49) 5.7 (0.9; 21.1) n=34	9.16 (6.77) 5.6 (1.4; 26.8) n=19	0.25	−2.01 (−5.45; 1.52)	0.337
Mean GH dose$_{Total}$ **(µg/kg/day)**	34.6 (3.6) 33.2 (30.4; 46.1) n=34	57.7 (6.7) 59.1 (41.9; 69.1) n=19	<.0001	−23.1 (−26.0; −20.3)	4.67
Time on GH (years)	5.19 (1.69) 5.55 (1.99; 8.29) n=34	5.28 (1.15) 5.37 (2.62; 7.14) n=19	0.85	−0.089 (−0.961; 0.774)	0.059
Duration puberty (years)	3.30 (1.87) 3.33 (0.61; 8.7) n=34	3.07 (1.04) 2.77 (1.61; 5.37) n=19	0.65	0.225 (−0.689; 1.178)	0.139
Gain in height$_{SDS}$ **GH start—adult height**	0.98 (0.89) 1.04 (−1.32; 2.66) n=34	1.55 (0.55) 1.61 (−0.19; 2.23) n=19	0.012	−0.570 (−1.031; −0.125)	0.721

For continuous variables Mean (SD)/Median (Min; Max)/n= is presented.
For comparison between groups the Fisher's non-parametric permutation test was used for continuous variables.
The confidence interval for the mean difference between groups is based on Fishers non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
AH, adult height; GH, growth hormone; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SD score between the height of the girl and the heights of her parents.

When age at AH was compared between the groups starting treatment when young versus old in the PP population, the GH$_{young}$ reached AH at a mean age of 16.8 years, while the corresponding age for the GH$_{old}$ was 17.9 years (p=0.0002) (Supplementary Table S7C).

All analyses made in the ITT population were also performed in the PP population and presented in Supplementary Tables S1, S2, S5–S7.

4.3 Multivariable linear regression analyses

In this study, we selected as height outcomes gain in height$_{SDS}$ and attained AH in both SDS and centimeters, and as age outcomes, age at onset of puberty, and age at attained AH. Stepwise forward regression models were used to explain the variation in these outcomes using independent available variables at two time points of interest, at GH start, and at onset of puberty. The age of the girls with TS at GH start was the only common independent variable in all models, followed by the selected GH dose in the models that explain variation in height gain and the ages at pubertal onset and AH but not for AH. For the height outcomes, the parental heights, *per se* or as the difference to the height of the girl, were selected for as an informative variable. No major differences were found when using the two populations, either the entire ITT group of 132 girls or only those in the PP population of 89 girls, who followed the protocol. See Table 5, for the ITT population and Table 6 for the PP population.

4.3.1 Height outcomes

4.3.1.1 Gain in height SDS

The prepubertal gain was to 25% (ITT) and to 42% (PP) explained by three variables at GH start: age, height, and the selected GH dose for ITT, with mother height added for PP population.

The pubertal gain was to 53% (ITT) and to 48% (PP) explained by two variables at pubertal onset: age at GH start and diffSDS.

The total gain was to 27% (ITT) and to 41% (PP) explained at GH start for ITT with age, height, and diffSDS at GH start, and at pubertal onset to 69% with also prepubertal gain.

PP at GH start was to 41%, with age, diffSDS, and chromosomes (the only model), and at pubertal onset to 71%, using age and height at GH start with the selected GH dose and prepubertal height gain.

4.3.1.2 Adult height$_{SDS}$

At GH start, the variation was explained to 40% (ITT) and to 43% (PP) and explained by three available variables, namely, age, height, and diffSDS (only ITT).

At pubertal onset, the variation could be explained to 75% (ITT) and to 77% (PP) by age at GH start, diffSDS [and for PP addition of parental heights (MPH, mother height or father height)], and height at pubertal onset.

4.3.2 Age outcomes

4.3.2.1 Age at pubertal onset

Age at pubertal onset could at GH start be explained to 23% (ITT) and to 28% (PP) by age and the selected GH dose.

4.3.2.2 Age at adult height, AH

Age at AH was to 30% (ITT) and to 32% (PP) explained by age at GH start and the selected GH dose, and at pubertal onset to 51% (ITT) and to 45% (PP) by age at GH start and age and height at pubertal onset (ITT).

4.4 Safety

Oxandrolone was started at dose 0.05 mg/kg/day with cautious monitoring according to voice deepening and other androgen effects. Dose reduction was allowed by the investigator, no girl treated with oxandrolone received a daily dose lower than 0.025 mg/kg. No girl was diagnosed with diabetes mellitus. If signs of celiac disease or thyroid disturbance became evident, proper treatment was instantly instituted and occurred after enrollment in two girls. FSH and LH were followed yearly from age 7 years (or at diagnose) for early diagnose of possible gonadal failure. A total of 10 girls with spontaneous onset of puberty needed supportive estrogen substitution.

5 Discussion

5.1 Principal findings

The major finding from this study based on a temporal design of multicentre clinical trials was that a normal adult height can be attained for girls with TS when treated with rhGH prior to puberty, irrespective of age when the diagnosis of TS was made, from early childhood through to ~16 years of age. This can be achieved by individualized treatment with GH (and possibly oxandrolone for growth support) with doses adapted according to individual GH responsiveness, as revealed by the first-year growth response. Pubertal growth and development can also be optimized using the addition of a combination of ERT and, where needed, androgens. Transdermal 17β-estradiol is preferred as possible to mimic physiology regarding onset, progress, and duration.

A GH-dose effect on growth was found in both early and late diagnosed girls relative to puberty, even though the between-group difference in mean GH dose was narrower than intended, 35 versus 57 μg/kg/day, respectively, with broad ranges indicating dose individualization. Total height gain was lowest for the young age group on the low GH dose (0.5 SDS) and greatest for the old age group on the high GH dose (1.55 SDS). A substantial prepubertal gain in height was achieved. However, this relative height gain was partly lost during puberty through subnormal pubertal growth. Although these findings suggest that GH dose is a key factor, later age at diagnose is known to be associated with more subtle features of TS, and therefore, comparisons within each age group will be the most relevant. AH was within acceptable ranges for all four groups, since the great gain achieved before puberty overcame the subnormal pubertal growth. Mean AH for all groups were close to or within the reference range for the normal population; height$_{SDS}$ was −2.28 for the group with the lowest mean AH of 153.7 cm ($GH_{young33}$) and −1.27 for the group with the greatest mean AH of 159.9 cm (GH_{old67}). It is of note that although girls who started treatment later with the lower GH dose did attain a mean AH_{SDS} within normal range (−1.82; 156.5 cm), this was only achieved when they were 18.5 years of age. In comparison, AH was attained at the age of 17.2 years in the GH_{67old} group. Irrespective of which GH dose they received, most girls in the old age group had adjuvant oxandrolone treatment from the first or second year on GH treatment before start of ERT. Thus, as shown in a previous study, GH treatment may increase AH, although delayed as in the present study, even when started late relative to the onset of puberty (31). Both pubertal duration and gain in height was low for all groups, resulting in that the prepubertal gain was partly lost during puberty. Thus, with improved treatment during puberty, initially using physiologically low transdermal 17β-estradiol available nowadays (38, 57, 59), the intentional delay in ERT and age for AH in these trials might not have been necessary (58).

5.2 GH treatment for growth

5.2.1 GH secretion and GH dose

In the late 1980s when the Swedish trials were planned and initiated, rhGH was only approved for use in children with GHD, and very little was known about the effects of GH dose on height gain. At this time, the parallel study by the group of Hintz and Rosenfeld that led to the approval of GH treatment (50 μg/kg/day) for TS were ongoing (60, 61). During the years to come, the effect of GH dose on girls with TS was studied in several trials in many countries (10, 22–31) and in international outcome databases (32–36). Results have convincingly shown the importance of GH dose during prepuberty; low doses are not enough (26, 28, 62, 63), while higher doses are (29). The present Swedish trial results strongly support these findings.

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TABLE 4 Growth from birth to adult height in the ITT study subgroups; expressed in SDS versus the Turner reference (5).

	Group									
	GH start 3- <9					GH start 9-				
Variables	Dose 33 µg	Dose 67 µg	p-value	Difference between groups Mean (95% CI)	Effect Size	Dose 33 µg	Dose 67 µg	p-value	Difference between groups Mean (95% CI)	Effect Size
	n=33	n=46				n=34	n=19			
At birth										
Height SDS	−0.58 (1.08) −0.24 (−3.04; 0.96) n=33	−0.21 (0.95) −0.24 (−2.24; 1.76) n=46	0.12	−0.365 (−0.824; 0.089)	0.364	−0.01 (0.86) 0.16 (−2.24; 1.56) n=34	−0.24 (1.11) −0.16 (−2.24; 0.96) n=18	0.41	0.235 (−0.333; 0.785)	0.248
At GH start										
Height SDS	−0.24 (0.88) −0.30 (−1.55; 1.49) n=33	−0.26 (0.86) −0.25 (−3.15; 1.45) n=46	0.94	0.015 (−0.378; 0.407)	0.017	0.21 (0.96) 0.14 (−1.81; 2.02) n=34	0.13 (0.67) −0.01 (−0.95; 1.93) n=19	0.78	0.071 (−0.425; 0.573)	0.082
+ 1 year all prepubertal										
$Height_{SDS}$	0.37 (0.80) 0.32 (−0.98; 2.16) n=32	0.81 (0.86) 0.89 (−1.85; 2.74) n=46	0.026	−0.441 (−0.829; −0.056)	0.528	0.82 (0.88) 0.77 (−1.06; 2.61) n=27	1.43 (0.70) 1.43 (0.39; 3.05) n=19	0.016	−0.605 (−1.084; −0.121)	0.748
At puberty start										
Height SDS	2.30 (0.97) 2.33 (0.17; 3.79) n=33	3.05 (1.07) 3.1 (−0.1; 4.92) n=46	0.0016	−0.753 (−1.219; −0.282)	0.730	1.90 (0.75) 1.88 (−0.02; 3.64) n=34	2.64 (0.82) 2.86 (1.07; 3.83) n=19	0.0018	−0.742 (−1.196; −0.292)	0.952
At adult height										
Height SDS	1.85 (1.01) 1.83 (−0.07; 3.62) n=33	2.81 (1.01) 2.9 (0.77; 4.86) n=46	<.0001	−0.962 (−1.419; −0.503)	0.952	2.04 (0.80) 2.2 (−0.45; 3.74) n=34	2.87 (0.50) 2.97 (1.78; 3.85) n=19	0.0002	−0.822 (−1.234; −0.427)	1.16
Height gain										
Gain in $height_{SDS}$ GH start—Puberty onset	2.54 (0.69) 2.42 (1.54; 4.48) n=33	3.31 (0.78) 3.34 (1.74; 5.3) n=46	<.0001	−0.767 (−1.105; −0.429)	1.03	1.70 (1.08) 1.78 (0; 4.06) n=34	2.51 (0.82) 2.64 (1.08; 3.69) n=19	0.0066	−0.812 (−1.381; −0.233)	0.815
Gain in $height_{SDS}$ Puberty onset—adult height	−0.45 (0.28) −0.43 (−1.20; −0.01) n=33	−0.24 (0.50) −0.27 (−1.18; 0.92) n=46	0.032	−0.209 (−0.401; −0.020)	0.499	0.14 (0.49) 0.04 (−0.59; 1.72) n=34	0.22 (0.66) 0.16 (−0.85; 2.17) n=19	0.61	−0.080 (−0.397; 0.246)	0.143
Gain in $height_{SDS}$ GH start—adult height	2.09 (0.72) 2.07 (0.69; 3.44) n=33	3.07 (0.76) 3.01 (1.47; 5.08) n=46	<.0001	−0.977 (−1.321; −0.633)	1.31	1.84 (1.03) 1.99 (−0.07; 3.99) n=34	2.73 (0.62) 2.91 (1.41; 3.63) n=19	0.0010	−0.892 (−1.416; −0.372)	0.983

For continuous variables, Mean(SD)/Median (Min; Max)/n= is presented.
For comparison between groups, the Fisher's non-parametric permutation test was used for continuous variables.
The confidence interval for the mean difference between groups is based on Fisher's non-parametric permutation test.
Effect size is absolute difference in mean/pooled SD.
GH, growth hormone; SDS, standard deviation score.

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TABLE 5 Multivariable linear regression analyses, ITT population 132 girls with TS.

		Independent variables Beta/(95% CI)/p-value								
		Before GHstart	At GHstart			1st year	Prepub period	At Pubertal onset		
		MPH (SDS)	Age (years)	Height (SDS)	Diff MPH (SDS)	Mean GH dose (µg/kg/day)	Gain in height (SDS)	Age (years)	Height (SDS)	Diff MPH (SDS)
Dependent	R-square									
Gain in height										
Prepub gain SDS @GH	0.2521		−0.05 (−0.09; −0.01) p=0.012	−0.26 (−0.44; −0.08) p=0.0049		0.02 (0.01;0.04) p<0.0001				
Pub gain SDS @puberty	0.5278		0.11 (0.09;0.14) p<0.0001							−0.21 (−0.29; −0.12) p<0.0001
Total gain SDS @GH	0.2714		0.06 (0.03;0.10) p=0.0008	−0.21 (−0.39; −0.03) p=0.024	−0.23 (−0.38; −0.08) p=0.0034	0.02 (0.01;0.03) p=0.0015				
Total gain SDS @puberty	0.6916		0.11 (0.08;0.13) p<0.0001		−0.14 (−0.24; −0.05) p=0.0032		0.74 (0.64;0.85) p<0.0001			
Adult height										
Adult height SDS @GH	0.3966	0.28 (0.12;0.43) p=0.0005	0.05 (0.02;0.09) p=0.0059	0.51 (0.31;0.70) p<0.0001						
Adult height cm @GH	0.3966	1.68 (0.75;2.61) p=0.0005	0.32 (0.09;0.55) p=0.0059	3.07 (1.86;4.28) p<0.0001						
Adult height SDS @puberty	0.7471	0.17 (0.07;0.26) p=0.0008	0.11 (0.09;0.14) p<0.0001						0.75 (0.65;0.85) p<0.0001	
Adult height cm @puberty	0.7471		0.68 (0.53;0.84) p<0.0001						5.58 (4.94;6.23) p<0.0001	−1.01 (−1.59; −0.43) p=0.0008
Age at Pubertal onset										
Age Puberty @GH	0.2258		0.18 (0.10;0.26) p<0.0001			−0.04 (−0.06; −0.01) p=0.0022				
Age at Adult height										
Age Adult height @GH	0.2999		0.23 (0.15;0.30) p<0.0001			−0.03 (−0.05; −0.01) p=0.0032				
Age Adult height @puberty	0.5097		0.13 (0.06;0.20) p=0.0002					0.42 (0.28;0.56) p<0.0001	−0.49 (−0.72; −0.26) p<0.0001	

GH, growth hormone; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SDS between the height of the girl and the heights of her parents. SDS calculated versus the Swedish population for the girls (6, 57) and for the parents (58). ITT, intention to treat population; PP, per protocol population; @GHstart, model with available independent variables at GHstart; @puberty, models with available independent variables at pubertal onset.

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TABLE 6 Multivariable linear regression analyses, PP population, 89 girls with Turner syndrome.

		Independent variables Beta/(95% CI)/p-value										
		Before GHstart				At GHstart			1st year	Prepub period	At Pubertal onset	
		Karyotype	MPH (SDS)	Mother height (SDS)	Father height (SDS)	Age (years)	Height (SDS)	Diff MPH (SDS)	Mean GHdose (µg/kg/day)	Gain in height (SDS)	Age (years)	Height (SDS)
Dependent	R-square											
Gain in Height												
Prepub gain SDS @GH	0.4212			0.25 (0.11;0.40) p=0.0006		−0.05 (−0.09; −0.01) p=0.026	−0.45 (−0.64; −0.27) p<0.0001		0.02 (0.01;0.03) p=0.0003			
Pub gain SDS @puberty	0.4747					0.13 (0.10;0.16) p<0.0001						
Total gain SDS @GH	0.4131	−1.17 (−1.91; −0.42) p=0.0024				0.07 (0.03;0.11) p=0.0007	−0.24 (−0.43; −0.04) p=0.017	−0.28 (−0.44; −0.11) p=0.0013	0.02 (0.01;0.03) p=0.0050			
Total gain SDS @puberty	0.7071					0.12 (0.09;0.15) p<0.0001				0.83 (0.71;0.95) p<0.0001		
Adult height												
Adult height SDS @GH	0.4245		0.28 (0.11;0.46) p=0.0019			0.07 (0.02;0.11) p=0.0034	0.45 (0.22;0.67) p=0.0002					
Adult height cm @GH	0.4245		1.73 (0.66;2.80) p=0.0019			0.41 (0.14;0.67) p=0.0034	2.72 (1.35;4.10) p=0.0002					
Adult height SDS @puberty	0.7726				0.10 (0.00;0.20) p=0.047	0.12 (0.09;0.14) p<0.0001						0.83 (0.72;0.95) p<0.0001
Adult height cm @puberty	0.7726				0.60 (0.01;1.19) p=0.047	0.71 (0.53;0.88) p<0.0001						5.06 (4.37;5.75) p<0.0001
Age at Pubertal onset												
Age Puberty @GH	0.2841					0.17 (0.08;0.25) p=0.0004			−0.05 (−0.08; −0.03) p=0.0002			
Age at adult height												
Age Adult height @GH	0.3244					0.18 (0.10;0.26) p<0.0001			−0.04 (−0.07; −0.02) p=0.0004			
Age adult height @puberty	0.4476					0.10 (0.02;0.18) p=0.019				−0.46 (−0.77; −0.14) p=0.0046	0.42 (0.26;0.58) p<0.0001	

GH, growth hormone; SDS, standard deviation score; MPH, mid-parental height; DiffMPH, difference in SDS between the height of the girl and the heights of her parents. SDS calculated versus the Swedish population for the girls (6, 57) and for the parents (58). ITT, intention to treat population; PP, per protocol population; @GHstart, model with available independent variables at GHstart; @puberty, models with available independent variables at pubertal onset.

The need for a different GH dose in girls with TS compared with children with GHD is today not surprising. The GH dose approved for treatment of GHD was estimated based on the GH secretion rate of healthy children (64). However, compared with non-TS girls, girls with TS have a different GH secretory pattern (51) due to that they mainly secrete the 20-kDa isoform rather than the normally more abundant 22 kDa isoform (65, 66). The 20-kDa isoform has a longer half-life and is associated with greater metabolic and less longitudinal growth-promoting effects (65, 66). The longer half-life of the 20 kDa form will also result in higher GH trough levels, resulting in constant serum GH concentration, something known to be negative for growth (67). Furthermore, it is known that administration of exogenous rhGH (the 22-kDa isoform) reduces endogenous GH secretion (of any isoform) for hours, owing to the well-known negative feedback mechanism by GH on its own secretion, with the duration of the reduction depending on the amount and depth of the injection (56).

In our studies, the intended GH doses were not adhered to by many of the investigators: the low dose, which was intended to be 33 μg/kg/day, instead became on average 37 μg/kg/day, whereas the high dose of 67 μg/kg/day became on average 57 μg/kg/day, thereby reducing the study dose–response range. Thus, the GH doses in at least a quarter of the 132 girls with TS were actually individualized, which resulted in the exclusion of data from these girls from the PP analysis. This high degree of deviation from protocol is of note, as it may indicate that the range of responsiveness was narrower than expected and that signs of overdose, such as water retention or the development of acromegalic features, may have occurred at a lower-than-expected dose in this patient group.

5.2.2 GH response and GH responsiveness

Growth response varies considerably, even between individuals with the same diagnosis and general characteristics who have received comparable GH doses. In this context, it may be helpful to imagine that, for each individual child, there is a set point of balance between GH secretion and GH responsiveness (68), and that this balance differs during the different growth phases (68, 69) and between tissues. We know, for example, that the bone tissue seems to be the least sensitive tissue in the body to GH, whereas the brain is the most sensitive (70, 71).

Responsiveness to GH can be estimated using the first year growth response to a specific GH dose (15) or using a prediction model for estimation of growth response (16, 17, 72). According to the KIGS TS prediction model (18), which estimates first year prepubertal GH growth response in centimeters, the most important variable was GH dose (studied dose range, 23–52 μg/kg/day) followed by age, weight, and oxandrolone treatment. This is consistent with the present findings. The fact that the model showed that the observed first year growth response explained most of the following second year growth response highlights the importance of considering individual GH responsiveness. In the present study, by multivariable regression model analysis, we identified GH dose to be an important variable to explain the variation in prepubertal gain in $height_{SDS}$: belonging to the high-dose group was associated with a greater gain. Additionally, being young at GH start was associated with a greater growth response. Together, these data suggest that the growth deficit associated with a late GH start can, at least partly, be compensated for by a higher GH dose during the remaining or extended prepubertal growth period. The association of young age at GH start and thereby greater growth response/responsiveness has previously been reported in girls with TS (18, 73), as in other diagnostic groups including children with GHD and ISS (16, 74, 75). When calculating growth response versus the TS growth reference, GH dose *per se* was, together with young age, found to explain most of the variation in total growth response, indicating the individual GH responsiveness (32). Young age at GH start also prevents short stature already from childhood by allowing more prepubertal years for growth (30, 32, 73).

5.3 Estrogen replacement therapy for growth and pubertal development

This analysis showed height gain during puberty to be low or absent, with many girls experiencing a loss in relative height during this period despite growing well when receiving GH treatment prior to puberty. As per the protocol, ERT was used to initiate puberty at an appropriate time from 13 years onwards as determined jointly by the physician and girl; data showed that puberty was initiated between 13 and 15.2 years of age, and that in all groups, girls were of an average height close to 150 cm at the time. This later than normal start of ERT may be due to prior clinical experience of little further growth after the start of estrogen therapy with EE2 at the doses used (33). Even though the EE2 dose used at that time was estimated to be low, it could still have had a higher-than-expected estrogen effect resulting in growth plate maturation being too rapid, leaving too little time for pubertal growth. Consistent with this, the mean duration of puberty was only approximately 3 years (2.6–3.3 years). Breast development also suffers when ERT dose increments are too rapid; both breast size and shape often did become abnormal when EE2 was used. However, this occurred only rarely when the more recent transdermal estradiol low-dose regime was used (personal communications and observations).

Treatment regimens for both GH and estrogen have changed since these trials were planned and initiated. The dose of GH given routinely to girls with TS is now closer to the range observed in the present study. Moreover, ERT is now primarily given in the form of 17β-estradiol, and the administration route is usually transdermal. Furthermore, the dose of estrogen used to induce puberty has reduced substantially, dose and tempo of administration should ideally mimic the initially very low serum estradiol levels of normal female puberty (38, 39). For maintenance, a dose should be used sufficient to result in serum levels appropriate for the young adult women, a dose that is twice that recommended for post-menopausal women (40–42).

Of all 144 girls with TS enrolled in this study, 37 girls experienced onset of puberty spontaneously; 5 of those received LHRH analogue treatment for a period (note: the data of these five girls were excluded before any analyses), and 10 other girls needed after some time ERT in order to undergo full puberty with menarche and sustained development of secondary sexual characteristics. Thus, 22 girls went through a full spontaneous puberty with regular menses. They started puberty at age mean 11.8 years, significantly younger than those who started puberty spontaneously but later needed ERT. Pubertal height gain was also greater in the former group, 12.0–17.5 cm, when receiving the high GH dose. Most of the girls who underwent full spontaneous puberty had a karyotype labelled as “other” (i.e., structural aberrations); this karyotype was also found to be positively associated with pubertal height gain, possibly indicating more subtle features of TS. In contrast, girls with TS with later spontaneous puberty grow less during infancy and mid-childhood, probably estrogen dependent (76). The height gain observed in those undergoing spontaneous puberty in these studies could serve as a future treatment goal for girls with TS needing ERT. Generating serum estradiol concentrations that mirror those seen physiologically during puberty in girls without TS would theoretically be optimal (38, 59). This approach has been tested in girls with TS receiving ERT in combination with GH, and it resulted in a growth-promoting effect (26, 27).

5.4 Oxandrolone as adjuvant growth-promoting treatment

Almost all girls, 94%, in GH_{old} groups received adjuvant oxandrolone treatment compared with only half of the GH_{young} group. The growth stimulating effect of oxandrolone is well documented (10). In recent reviews and a meta-analysis, the additive effect on growth was calculated to be 2.3–4.6 cm (77) and 2.06 cm (78), respectively. The dose used in the present studies was consistent with those described in these more recent publications. However, in the multivariable analysis, oxandrolone treatment was not found to be a predictor of growth in SDS, possibly due to the high proportion of study subjects receiving treatment. When calculating growth in centimeters, there was a small negative impact on growth, indicating interaction with the maturation tempo or, more likely, a selection bias owing to the use of oxandrolone. A possible long-term negative effect of oxandrolone treatment in adolescence on QoL and socio-emotional functioning in adulthood has been identified (79), which may oblige us to optimize GH treatment to minimize oxandrolone treatment, thereby also avoiding negative effects on mammary development and the risk of voice deepening and other virilising effects (77). Thus, early diagnosis of TS and initiation of GH treatment at a suitable dose could allow us to normalize height in time to allow ERT to be initiated in harmony with the onset of puberty in peers (7, 37).

5.5 Methodological aspects on evaluation of height during childhood and puberty

The goal for height-promoting therapy is normalization. The girls themselves compare heights both with their family and their non-TS peers. Therefore, we primarily used the non-TS reference when calculating SDS (6), which includes the childhood component calculated from the ICP-growth model (3). This prepubertal growth function within this model allows height gain calculations in SDS to be made separately for growth related to the prepubertal phase, from the specific pubertal growth. Thereby, the influence of any normal early pubertal growth in the reference population was omitted.

As age at start of puberty/ERT and age at AH have great variations within the groups and this interferes with SDS calculations, it is necessary to use centimeter when discussing total pubertal height gain. The effect of different timing of pubertal growth in the reference population and in our treated TS girls is visualized in Figure 5. Until recently, only data on the entire pubertal period could be used for calculations of pubertal change in $height_{SDS}$ (6, 80). However, a novel type of pubertal growth reference, aligned for height at the onset of puberty, is now available (81). This height reference will allow comparisons in SDS and centimeter at any timepoint throughout the pubertal period and serve as a tool for monitoring the impact of treatment with GH on growth during puberty, for both total pubertal growth and separating ongoing basic growth from the specific pubertal growth, including also references for weight and BMI (82, 83). However, for this report, calculation of AH_{SDS} was adjusted by aligning AH for girls with TS in centimeters with height at 18 years for the reference population (6). When we also compared heights with our TS height reference (4, 5), the entire treatment effect, the total height gain, was approximately 2 SDS for girls with TS on the low GH dose and approximately 3 SDS for those on the high GH dose. Thus, reducing on the low dose approximately half of the deficit relative to their parental heights and almost all deficit on the high GH dose (Table 4, Figures 2, 3).

5.6 Strengths and limitations

The major strength of these studies was that they are based on national-level data collected over a considerable time period. All Swedish pediatric endocrinologists and pediatricians caring for patients with TS participated, resulting in over 25 years of clinical follow-up from a range of healthcare professionals within the multidisciplinary team. The setting also allowed researchers to gather knowledge on the management of TS and led to the development of tools for monitoring treatment efficacy and safety in clinical practice.

5.6.1 Study population

Out of all those diagnosed in Sweden with TS 1987–1998, a "homogeneous" study population was obtained: with known karyotype for the participants and with inclusion/exclusion criteria narrowing the study group by avoiding those with Y-line and those with severe organ diseases.

5.6.2 The study design

The study design, being an open temporal design, allows generalization of the results, as it includes all girls diagnosed with TS in the country fulfilling the criteria. All university and regional pediatric clinics in Sweden and most local ones were involved, thus making the study as close as possible to the real-world situation. While this was beneficial in many ways, and the results obtained in this situation highlight the clinical need for GH dose to be individualized, it was not possible to control dosing levels or to control for unknown events over time, possible with other types of design. However, it is important to remember that there were other ongoing studies looking at individualization of GH dose at the time of these investigations (84, 85). Swedish pediatric endocrinologists were therefore well acquainted with working clinically according to this dose-changing concept. This experience may have influenced the dose adaptations made for the TS girls.

5.6.3 National knowledge gathering led to development of tools for monitoring safety and efficacy

5.6.3.1 Centralized laboratory analyses

Centralized laboratory analyses were used for all hormonal pre-investigation and follow-up of efficacy and safety variables. Extensive laboratory monitoring throughout the studies ensured

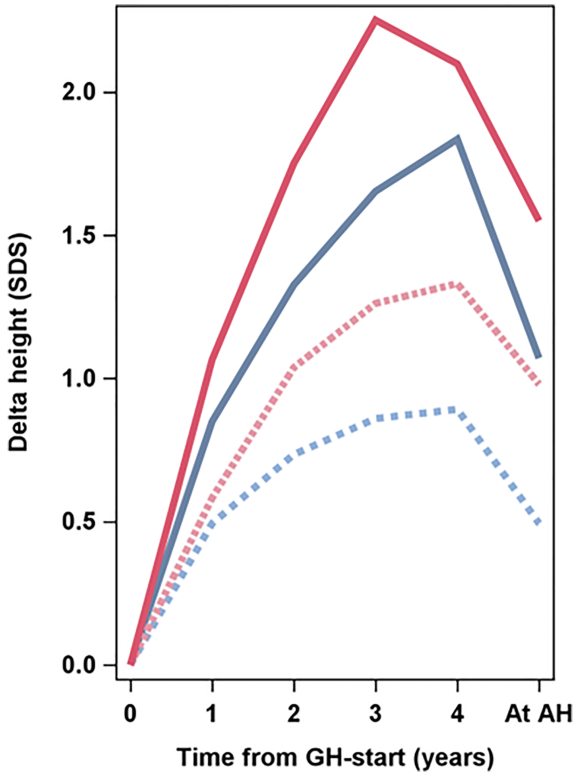

FIGURE 5
Average delta height$_{SDS}$ in relation to Swedish reference for healthy girls (6) versus time from start of GH treatment to adult height (AH) (years). The four lines present the four groups: GH$_{33young}$ (blue dotted line), GH$_{67young}$ (blue solid line), GH$_{33old}$ (red dotted line), GH$_{67old}$ (red solid line). The Swedish reference used for SDS (6).

that care could be changed as needed and made it possible to gather unique new knowledge about girls with TS: different GH profiles (51), different GH forms (65, 66), GHBP (52), different FSH forms (54, 86, 87), and more to come through the biobank, and for autoimmune diseases as celiac disease (55) and thyroid hormone disturbances (53).

5.6.3.2 Proper growth evaluation

To make it possible, we developed both a height reference (5) and an ICP growth model (4) by using data on spontaneous growth in girls diagnosed with TS. Both items were developed using methods comparable to those used to create the reference and growth model developed from healthy girls (6, 88).

5.6.4 Variables followed within the study

All yearly follow-up visits were at the university hospitals, with yearly X-ray bone age (10). Most girls were visiting the GP-GRC as the national center for TS, and QoL and psychological functioning of the girl with TS (89, 90) and the psychosocial impact on her family (91, 92) were explored.

5.6.4.1 Voice frequency

Voice frequency was recorded, and follow-up showed speech frequency to be normalized on GH and oxandrolone treatment (93, 94).

5.6.4.2 Hearing function and ear

Also followed were ear and hearing problems, ranging from external morphological abnormalities to sensorineural or conductive hearing loss, known to constitute major medical issues affecting the QoL and wellbeing in girls and women with TS (95). The prevalence of otological disease as external ear deformities (20–62%), recurrent otitis media (24–48%), and hearing loss (36–84%) is high in TS (96). When subdivided according to karyotype, 45,X and "45,X/46,iso(X) and equivalents" (i.e., TS harboring an isochromosome) experience hearing loss, middle ear infections, and external ear malformations more often than other karyotypes (97).

Hearing correlate to height and IGF-1 concentrations (98). Thus, it was hypothesized that lacking the p-arm is detrimental for the TS phenotype, which could be due to growth defects, attributed to a combination of the generally prolonged cell cycle time in abnormal chromosomal cells and the haploinsufficiency of growth-regulating Xp-linked genes, such as SHOX (98). So far, no impact of ERT nor of GH on these conditions has been shown (99, 100), as supported by the results of our study. The hearing loss with age did not differ between the two GH dose groups (101).

5.6.5 Variables not followed within the study

Despite the collection of these many variables, bone quality and uterine growth were not followed as part of the trials. Initiated by those who were enrolled in these trials, there was structured follow-

up by the Turner Academia teams at the university hospitals for young women with TS after transition to adult care. Thus, we will be able to investigate the impact of the different treatment regimens on these variables.

5.6.5.1 GH and ERT for bone mineralization and bone health

A more physiological endocrine milieu would favor both normal growth and optimal bone mineralization, helping girls to attain a normal peak bone mass (PBM). Today, we are aware of the favorable effect of GH on amplitude and timing of PBM (102) and the role this has in reducing the risk of later osteoporosis. Unfortunately, GH therapy was ended prematurely in the girls participating in our studies; one-third ended GH treatment too early for growth, and all girls ended according to the protocol when growth velocity was still 2 cm/year, which was before the attainment of PBM. In addition, it is known that starting ERT late delays bone mass accrual (103, 104) and note that the "pediatric" adult ERT dose at that time of these studies was only 25 µg/kg/day of transdermal estradiol, i.e., a suboptimal dose for a young adult woman. However, the route of administration was beneficial: compared with oral EE2, transdermal estradiol was found to result in faster bone accrual in the spine (105).

5.6.5.2 GH and ERT for growth of uterus

There are doubts about estrogen dosing in these trials. Was it too high in the beginning hampering pubertal growth and was it too low after attained AH with insufficient effects on uterus and bone? Could uterine growth and size have guided us to identify optimal individual dose of ERT? Our rational for this question is that both GH and estrogen are uterine and endometrium growth promoters (106–108). Prepubertal GH treatment increases uterine size (109) and may positively prepare the uterus for the effects of pubertal ERT. Estradiol dose has implications for uterine size: in a post-menarcheal group uterine length reflected estrogen dose (110). In addition, in girls with TS for whom puberty was induced, uterine size was small compared with those who underwent spontaneous puberty (111), as was uterine size in estradiol-treated TS small women compared with non-TS healthy women (112), while a more normal size was found by others (113). Thus, uterine size reflects the estrogen effect on uterus and possibly could monitor and guide individual dosing. However, there is a conflict between the low estradiol dose promoting pubertal growth and the higher dose stimulating uterine growth. Thus, we should aim for a low enough growth-stimulating estradiol dose in early puberty, to be followed during puberty by an estradiol dose high enough to promote normal uterine size. If uterine size post-menarche is found too small, an increased estradiol dose can stimulate uterine growth to obtain normal adult size (44, 110, 114), as increasing fertility possibilities makes uterine size and shape important (115–117).

5.6.6 Safety aspects

We aimed to mirror normal physiology using the hormones given and thereby minimize the occurrence of under- and overtreatment effects. There were no SAEs of diabetes, thrombosis, or increased intracranial pressure reported in association with treatment. Five girls withdrew from the study after enrollment owing to other diseases that were reported as AEs.

GH. Girls who were planned to receive GH dose 67 µg/kg/day started treatment with the lower dose, with dose escalations being made over 1–3 months; in a few cases, dose escalation was slower than planned, mostly due to a tendency towards edema. In practice, both GH doses were not always maintained; the GH_{67} dose was often reduced, while the GH_{33} dose was increased. Probably when the investigators observed signs of overdose or lack of efficacy on growth, then the dose was individually adapted.

Oxandrolone. Oxandrolone was used from 11 years age in almost all girls in the GH_{old} group and in about half of the GH_{young} group. Voice deepening is a well-known effect of oxandrolone (77) and if observed/reported, led to prompt dose reduction as was also done when virilization and delayed breast development were seen (118); however, these effects were rarely seen in our study.

Oral estrogen. Oral estrogen, EE2, was used for many years in the present studies. Even though this synthetic, long-acting form is known to impact on coagulation factors and metabolism due to its liver passage and that levels in serum cannot be reliably estimated to protect against overdosing, no clinically significant AEs were seen. Similarly, no AEs were reported in connection with the use of 17β-estradiol in the present study; 17β-estradiol was administered via the transdermal route, which is known to circumvent liver passage and thereby avoids cardiometabolic side effects (105, 119).

5.7 Transition

5.7.1 Pediatric studies highlighted the need for organized care in a lifelong perspective

Before clinical trials on GH treatment, healthcare for girls and women with TS was managed by various different clinics and primary care practitioners, resulting in late diagnoses and high study/treatment dropout rates.

The GH trials led to a worldwide shift towards the centralization of care for children with growth disorders, including those with TS to pediatric endocrinologists working within university hospitals and national centers such as GP-GRC in Sweden, responsible for both clinical care and research. Here, multidisciplinary teams for the girls with TS were formed, including both organ, function, and growth phase specialties, during the transition years closely together with the fertility gynecologist.

During these long-term studies, a need was identified for a lifelong, patient-centered approach to the care of these individuals, which saw the extension of care from childhood through adolescence to adulthood. The fertility gynecologist, who had gained knowledge by participating during transition, as the key person working closely with the endocrinologist formed the multidisciplinary team with specialists in essential disciplines after transition to maintain a lifelong perspective (120). The practical approach with "TS days" is that it should be both convenient for the TS women to meet the specialists they need and for the specialists in different areas to meet for sharing knowledge, which is proven to be a successful concept (51, 52). This has been advocated within the

Swedish Turner Academy organization, initiated after the 4th TS meeting in Gothenburg in 1995, devoted to TS in a lifespan perspective (121). The concept of international TS consensus meeting was initiated (121), with subsequent meetings being held in 2001 (122), 2007 (104), and most recently 2017 (37). As a result, care for girls and women with TS continues to improve, and international guidelines and recommendations for the management of TS are continuously updated.

5.7.2 Education and independence training during the transition phase

Today, the pediatric endocrinology team often plays a key role in coordinating care provided by other specialists during childhood. With improvements in medical knowledge, the number of areas and therapies that this multidisciplinary team needs to monitor has increased considerably. Not least important are the social and educational aspects, including QoL and self-esteem obtained during childhood and adolescence, as they will have lifelong implications.

The transition between pediatric and adult care is a critical process that takes place over several years. Both the pediatric endocrinologist and adolescent/fertility gynecologist are involved, and alongside this, each young girl will take on increasing responsibilities for herself. This "independence" training is important if we are to prevent the well-documented high treatment dropout rates in young adulthood and needs to start years before the girl becomes a legal adult. In dialogue with her future health providers, discussions with each girl about transition should cover not only all aspects of her present health and social situation but also possible problems that may arise with age, thereby minimizing the risk of low therapy adherence in adulthood.

Patients typically request a known contact path, with easy access to the team and a key specialist within the multidisciplinary team who is well trained in TS healthcare. This person must be able to guide the individual and facilitate connectivity within the healthcare system if or when a new sign or symptom presents. Because TS health issues are complex, the involvement of various kinds of specialists and their multidisciplinary expert team is crucial. Easy interdisciplinary communication is of foremost importance not only for care but also for the continued expansion of knowledge about this syndrome, which will bring further benefits to the TS girl or woman.

Thus, bearing in mind the complexity of TS, with the potential for major health issues impacting most organ systems, the management should not sit within primary care.

5.8 Future challenges

We identified three essential challenges.

First is early diagnosis. Still, early diagnosis of TS remains a challenge (123). Early identification is essential if we are to normalize growth during childhood, with the benefits that this will bring in terms of QoL and wellbeing. Thus, the observation of a significant difference in height$_{SDS}$ relative to mid-parental height (diffSDS) should raise the suspicion of TS and prompt referral for karyotyping

Although we have shown using current treatment regimens that most girls with TS can achieve a normal prepubertal height, and an AH within the normal range, it is obvious that growth during puberty remains suboptimal. As a result, the induction of puberty in girls with TS is often delayed until a point at which the individual is happy with her current height; this may be quite a bit later than the spontaneous onset of puberty in her peers. If we can diagnose and start GH treatment at an earlier age, it will provide the opportunity to normalize height while the individual is younger, thus allowing normal height during childhood and puberty to be induced at a more normal age.

Second is normalize puberty. Finding a way to improve pubertal growth for girls with TS remains also as a challenge. How should this be improved? The GH dose may need to more closely mimic the three–fourfold increment seen in puberty in non-TS girls (64). ERT may be optimized for better growth stimulation regarding gain and tempo/duration while still ensuring normal development of secondary sex characteristics (58). There are also other unanswered questions, such as possible substitution of the androgen deficiency (124). Whatever the direction of future studies on pubertal growth, evaluations will be facilitated from the newly developed growth models on pubertal growth references that enable individual monitoring of growth, such as height (81) weight, and BMI (82, 83), in an individual during the pubertal period relative to growth of individuals of a healthy population, aligned for the onset of puberty, spontaneously or at start of ERT.

Third is the lifelong structured follow-up. The challenge will be to study the long-term effects during lifespan of the combined administration of GH and ERT in women with TS. In particular, it is of interest to study possible hormone-sensitive age-related conditions such as hearing and balance, fracture risk, metabolic or cardiovascular diseases, and dementia. Follow-up investigations are already being conducted in women with TS, and hopefully, these will provide insights that will help us to shape future care.

5.9 Conclusion

This study of different GH doses on growth and puberty in girls with TS shows a clear dose effect on not only increased heights but also earlier ages for pubertal onset and AH. It highlights the importance of using a high GH dose when starting treatment to maximize prepubertal height gain and normalize childhood growth for optimal QoL and self-esteem from childhood onwards. Although the successful prepubertal gain was partly lost during puberty, an AH within the normal range was achieved, however, at the cost of both delayed pubertal development and attained AH. Of note, pubertal height gain was substantially lower in estradiol-treated girls with TS compared with those who underwent a spontaneous puberty. These findings highlight a need to improve pubertal GH and ERT regimens and to monitor changes during puberty more closely to this end, to achieve a more normal pubertal growth spurt, peak bone mass, and uterine size before transition. Future studies including individual GH dosing and growth-promoting pubertal induction and maintaining strategies in girls with TS are warranted, during the critical window possible in time,

knowing the importance for a pubertal development as normal as possible for psychosocial health, hearing, possible future fertility intervention, bone, and cardiovascular health.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committees of Sweden at the university hospitals in Lund (221/87), Gothenburg, Linköping, Umeå, Uppsala (all 76-88) and the Karolinska Institute (88-40). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

KON was the initial principal investigator; in 2014, he handed over to KA-W as principal investigator for these national TS investigator-initiated clinical trials (TRN 87-052-01 and TRN-072). Co-investigators from 1987 onwards were KA-W and from 1991 BK. KON and KA-W contributed to the study design. CA-L and KA-W wrote the statistical analysis plan and the first draft of the manuscript. BK, CA-L, M-LB, and KA-W contributed to the interpretation and analysis of data. BK, CA-L, M-LB, and KA-W contributed to the writing and revising of the manuscript for important intellectual content. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1197897/full#supplementary-material

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George Paltoglou,
National and Kapodistrian University of Athens, Greece

REVIEWED BY
Dimitrios T. Papadimitriou,
National and Kapodistrian University of Athens, Greece
Daisuke Areiyasu,
Kawasaki Municipal Hospital, Japan

*CORRESPONDENCE
Jenny Lam
jenny.lam40@gmail.com
Kanetee Busiah
Kanetee.Busiah@chuv.ch

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Turner syndrome: skin, liver, eyes, dental and ENT evaluation should be improved

Jenny Lam[1]*, Sophie Stoppa-Vaucher[2,3], Maria Cristina Antoniou[3], Thérèse Bouthors[3], Inge Ruiz[3], Nicole Sekarski[4], Tobias Rutz[5], Sophie Fries[6], Pierre Alain Binz[7], Florence Niel Bütschi[8], Nicolas Vulliemoz[9], Aneta Gawlik[10], Nelly Pitteloud[1,11], Michael Hauschild[1,3] and Kanetee Busiah[1,3]*

[1]Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland, [2]Department of Pediatric, Neuchatel Regional Hospital, Neuchâtel, Switzerland, [3]Pediatric Endocrinology, Diabetology and Obesity Unit, Women-Mothers-Children Department, Lausanne University Hospital, Lausanne, Switzerland, [4]Pediatric Cardiology Unit, Women-Mothers-Children Department, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland, [5]Cardiology Unit, Heart-Vessels Department, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland, [6]Pediatric Ear, Nose and Throat Unit, Surgery Department, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland, [7]Clinical Chemistry Service, Laboratory Medicine and Pathology Department, Lausanne University Hospital, Lausanne, Switzerland, [8]Laboratory Medicine and Pathology Unit, Genetic Labs Department, Lausanne University Hospital, Lausanne, Switzerland, [9]Centre de Procréation Médicalement Assistée (CPMA), Lausanne, Switzerland, [10]Department of Pediatrics and Pediatric Endocrinology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland, [11]Endocrinology, Diabetology and Obesity Unit, Medicine Department, Lausanne University Hospital, Lausanne, Switzerland

Introduction: Turner syndrome association with multi-organ system comorbidities highlights the need for effective implementation of follow-up guidelines. We aimed to assess the adequacy of care with international guidelines published in 2007 and 2017 and to describe the phenotype of patients.

Methods: In this multicenter retrospective descriptive cohort study, we collected growth and pubertal parameters, associated comorbidities, treatment, and karyotype in patients diagnosed at age <18 years between 1993 and 2022. We assessed age-appropriate recommendation follow-up (children, adolescents and adults) according to the 2007 guidelines if the last visit was before 2017 (18 recommendations) and the 2017 guidelines if the last visit was after 2017 (19 recommendations).

Results: We included 68 patients followed at Lausanne University Hospital (n=64) and at Neuchatel Regional Hospital (RHNe) (n=4). 2.9% of patients underwent all recommended investigations.Overall, 68.9 ± 22.5% and 78.5 ± 20.6% of the recommendations were followed, before and after 2017 respectively. High implementation rates were found for height, weight and BMI (100%), cardiac (80 to 100%) and renal (90 to 100%) imaging. Low implementation rates were found for Ear, Nose and Throat (ENT) (56.5%), skin (38.5%), dental (23.1%), ophthalmological (10%) and cholestasis (0 to 29%) assessments, depending on age and time of visit. In adults (n=33), the mean proportion of followed recommendations was lower before than after 2017: 63.5 ± 25.8% vs. 78.7 ± 23.4%, p=0.039.

Conclusion: Growth parameters, cardiac and renal imaging are well followed. However, efforts should be made for dental, ENT, ophthalmological, skin and cholestasis assessments. Adequacy of follow-up improved with the quality of transition to adult care.

KEYWORDS

Turner syndrome, international guidelines, follow-up, transition, recommendations, care coordination, comorbidities

1 Introduction

Turner syndrome (TS), caused by the complete or partial absence of one of the two X chromosomes, is the most common sex chromosome disorder in females, affecting approximately 1 in 2,000 live-born females. Comorbidities of TS may involve the endocrine system (the main clinical features are short stature, hypogonadism due to ovarian dysgenesis, and thyroid disease), the cardiovascular system (congenital heart disease, aortopathy, vasculopathy, arterial hypertension) and neuropsychocognitive development (e.g. learning difficulties). Other possible manifestations include hearing loss, orthopedic disorders (hip dysplasia, scoliosis, osteoporosis), renal and urinary tract disorders, (metabolic syndrome (hypertension, insulin-resistance, diabetes, overweight) or autoimmune disorders (hypothyroidism, celiac disease). This explains the need for screening and lifelong multidisciplinary follow-up of these patients (1). As growth and pubertal disorders are the main complaints of patients with Turner syndrome, the pediatric endocrinologist is usually the first to start the workup. The transition of care to adult specialists will most often occur at the end of puberty.

International clinical practice guidelines for TS were first published in 2007 (2) and updated in 2017, with recommendations for care across the lifespan and covering all health issues and comorbidities (3). Based on these recommendations, the pediatric and adult endocrine units of the Lausanne University Hospital developed an in-house clinical care guideline in 2011. This document was upgraded in 2019 into a mobile, electronic health (m-health) tool called the TS health transition passport (Supplementary Data Sheet 1). The aim is to support patients' understanding of their condition and improve effective transition to adult-oriented care.

The aims of our study were, first, to assess the adequacy of care according to published international recommendations in a tertiary center with pediatric and adult endocrine units (Lausanne University Hospital) and in a general hospital pediatric service (Neuchâtel Regional Hospital) and, second, to describe the clinical, biological and radiological phenotype of patients.

Abbreviations: AMH, Anti-Müllerian Hormone; Anti-TPO, Anti-Thyroperoxydase; BMI, Body Mass Index; CMR, Cardiac Magnetic Resonance; ECG, Electrocardiogram; ENT, Ear, Nose and Throat; FSH, Follicle-Stimulating Hormone; GH, Growth Hormone; IGF-1, Insulin-like Growth Factor One; IGFBP-3, Insulin-like Growth Factor Binding Protein-3; LH, Luteinizing Hormone; MRI, Magnetic Resonance Imaging; SD, Standard deviation; TTE, Transthoracic Echocardiography; TS, Turner Syndrome; US, Ultrasound.

2 Methods

We conducted a retrospective study on patients affected with Turner syndrome.

2.1 Patients' selection

We included patients with karyotype-confirmed TS, diagnosed before the age of 18 years and followed between 1993 and 2022 at the pediatric and adult endocrinology units of Lausanne University Hospital (CHUV) and the pediatric endocrinology unit of Neuchatel Regional Hospital (RHNe). No adult patients were followed at the RHNe. We excluded patients with less than 5% mosaic cells or with a written refusal.

2.2 Data collection

We collected clinical, radiological and biological data obtained during follow-up from medical records including karyotype, growth parameters, growth laboratory tests (IGF-1 and IGFBP-3 concentrations), growth treatment information and pubertal parameters.

Height was expressed as standard deviation (SD) using healthy female growth charts. Serum IGF-1 and IGFBP-3 were routinely assayed using IGF-1 and IGFBP-3 immunoassay kits: Nichols Institute Diagnostics from 1995 to 2005; Immulite by Siemens thereafter. Reference values were from Le Bouc Y for the Nichols Institute Diagnostics kit (4) and from Elminger et al. (5) for the Immulite kit.

We recorded comorbidities as follows: number of surgical treatments, presence of heart disease, hearing impairment, liver disease, dysthyroidism, renal disease, bone disease and celiac disease, transthoracic echocardiography (TTE) and cardiac magnetic resonance (CMR), abdominal-pelvic US, total body bone mineral density and laboratory tests such as creatinine, urea, anti-transglutaminase antibody, Thyroid Stimulating Hormone (TSH),

free Thyroxine (free T4), anti-TPO antibody, glycated hemoglobin (HbA1C), Alanine Aminotransferase (ALAT), Aspartate Aminotransferase (ASAT), Gamma-Glutamyl Transpeptidase (γGT) and Alkaline phosphatase (ALP) concentrations.

2.3 Adequacy of follow-up

We defined the last visit as the last endocrinology consultation and if there was none, the last specialist consultation for patients who were no longer followed up in the pediatric unit. The last visit was the date for assessing adequacy of follow-up. We defined good adequacy of recommendation if ≥65% of patients followed it.

To assess the adequacy of follow-up, patients were divided into 2 groups: those whose last visit was before 2017 and those whose last visit was after 2017. Their follow-up was compared with the appropriate guidelines at the time, i.e. the 2007 guidelines and the 2017 guidelines, according to their age and pubertal status. The results were as follows: children, adolescents (girls with a Tanner stage S2 stage or higher -spontaneous or with estrogen therapy- and <18 years old), and adults.

2.4 Karyotype and cohort description

The diagnosis of TS was confirmed in all patients by karyotype using routine G-banding, including counting of at least 30 metaphases. We divided patients into 4 groups according to their karyotype: complete monosomy X (45,X); 45,X mosaicism (45,X/46,XX; 45,X/47,XXX; 45,X/46,XX/47,XXX; 45,X/46,XY); X structural rearrangement [45,X/46,X,del(Xp), 46,X,del(Xp); 45,X/46,X,del(Xq), 46,X,del(Xq); 45,X/46,X,i(Xq), 46,X,i(Xq); 45,X/46,X,r(X)], and Y structural rearrangement [45,X/46,X,idic (Y)]. We then described clinical and biochemical profiles according to karyotype.

2.5 Statistical analyses

Qualitative data were expressed as absolute number (percentage) and quantitative data as median (interquartile range - IQR) or as mean ± Standard Deviation (SD). We compared groups with Kruskall-Wallis, Chi^2 or Fisher's exact tests, using R statistical software. Values of p smaller than 0.05 were considered statistically significant.

This study was approved by the Local Ethics Committee (N° 2021-00229).

3 Results

We included 68 patients (Figure 1). Mean age at diagnosis was 6.3 ± 5.3 (range: 0 to 16.7) years. At last visit, there were 13 children, 22 adolescents and 33 adults. Mean age at last visit was 17.6 ± 7.5 (range: 1.2 to 40.5) years. We identified 17 patients (25%) who had their last visit before 2017 and 51 (75%) patients who had their last visit after 2017 (Table 1). All children had their last visit after 2017.

In our clinic, we implemented 3 additional recommendations: IGFBP-3, thyroid US and bone age assessment, that are usually performed in children affected with growth retardation or dysthyroidism (Supplementary Table 1).

3.1 Follow-up adequacy

Overall, 2.9% (n=2/68, one child and one adult last seen after 2017) of the patients underwent all recommended investigations. The overall mean proportion of recommendations followed was 76.1 ± 21.4%: 68.9 ± 22.5% of the 2007 recommendations for patients last seen before 2017 and 78.5 ± 20.6% of the 2017 recommendations for patients last seen after 2017 (Table 1 and Supplementary Table 1).

3.1.1 Adequacy of follow-up according to recommendations

The recommendations with the highest implementation rate were height, weight and BMI (100%), and cardiac (range: 80 to 100%) and renal (range: 90 to 100%) imaging. The recommendations with the lowest implementation rate were bone mineral density (in adults last seen before 2017: 30%), skin examination (in children: 38.5%), ENT (in adolescents last seen before 2017: 57% and in adults last seen after 2017: 56.5%), ophthalmological (in adolescents and adults last seen before 2017: respectively: 0% and 10%, and children: 61.5%) and dental consultations for the whole cohort (Table 1). Liver function biomarkers were often not assayed, especially ASAT and ALAT for adolescents last seen before 2017 (43%), and γGT and ALP for the whole cohort (Table 1).

We found a difference for HbA1C between adolescents and adults last seen before 2017 (100% vs. 40% p=0.035) and for fertility counseling among all patients last seen after 2017 (38.5% of children, 93.3% of adolescents and 91.3% of adults, p<0.001).

3.1.2 Adequacy of follow-up according to age

In children (n=13), the overall mean proportion of recommendations followed was 75.5 ± 19.1% (Table 1). We found good adequacy (i.e., ≥65% of recommendations followed) for 11/16 (69%) of the recommendations in children. In adolescents (n=22), we found no difference between overall followed recommendations for patients last seen before 2017 compared to patients last seen after 2017 (76.6 ± 15.1% vs. 80.9 ± 18.3%, p=0.306). We found good adequacy for 9/15 (60%) of the recommendations for adolescents last seen before 2017 and 14/17 (82%) of the recommendations for adolescents last seen after 2017. In contrast, in adults (n=33), the mean proportion of overall followed recommendations was lower before than after 2017: 63.5 ± 25.8% vs. 78.7 ± 23.4%, respectively, p=0.039. We found good adequacy for 8/16 (50%) of the recommendations for adults last seen before 2017 and for 13/17 (76%) of the recommendations for adults last seen after 2017.

All children and adolescents had cardiac imaging, whereas 20% and 13% of adults last seen before and after 2017 respectively did not have any cardiac imaging; the difference was not significant.

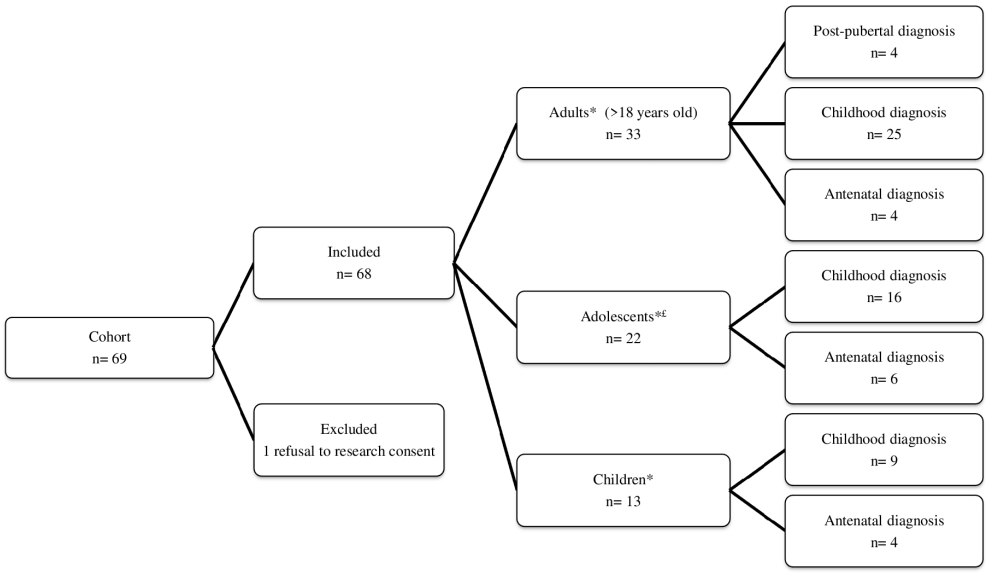

FIGURE 1
Patient flow chart. *Age at last visit: £Girls with a Tanner S2 stage or above (spontaneously or with estrogen therapy) and aged below 18 years old.

TABLE 1 Adequacy of recommendations of care according to the 2007 and the 2017 international guidelines.

Items	2007 recommendations[1]				2017 recommendations[2]				
	Monitoring	Adolescents	Adults	P-value [3]	Monitoring	Children	Adolescents	Adults	P-value [3]
Number of patients total n=68 (100%)		7	10	NA		13	15	23	NA
Clinic (%)									
Height/Weight/ BMI	Not mentioned	NA	NA	NA	Annually	100	100	100	1
Fertility counseling	At least once	100	90	1	At least once	38.5	93	91	<0.001
ENT and audiology	Each 1-5 Y	57	80	0.6	Each 3 Y (children and adolescents), each 5 Y (adults)	77	80	56.5	0.2
Ophthalmology	Between 0-4 Y (If age > 1Y)	0	10	1	At least once	61.5	67	78	0.5
Skin examination	Not mentioned	NA	NA	NA	Annually	38.5	67	70	0.2
Dental specialist	At least once	43	30	0.6	At least once	23	53	48	0.2
Biology (%)									
Fasting glucose	Annually (> 10Y)	100	90	1	Annually (> 10Y)	100[4]	87	91	1
HbA1c	Annually (> 10Y)	100	40	**0.035**	Annually (> 10Y)	100[4]	87	61	0.2
IGF-1 (on GH treatment)	Not mentioned	NA	NA	NA	Annually (< 18 Y)	70[5]	64[5]	NA	1

(Continued)

TABLE 1 Continued

Items	2007 recommendations[1]				2017 recommendations[2]				
	Monitoring	Adolescents	Adults	P-value [3]	Monitoring	Children	Adolescents	Adults	P-value [3]
TSH and Free T4	Annually (> 4 Y)	86	70	0.6	Annually (no starting age)	69	100	83	0.066
Anti-TPO antibody	Not mentioned	NA	NA	NA	If dysthyroidy	No dysthyroidy	100 [7]	100 [7]	1
Celiac screen	Each 2-5 Y (> 4Y)	86	60	0.4	Each 2 Y (from 2 to 18 Y)	69	93	NA[6]	0.2
Total cholesterol, HDL, LDL, TG	Annually (> 10 Y)	86	70	0.6	Annually (> 18 Y if cardiovascular risk factors[8])	NA	NA	87	NA
Creatinine/Urea	Each 1-2 Y	29	40	1	Not mentioned	NA	NA	NA	NA
ASAT/ALAT	Annually (> 10Y)	43	60	1	Annually (> 10Y)	100[4]	80	83	1
γGT/ALP	Annually (> 10Y)	29	20	0.6	Annually (> 10Y)	0[4]	27	52	0.2
Imaging (%)									
Renal US	At least once	100	90	1	At least once	100	93	96	1
ECG	At least once	86	70	0.6	At least once	92	100	91	0.6
TTE or CMR	Each 5-10 Y[9]	100	80	0.5	Each 5 Y	100	100	87	0.2
Bone mineral density	No specific time interval (>18 Y)	NA	30	NA	Each 5 Y (> 18 Y)	NA	NA	78	NA

ALAT, Alanine Aminotransferase; ALP, Alkaline phosphatase; ASAT, Aspartate Aminotransferase; BMI, Body Mass Index; CMR, Cardiac magnetic resonance; ECG, Electrocardiogram; ENT, Ear, Nose and Throat; γGT, Gamma-Glutamyl Transpeptidase; HBA1C, Glycated hemoglobin; IGF-1, Insulin-like Growth Factor 1; NA, Non-applicable; T4, Thyroxin; TG, Triglyceride; TSH, Thyroid Stimulating Hormone; TTE, Transthoracic echocardiography; US, Ultrasound; Y, years.
All children had their last follow up visit after 2017.
[1]From Bondy et al. (2007); [2]From Gravholt et al. (2017);[3]The p-values were calculated across groups: "Children", "Adolescents" and "Adults" with the Fisher's Exact Test; [4]1 patient > 10 Y; [5]10 children and 11 adolescents with GH treatment, [6]To do when suggestive symptoms [7]2 adolescents and 10 adults with dysthyroidsm, [8]Considered to be present in all adults in our cohort, [9]if normal anatomy, otherwise according to the opinion of the cardiologist.

3.2 Growth, puberty and comorbidities

We then compared patients' clinical, biological and radiological findings according to karyotypes, that were as follows: monosomy 45,X (n=24, 35.3%); 45,X mosaicism (n=18, 26.5%); X chromosome structural rearrangement (n=24, 35.3%); and Y chromosome structural rearrangement (n=2, 2.9%).

Height and height velocity at diagnosis were significantly different across the groups (p=0.031 and p=0.019, respectively) (Table 2). Nevertheless, final height was similar across the groups (p=0.106).

Among the 86.8% of patients who had GH treatment, height and height velocity at the start of GH were significantly different across the groups (p=0.017 and 0.006 respectively) (Table 3). One year after the start of GH treatment, height velocity was no longer different across the groups (p=0.971) while the height remained significantly different (p=0.021). Bone age delay of more than 1 year was found in 23/46 (50%) of patients at the start of GH treatment and in 12/46 (26.1%) of patients at the end of GH. In these 12 patients, GH therapy was discontinued despite the bone delay, because of patients' willingness.

Puberty was spontaneous in 3 (13.6%) 45,X patients (Table 4). Puberty was induced in 28 (50.9%) patients, mainly in the 45,X group (p<0.001), and after 12 years of age for 20/28 (71.4%) patients. 40 patients received estrogen therapy, 29 by oral administration route and 11 by transdermal administration route.

Heart disease, kidney disease, celiac disease and surgery were significantly different according to their karyotype (Table 5). Osteopenia, defined as a BMD Z-score <-1, was present in 7/24 (29%) patients.

4 Discussion

In this cohort of children, adolescents and adults patients with TS, a small minority of patients had a complete follow-up according to the international guidelines. However, the most important and potentially serious comorbidities were well followed-up, especially growth parameters, cardiac assessment and renal ultrasound. Adequacy of follow-up improved with quality of transition to adult care.

Four studies evaluated the adequacy of care according to international guidelines: three compared the adequacy of follow-up with the 2007 guidelines, and one with the 2017 guidelines (6–9). Two studies from France and Poland found that less than 5% of adult patients received all the medical investigations recommended

TABLE 2 Anthropometric characteristics according to karyotype.

	Total	Complete Monosomy X	45,X Mosaicism	X Structural Rearrangement	Y Structural Rearrangement	p-value*
Turner syndrome *n* (%)	68 (100%)	24 (35.3%)	18 (26.5%)	24 (35.3%)	2 (2.9%)	NA
At diagnosis						
Age at diagnosis, years	5.3 (0.8 to 9.9)	4.9 (0.0 to 10.5)	2.15 (0.0 to 6.1)	8.1 (4.5 to 11.2)	9.0 (6.8 to 11.3)	0.015
Height at diagnosis, SD	-2.2 (-2.5 to -1.6)	-2.3 (-2.4 to -1.6)	-1.8 (-2.1 to -0.6)	-2.4 (-3.0 to -1.9)	-2.5	0.031
Height velocity at diagnosis, SD	-1.1 (-2.2 to 1.3)	-2.4 (-2.7 to -2.1)	1.9 (0.3 to 2.0)	-0.3 (-1.0 to 2.0)	Not available	0.019
At last visit						
Age at last visit, years	17.9 (13.6 to 21.7)	19.4 (16.5 to 25.9)	17.6 (12.7 to 21.8)	16.2 (12.8 to 19.9)	12.1 (10.7 to 13.6)	0.084
Height at last visit, SD	-1.6 (-2.3 to -1.1)	-1.8 (-2.2 to 1.4)	-0.8 (-1.6 to -0.2)	-2.2 (-2.7 to -1.3)	-0.7 (-1.1 to -0.2)	0.002
Final height, SD	-1.7 (-2.4 to -1.1)	-1.8 (-2.2 to -1.3)	-1.4 (-1.9 to -0.2)	-2.3 (-2.8 to -1.3)	Not available	0.106

Quantitative data are expressed as median and interquartile range (IQR).
SD, Standard Deviation.
*The p-values were calculated across 3 groups: "Complete Monosomy X", "45,X Mosaicism" and "X Structural Rearrangement" with the Kruskal-Wallis test.

in the 2007 guidelines. They showed that liver enzymes were often not assayed (6, 7). The prevalence of liver disease is higher in adults with TS, especially with elevated γGT rather than transaminases (10). Reported complications include non-alcoholic steatohepatitis, hepatic architectural changes such as cirrhosis, and biliary lesions such as sclerosing cholangitis (1, 11). An American study evaluated the medical care of girls with TS compared to the 2007 guidelines. In our study, adherence to recommendations was higher for follow-up of lipid levels, liver enzymes, blood glucose, thyroid function, ENT assessments, fertility counselling, celiac screening and bone mineral density and cardiac imaging, depending on the study (6–8). However, celiac screening, ENT and ophthalmological assessments were lower than in the study published by Hoag and colleagues (9). This suggests an improvement in the management of patients with TS. There is still room for improvement in care coordination. The implementation of new models of care coordination could help (12).

We focused on more recommendations from the 2007 and 2017 guidelines than these studies, especially dental, eye and skin examinations. Lack of compliance with these follow-up visits can lead to reduced quality of life. This highlights the importance of awareness among clinicians. Dermatological screening aims to detect lymphedema, dermatitis, eczema, psoriasis and multiple pigmented nevi (1, 13). Eye disorders include high rates of strabismus, visual impairment such as myopia, or sight-threatening abnormalities such as papilledema (14). The time interval recommended by the 2007 guidelines for seeing an ophthalmologist was very restrictive. This might explain the poor follow-up adequacy of ophthalmic consultations for patients with last visit before 2017. Dental disorders include a wide range of manifestations from micrognathia to abnormal dental development (1). The fact that dental consultations are not covered by the Swiss National Health Insurance may explain the low number of dental consultations in our cohort. The distribution of karyotypes in our cohort showed slightly higher proportion of X structural rearrangement than previously published (15). The comorbidities and their distribution between the different karyotypes in our cohort were globally consistent with the literature (1, 16–18). In our study, we found lower proportions of heart and liver diseases but similar proportions of thyroid and celiac diseases (15). Nevertheless, the number of comorbidities should be correlated with the adherence to recommended follow-up.

All recommendations, except HbA1c for patients last seen before 2017 and fertility counselling for patients last seen after 2017, were monitored equally between children, adolescents and adults. This may reflect the structured transition clinic between pediatric and adult endocrinological care that we have developed at the CHUV. This transition endocrine clinic has improved, as suggested by a better follow-up of adults last seen before 2017 compared to those last seen after 2017. As all RHNe patients are children or adolescents, they did not have transition. Our group has shown the usefulness of integrating transition passports as a usable, understandable health tool for patients and physicians, to reduce gaps in transition from pediatric to adult-oriented care (19). Our study suggests that the CHUV pediatric and adult TS transition passport could provide patients with a better understanding of their follow-up, of treatment, fertility care and comorbidities.

GH treatment followed the 2017 guidelines in terms of starting age, doses and IGF-1 monitoring. Estrogen treatment was started at low doses and increased over 2 to 3 years as recommended. However, the majority of patients started later than recommended. This discrepancy may be explained by the fact that only five patients started treatment after the publication of the 2017 guidelines (3). Late initiation of estrogen therapy can be detrimental to bone and uterine health (20). Data are consistent with no change in adult height when low-doses estrogen is started before the age of 12, as recommended.

The strengths of our study include the comparison of long-term medical follow-up of girls and women with TS between the two published international guidelines. Studies on the adequacy of medical follow-up in girls and women with TS are limited (6–9), and most have compared the adequacy of follow-up with the 2007 guideline. We divided our cohort into 3 different age groups, as in the study by Hoag and colleagues (9). We also included additional important recommendations such as dental and ophthalmological

TABLE 3 Main clinical and laboratory characteristics on growth hormone treatment according to karyotype.

	Total	Complete Monosomy X	45,X Mosaicism	X Structural Rearrangement	Y Structural Rearrangement	p-value*
Growth hormone treatment n (%)	59 (86.8%)	23 (95.8%)	12 (66.7%)	22 (91.7%)	2 (100%)	0.094
At start of GH						
Age, years	7.4 (5.0 to 10.6)	6.5 (4.5 to 10.5)	7.0 (5.4 to 9.8)	8.9 (6.0 to 11.2)	9.07 (6.9 to 11.3)	0.554
Starting dose of GH, µg/kg/day	34.0 (22.9 to 41.4)	35.71 (22.9 to 41.4)	38.6 (24.3 to 41.4)	25.7 (22.9 to 37.1)	31.4 (30.0 to 31.4)	0.584
Height, SD	-2.3 (-2.6 to -1.6)	-2.4 (-2.5 to -2.0)	-1.5 (-2.1 to -1.3)	-2.4 (-3.0 to -2.1)	-1.6 (-2.1 to -1.2)	0.017
Height velocity, SD	-1.4 (-1.9 to 0.0)	-1.8 (-2.8 to -1.6)	0.0 (-0.2 to 1.9)	-1.4 (-2.1 to -1.1)	Not available	0.006
IGF-1, SD	-0.8 (-1.4 to 0.3)	-1.2 (-2.2 to -0.3)	-0.5 (-1.2 to 0.3)	-0.8 (-1.3 to 0.1)	-0.6	0.393
IGFBP-3, SD	0.5 (-0.0 to 1.3)	0.3 (-0.2 to 0.8)	1.1 (0.2 to 1.8)	0.5 (0.2 to 0.8)	-1.8	0.382
One year after start of GH						
Height, SD	-1.9 (-2.2 to -1.1)	-1.8 (-2.3 to -1.3)	-1.0 (-1.8 to -0.7)	-2.1 (-2.5 to -1.8)	-1.4 (-1.7 to -1.0)	0.021
Height velocity, SD	1.4 (0.6 to 2.5)	1.5 (0.3 to 3.8)	1.7 (1.0 to 2.1)	1.4 (0.7 to 2.3)	1.1 (1.1 to 1.1)	0.971
At maximum dose of GH						
Maximum dose of GH, µg/kg/day	45.7 (42.9 to 50.0)	47.1 (47.1 to 58.6)	45.7 (42.9 to 50.0)	42.9 (42.9 to 48.6)	31.4	0.060
Age, years	11.6 (8.9 to 13.4)	12.0 (8.6 to 14.0)	11.5 (9.7 to 12.6)	10.8 (8.9 to 13.3)	15.0	0.895
IGF-1, SD	0.9 (0.1 to 1.8)	0.3 (-0.5 to 1.1)	0.7 (0.3 to 1.2)	1.4 (0.9 to 2.2)	0.6	0.018
IGFBP-3, SD	0.9 (0.2 to 1.3)	0.9 (0.1 to 1.3)	1.2 (0.7 to 1.4)	0.8 (0.4 to 1.2)	0.2	0.695
At the end of GH						
Age, years	15.6 (14.9 to 17.0)	16.3 (15.5 to 17.4)	14.9 (14.7 to 15.2)	16.6 (14.8 to 17.2)	Non applicable	0.011
Dose of GH, µg/kg/day	42.9 (41.4 to 45.7)	42.9 (41.4 to 45.7)	41.4 (40.0 to 45.7)	41.4 (38.6 to 45.7)	Non applicable	0.458
GH duration, years	8.0 (4.6 to 10.1)	8.9 (6.0 to 11.2)	8.0 (5.2 to 9.8)	7.2 (2.6 to 8.1)	Non applicable	0.192

Quantitative data are expressed as median and interquartile range (IQR).
GH, Growth Hormone; IGF-1, Insulin-like Growth Factor One; IGFBP-3, Insulin-like Growth Factor Binding Protein-3; SD, Standard Deviation.
*The p-values were calculated across 3 groups: "Complete Monosomy X", "45,X Mosaicism" and "X Structural Rearrangement" with the Kruskal-Wallis test.

consultations and skin examinations, which are rarely investigated, and we could perform a karyotype in all patients. This study looked at patients with TS followed at a large university center (CHUV) with many experienced specialists, including cardiologists, radiologists, ear, nose and throat specialists, adult endocrinologists, and others. As a result, follow-up of these patients may not be the same throughout Switzerland. Moreover, in other studies, most adult patients with TS were followed up by general practitioners, who were sometimes unaware of the TS diagnosis. For this reason, we developed a patient oriented electronic health (m-health) TS health transition passport, to avoid loss of medical information (Supplementary Data Sheet 1).

A limitation of our study is its retrospective design, which is justified by the rarity of the disease. However, we minimized missing data through in-depth analysis of medical records.

Our study highlights the importance of improving awareness among patients themselves and primary care physicians of the broad spectrum and variability of TS presentation at different ages. We should aim to reduce health inequalities by making multidisciplinary clinics and comprehensive care available and accessible. It is also important to ensure adequate medical and social support for transition of young adults and care of adults with TS. We also should involve the patient, who gains autonomy and responsibility for her health care during adolescence and young adulthood. Our TS education program, launched in 2011 and improved in 2019, aims to address these challenges. We aim to serve as a regional resource for the community and for physicians in our community.

In conclusion, complete guideline adherence in TS patient care and follow-up should be improved, especially in bone mineral density, liver, ophthalmic, ENT, dermatological and dental assessment. Our results open a field for possible future research on patient education and healthcare organizations: how to understand the lack of awareness in TS, how to improve structural problems, how to implement a complete work-up or how to spread these guidelines among non-endocrinologists, especially pediatricians and general practitioners. A better follow-up has already been observed compared to the studies before 2017, which makes us optimistic for the future.

TABLE 4 Clinical and biological puberty characteristics according to karyotype.

	Total	Complete Monosomy X	45,X Mosaicism	X Structural Rearrangement	Y Structural Rearrangement	p-value*
Spontaneous puberty onset n (%)	27 (49.1%)	3 (13.6%)	12 (85.7%)	12 (66.7%)	0 (0%)	<0.001
Induced puberty onset n (%)	28 (50.9%)	19 (86.4%)	2 (14.3%)	6 (33.3%)	1 (100%)	
Age at the onset of puberty, years	12.0 (11.1 to 12.7)	12.3 (11.6 to 13.5)	10.3 (10.2 to 11.6)	12.1 (11.8 to 13.0)	13.9	0.001
E2 therapy n (%)	40 (72.7%)	22 (100%)	3 (21.4%)	14 (77.8%)	1 (100%)	<0.001
Age at the start of E2, years	12.8 (12.1 to 15.0)	12.5 (11.9 to 14.6)	11.8 (11.7 to 14.2)	13.8 (12.8 to 15.4)	13.9	0.104
Starting dose of E2,µg/day	3 (2 to 20)	3 (2 to 10)	4 (3 to 502)	6 (2 to 813)	5	0.260
Progesterone therapy n (%)	32 (58.2%)	19 (86.4%)	2 (14.3%)	11 (61.1%)	0 (0%)	<0.001
Age at the start of Progesterone, years	16.5 (14.5 to 17.4)	15.8 (14.5 to 17.4)	15.4 (14.9 to 16.0)	17.1 (15.8 to 17.4)	Non applicable	0.524
Starting dose of Progesterone, mg/day	10	10	10	10	Non applicable	0.575
FSH at start of E2 or at start of puberty, U/l	27.4 (4.0 to 87.5)	81.9 (31.8 to 121.6)	3.9 (1.9 to 8.9)	47.0 (7.5 to 82.7)	98.6	0.001
LH at start of E2 or at start of puberty, U/l	13.1 (1.3 to 24.2)	23.7 (15.3 to 28.5)	2.0 (0.5 to 6.5)	12.7 (1.0 to 21.7)	12.6	0.010
AMH, pmol/l	13.1 (9.0 to 22.4)	8.8	16.9 (9.7 to 23.1)	12.0 (8.0 to 28.5)	Non applicable	0.777**
Spontaneous menstruation n (%)	24 (46.2%)	2 (9.5%)	12 (85.7%)	10 (62.5%)	0 (0%)	<0.001
Induced menstruation n (%)	24 (46.2%)	16 (76.2%)	2 (14.3%)	5 (31.3%)	1 (100%)	
Age at first menstrual period, years	14.4 (13.2 to 15.5)	15.7 (14.4 to 16.9)	12.7 (12.1 to 13.6)	14.4 (13.3 to 15.3)	Non applicable	<0.001
Total body bone densitometry, Z-score	-0.1 (-1.3 to 0.6)	-0.3 (-1.2 to 0.6)	1.3 (-0.1 to 1.8)	-0.3 (-1.4 to 0.2)	Non applicable	0.221
Age at bone densitometry, years	18.9 (14.9 to 23.3)	23.3 (18.9 to 27.2)	18.6 (14.7 to 22.1)	16.1 (12.9 to 21.4)	Non applicable	0.055

Quantitative data are expressed as median and interquartile range (IQR).
AMH, Anti-Müllerian Hormone; E2, Oestrogen; FSH, Follicle Stimulating Hormone; LH, Luteinizing hormone.
*These p-values were calculated across 3 groups "Complete Monosomy X", "45,X Mosaicism" and "X Structural Rearrangement" with the Kruskal-Wallis test.
**These p-values were calculated across 2 groups "45,X Mosaicism" and "X Structural Rearrangement". AMH was detectable in one patient in the "Complete Monosomy X" group.

TABLE 5 Frequency of co-morbidities and associated features according to karyotype.

	Total	Complete Monosomy X	45,X Mosaicism	X Structural Rearrangement	Y Structural Rearrangement	p-value***
Heart disease n (%)	31 (45.6%)	16 (66.7%)	8 (44.4%)	7 (29.2%)	0 (0%)	0.011
Hearing impairment n (%)	25 (36.8%)	11 (45.8%)	5 (27.8%)	8 (33.3%)	1 (50%)	0.377
Hypothyroidism n (%)	15 (22.1%)	7 (29.2%)	4 (22.2%)	4 (16.7%)	0 (0%)	0.576
Renal disease n (%)	13 (19.1%)	8 (33.3%)	2 (11.1%)	2 (8.3%)	1 (0%)	0.049
Bone disease* n (%)	6 (8.8%)	2 (8.3%)	1 (5.6%)	3 (12.5%)	0 (0%)	0.867
Celiac Disease n (%)	4 (5.9%)	4 (16.7%)	0 (0%)	0 (0%)	0 (0%)	0.016
Liver disease** n (%)	3 (4.4%)	1 (4.2%)	0 (0%)	2 (8.3%)	0 (0%)	0.771

*Bone disease referred to osteopenia, Léri-Weill dyschondrosteosis and cartilaginous protrusion of the ribs.
**Liver diseases were steatohepatitis with hepatomegaly for a 16 years and a 20 years old patient and inflammatory liver disease for a 30 years old patient.
***The p-values were calculated across 3 groups "Complete Monosomy X", "45,X Mosaicism" and "X Structural Rearrangement" with the Chi^2 test or with Fisher's exact test when Chi^2 test was not applicable.

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/s.

Ethics statement

The studies involving human participants were reviewed and approved by Commission cantonale (VD) d'éthique de la recherche sur l'être humain (CER-VD). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

JL collected the data, performed the data analysis and interpretation, performed the statistical analysis, wrote, and critically revised the manuscript. KB designed the study, was responsible for the data analysis and interpretation, and for the statistical analysis, wrote, and critically revised the manuscript. NV and MH interpreted the data and critically revised the manuscript. SS-V, MA, TB, IR, NS, TR, SF, NP, MH, and KB followed the patients, PB was responsible for hormonal assay, FB was responsible for genetic investigation. AG provided feedback on the study design. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1190670/full#supplementary-material

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EDITED BY
Tommaso Aversa,
University of Messina, Italy

REVIEWED BY
Roberto Lanes,
Hospital de Clinicas Caracas, Venezuela
Luisa De Sanctis,
University of Turin, Italy

*CORRESPONDENCE
Baryab Zahra
b.zahra@nhs.net

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Turner syndrome transition clinic in the West of Scotland: a perspective

Baryab Zahra[1]*, Aparna Sastry[2], Marie Freel[3], Malcolm Donaldson[4] and Avril Mason[4]

[1]Department of Paediatric Endocrinology, Queen Elizabeth University Hospital, Glasgow, United Kingdom, [2]Assisted Conception Service, Glasgow Royal Infirmary, Glasgow, United Kingdom, [3]Department of Endocrinology, Queen Elizabeth University Hospital, Glasgow, United Kingdom, [4]Section of Child Health, Glasgow University School of Medicine, Glasgow, United Kingdom

Introduction: Turner Syndrome (TS) is the commonest chromosomal abnormality in females. Establishing and maintaining long-term follow-up after transition to adult endocrine services, to allow for essential lifelong surveillance of hypertension and cardiovascular disease, and optimal hormone replacement, remains a challenge. A TS transition clinic was established with the aim of supporting successful transfer and establishing long-term follow-up in adult endocrine services. Our objectives are to evaluate the success of our TS transition service primarily in achieving and maintaining follow-up after transfer to adult services and to assess the adequacy of health surveillance post-transition with a specific focus on cardiac monitoring and hormone replacement.

Methods: A departmental database was used to identify young people whose care had transferred to adult endocrine services. An electronic case record was utilised to obtain clinic attendance and relevant clinical information on cardiovascular monitoring and hormone replacement therapy (HRT).

Results: Forty-six (n=46) young people transferred to adult endocrine services during the observed 20-year period, 1998-2017. Thirty-six (n=36) had transferred prior to 2015, of whom sixteen (n=16, 44%) are lost to long-term follow-up at 5 years. Overall, 41 (89%) patients have had cardiac imaging surveillance since transferring, However, only 30 (73%) of these were carried out at the recommended frequencies. All 20 women in established follow-up have had cardiac imaging. Five out of the 46 (11%) patients do not have any documented cardiovascular monitoring. Forty (86.9%) women have had a documented BP measurement. Nineteen of the 20 women who are in 5- year established follow-up have a documented blood pressure. Five (11%) women are not on HRT, while two (4%) remain on oestrogen-only HRT. Thirty-seven (80.4%) women are on combined HRT, only eight (21.6%) are on the recommended form of oestradiol. Two (4%) are not on HRT due to normal ovarian function.

Conclusion: A significant proportion of girls with TS are currently lost to adult endocrine services. Strategies to improve long-term endocrine follow-up are needed to ensure lifelong health needs and adequate hormone replacement are met. Whilst similar parameters are monitored in adult endocrine services a group of patients may be at risk of receiving inadequate HRT and developing cardiovascular complications.

KEYWORDS

Turner syndrome, cardiovascular, HRT, blood pressure, transition

Introduction

Turner Syndrome affects 1/2500 of live female births and is the commonest chromosomal abnormality in females (1). TS continues to be diagnosed across the lifespan with peaks during foetal life, infancy, late pre-pubescence (8–12years) and during late adolescence/early adulthood (2). The first presentation to healthcare services is often consequential to short stature or pubertal delay, however, the clinical presentation of TS ranges from a classic appearance with many physical differences to individuals who have no apparent or minimal observable features (1).

Optimal care in TS includes regular monitoring through childhood, adolescence and into adult life to screen for associated complications. In childhood and adolescence early screening and intervention are required to offset the following problems: short stature, delayed puberty, abnormal alignment of one or both eyes, hearing loss, heart and kidney abnormalities, thyroid dysfunction, and gluten intolerance. In late adolescence and early adulthood, screening and intervention are required to offset the following additional complications: hypertension, coarctation and aortic dissection (3), hypogonadism and infertility, osteoporosis, hearing impairment, increased risk of developing diabetes, and obesity (4).

Young women with TS have complex health needs which require life-long treatment through seamless transfer from paediatric to adult services. Management of any individual with TS requires support from an extensive and non-exhaustive list of specialties which may include the following: endocrinology, cardiology, gynaecology, audiology, ENT, urology, renal medicine, and clinical psychology. Establishing and maintaining long-term follow-up after transition to adult services remains a challenge to paediatric and adult services and requires active participation from both (5).

A TS transition clinic was set-up in 1998 at the Royal Hospital for Children Glasgow (RHCG), to coordinate transfer from paediatric endocrine to adult endocrine clinics. The TS Transition clinic serves to introduce girls with TS to members of the adult multi-disciplinary endocrine team in a paediatric setting with the aim of supporting a successful transition and establishing long-term follow-up in adult life. The primary aim of our study is to establish the success of the TS transition clinic, using the proportion of girls with TS who remain in established adult endocrine follow-up at three and five years after transfer as the primary outcome measure. Our secondary aim was to establish if the recommendations outlined in European Society for Endocrinology with respect to monitoring for cardiovascular complications (hypertension, aortic dilatation and dissection) and hormone replacement therapy (HRT) were adhered to following transfer to adult services. Recommendations include checklists, these can be used during the process of transitioning to ensure appropriate parameters are monitored. Cardiovascular complications are the commonest cause of early mortality and research has shown a reduced lifespan by 10 years in TS patients (6). Additionally, adequate HRT use has been shown to result in reduced hospitalisations with osteoporotic fractures and strokes (7). We therefore focused on assessing compliance with recommendations for these parameters. The current recommendations for cardiac monitoring suggest performing transthoracic echo (TTE) or Cardiac MRI (CMR) every 5 years in paediatrics and every 10 years in adult services in the absence of significant cardiac disease or abnormality. If cardiac abnormality such as Bicuspid Aortic Valve (BAV) or Coarctation of Aorta (CoA) exists then the recommendation is to perform TTE/CMR every 2-3 years or every 6 to 12 months, respectively. It is suggested to monitor BP annually and treatment should be commenced in a timely manner if indicated in the form of a beta- blocker, angiotensin receptor blocker or both (6). Hormone replacement therapy in Turner Syndrome is important for achieving adequate bone mass and maintaining optimal reproductive function, as well as facilitating development of secondary sexual characteristics. Pubertal induction, in the presence of ovarian insufficiency, should be commenced at the age of 11-12 years with incremental doses of oestradiol building to an adult dose of replacement over 2-3 years. 17 β-oestradiol (E2) is known to be more physiological compared to synthetic forms and is therefore recommended, with transdermal route preferred over the oral route to avoid first pass metabolism (8). A previous study has analysed oestrogen replacement trends in a large population of TS patients (n=627) and shows administration of oral and transdermal E2 increased collectively by 2010 in comparison to use before 1995, reinforcing recommendations of preferential use of E2 as set out by the European Society for Endocribology (9). Progesterone is added once tanner stage 4 is attained or after 2-3 years of unopposed oestrogen or after an initial breakthrough bleed has occurred. Hormone replacement therapy, with oestrogen and progesterone, should be continued until an age of natural menopause is reached (2).

Materials and methods

A paediatric endocrine departmental database containing information on all TS patients who had attended the TS transition clinic at the Royal Hospital for Children, Glasgow, and had subsequently had their care transferred to an adult endocrine service (between years 1998 and 2017) was used. Information collected from the database included the dates of attendance/non-attendance at the TS transition and adult Endocrine clinics. Relevant clinical data collected from the database included the following: information on co-morbidities, cardiovascular complications (hypertension, bicuspid aortic valve and previous surgery for coarctation), evidence of preserved ovarian function with spontaneous onset of puberty and regular menses, prescribed hormone replacement therapies. Additional clinical data, following transfer to an adult endocrine service, was gathered using access to a clinical electronic medical record. Information sourced from the clinical electronic medical record included the following: secondary/tertiary care clinical appointment letters, blood test results, current active prescriptions, upcoming clinic appointments with dates, previously attended and missed clinical appointments in the West of Scotland. The information collated from the clinical electronic medical record included the following:

frequency of blood pressure measurement in an adult clinical setting, values of systolic and diastolic blood pressures were compared to standards used to diagnose hypertension in an adult population, current treatment with an anti- hypertensive therapy, frequency of cardiac imaging in an adult setting (echocardiogram or cardiovascular MRI), available obstetric/pregnancy history and current hormone replacement therapy, oestradiol (E2) and progesterone are currently recommended (8)

Results

Follow-up

Overall, 53 girls had attended a Turner transition clinic and transferred to an adult endocrine clinic between 1998 and 2017 inclusive. Seven cases were excluded from further analysis because of: relocation outside the region (n=1), incomplete information (n=5) and deceased (n=1). Attendance was assessed in the remaining 46 girls, median 18.3 years (range, 16.0-21.5) at time of transfer. Of these 46 girls, 36 had their care transferred to an adult endocrine service before 2015 and their data were used to assess long- term follow up in an adult endocrine clinic at three and five years. Of these patients, 26/36 (72.2%) were in established follow-up at three years and 20/36 (55.5%) remained in established follow-up at 5 years.

Assessment of adequacy of hormone replacement therapy

Table 1 shows HRT preparation currently in use by 43 women. A combined form of progesterone and oestrogen is in use by 37/46 (80%), however only 8 (21.6%) are on the recommended form of oestradiol. Two out of 46 (4%) are only on one form of hormone replacement (Ethinylestradiol), and 2/46 (4%) are not on any form of replacement at present as deemed to have normal ovarian function and regular menses. Finally, 5/46 (10%) have no record of active prescriptions, all of whom are lost to long-term follow-up.

Transdermal preparation is used by 6/39 (15.4%) only, oral preparations are used by thirty-three (84.6%).

Cardiovascular monitoring

Blood pressure

Forty out of 46 women (86.9%) have a documented BP measurement in any tertiary clinics, with sixteen (40%) of these

TABLE 1 Details of different hormone replacement prescriptions given to 46 women with Turner syndrome who were transferred to adult care from 1998-2017.

Num. of Patients	HRT Brand name as prescribed by practitioner	Active Drugs	Method of Administration	Compliant with recommendation
12	Rigevidon	Ethinylestradiol & Levognesterol	Oral	n
7	Gedarel	Ethinylestradiol & Desogestrel	Oral	n
2	Ethinylestradiol	Ethinylestradiol	Oral	n
2	Norethisterone & Estradot Patches	Estradiol & Norethisterone	Transdermal	y
4	Ethinylestradiol & Levognesterol	Ethinylestradiol & Levognesterol	Oral	n
4	Evorel	Estradiol & Norethisterone	Transdermal	y
1	Millinete	Ethinylestradiol & Gestadone	Oral	n
4	Ethinylestrasiol & Norethisterone	Ethinylestrasiol & Norethisterone	Oral	n
1	Novefem	Estradiol & Norethisterone	Oral	y
1	Microgynon	Ethinylestradiol & Levognesterol	Oral	n
1	Femoston	Estradiol & Dydrogesterone	Oral	y
5	Unknown	not applicable	Not Applicable	n
2	Spontaneous pubery	not applicable	Not Applicable	Not Applicable

Both trade and chemical names for each preparation are given. Method of administration either given orally or transdermal patches.

having been monitored in the adult endocrine clinic and twenty-four (60%) monitored in the adult cardiology clinic. Nineteen of the 20 women who are in 5-year established follow-up have a documented blood pressure. The remaining 6 women who have no recorded measurement of blood pressure, of those only one (16%) is in established follow-up. Eight out of 40 women (20%) with a documented BP measurement have a BP in a hypertensive range and are currently on anti-hypertensive treatment.

Cardiac imaging with echocardiogram or cardiac MRI

Forty-one out of the 46 women (89%) have had cardiac imaging (Echocardiogram or Cardiac MRI) performed since transfer to adult healthcare services, of which 30 (73%) were performed as per recommendations. All 20 women in established follow-up have had cardiac imaging. Two women out of this cohort who are in established follow-up, have had successful pregnancies with no reported complications, only one had additional cardiac monitoring. Table 2 outlines any cardiac asnipbnormalities within our patient group and if this meets recommended monitoring standard. The remaining five women who have no documented cardiac imaging following transfer to adult care, only one (20%) is in established follow-up.

TABLE 2 Percentage of patients meeting required cardiac monitoring standards depending on cardiac anatomy in TS.

Anatomical Abnormality	*Num. of Patients & Percentage Meeting Recommendations*
Conarctation of Aorta	3 (0%)
BAV	7 (57%)
Tricuspid AV with fusion Aortic root dilation	1 (0%)
	1 (100%)
Nil	3 4(70%)

BAV, biscuspid aortic valve; AV, aortic valve.

Discussion

TS is a complex disorder requiring multi-disciplinary care throughout life and would benefit from seamless transition of medical care between paediatric and adult services. TS patients continue to have a 3-fold higher mortality risk than the general population in all major causes of death (10). Cardiovascular related deaths account for 40% of this excess risk, and therefore, attendance at an appropriate adult clinic beyond paediatric care is essential (11). Following the International Turner Syndrome Meeting in 2016, clear guidance has been issued suggesting optimal screening and timing of cardiovascular imaging during transition and into adult life and should be adopted for those transitioning after this period (2). In addition, regular monitoring of modifiable risk factors for cardiovascular disease, including hypertension, obesity, and metabolic syndrome, are imperative. The health burden imposed by the life- long risk of associated co-morbidities in TS is more difficult to quantify. Recommended surveillance for endocrine complications and other organ complications, with timely intervention, should continue at the recommended frequencies across the lifespan.

In response to international guidance and recommendations outlined by the Endocrine Society a TS transition clinic was initially set-up in 1998 at the Royal Hospital for Children Glasgow. The TS Paediatric and Transition clinic has subsequently co-located with the designated Adult TS clinic since 2015 providing care for girls and women in the West of Scotland at a single site. The process of transition is planned and staged and makes use of TS specific materials including checklists outlining the recommended frequency of investigations and clinical summary documents to aid communication between paediatric and adult teams.

This review of the TS Transition clinic highlights that a substantial number of girls in our clinic are not in established adult endocrine follow-up at three- and five-years following transfer and is higher than previously reported in other centres (12). However, we have observed that most girls not in established adult endocrine follow-up attend clinics in other specialties including Cardiology, Gastroenterology, ENT and Dermatology. We can infer that young women attending clinics other than adult endocrinology have not understood the need for lifelong follow-up in this service. It is not clear if this group of young women, not in regular adult endocrine follow-up whilst attending other specialty clinics, are receiving the necessary monitoring, hormonal replacement and surveillance recommended in adult Turner clinics. The young women who are lost to endocrine follow-up are not in receipt of sufficient oestrogen replacement or cardiovascular monitoring based on data available from a clinical electronic medical record. In addition, there is lack of information on their metabolic parameters such as weight, cholesterol status given the increased risk of cardio-metabolic complications in TS patients these need to be monitored and evaluated in further reviews of this service.

Cardiac monitoring beyond initial identification of congenital cardiac anomalies remains an integral aspect of TS patient care throughout life. The international consensus recommends cardiac imaging (either Transthoracic ECHO or Cardiac MRI) at the time of diagnosis and in the absence of cardiac anomaly to be performed at either 5-year interval in paediatric care and at a 10-year interval in adult care except for pregnant women who are recommended to undergo further imaging. In the presence of hypertension or cardiac anomalies (bicuspid aortic valve and previous coarctation) more frequent monitoring is recommended (2). The women who were in established follow-up were having regular measurements of blood pressure in either adult cardiology or endocrinology clinics and had cardiac imaging performed at recommended intervals based on a pre-determined risk of aortic dissection. Whilst guidance is to have additional cardiac monitoring during pregnancy, this was only the case for one patient.

Amongst the many complications of TS, gonadal failure occurs in almost all patients, resulting in infertility and in most cases absence of puberty (13). In a small number of patients, spontaneous puberty occurs but there is an increased risk of ovarian insufficiency therefore regular monitoring is recommended (14). Similarly, to

post-menopausal females, the risk of developing co-morbidities due to lack of oestrogen such as osteoporosis is the same in TS patients with ovarian failure therefore it is recommended to commence hormone replacement therapy from the age of 11 or 12 until age of menopause (2). Except for a small proportion of women who have had previous normal onset of puberty and have regular menses most women in our cohort are on a combined form of hormonal replacement. The recommendation is to use natural oestrogen, we find only a small number of women are on the recommended form of oestradiol, and an even smaller number use a recommended transdermal preparation. Most TS patients are receiving Ethinylestradiol which is a synthetic form, this is an issue that requires attention for both existing and future transitioning women. More recently the paediatric team has moved to using a physiological oestrogen with preference to transdermal route. Going forward this information should be shared with the adult team during transition to ensure appropriate HRT is used. Additionally, a proportion of women in our cohort are inappropriately maintained on oestrogen only HRT or without any prescribed HRT, with no evident reason identified for this from their clinical record. These women were all noted to be lost to long-term follow-up. Single-form oestrogen HRT in females increases the risk of endometrial cancer, therefore progesterone is routinely prescribed with oestrogen to counteract the proliferative effects on the endometrium (15)

We believe that various factors may explain why a substantial number of girls are lost to endocrine follow-up at our centre. The median age of transfer in our clinic at 18 years likely coincides with social and educational transitions. In addition to leaving the paediatric TS clinic, girls are likely to be making other life choices regarding further education and career which may necessitate leaving the family home (16). Attending an adult clinic may not take priority over these changes particularly if the opportunity to discuss ongoing health monitoring, beyond growth and puberty, has not been met whilst these girls are either in the paediatric or transition TS clinic (17).

This review of our service has highlighted that the current model of transition is unsuccessful in establishing and maintaining attendance in an adult endocrine clinic and therefore unsuccessful at providing the necessary monitoring and intervention to maintain health in women with TS beyond paediatric services. Changes to our current transition model could include using a transition coordinator, whose role would be to provide important information regarding health to young women with TS, sending reminders regarding upcoming appointments and remaining a point of contact until young women are in established adult endocrine follow-up. In addition, involving families and TS patients to address their specific concerns and providing them with written information and guidance prior to transition could improve retention. The services in the West of Scotland will introduce dedicated TS clinic in the adult setting with paediatricians attending multiple visits to remain a visible contact and link. It has previously been shown that the combination of a structured approach with an official transition coordinator and attendance of the paediatrician at a first adult clinic appointment led to a more efficient transition process and was preferred by families (18).

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

Authors BZ, AM conceived the idea, the script was written by BZ and received editorial input from AM and MD. AS, MF contributed to script idea as well as providing relevant patient information. All authors contributed to the article and approved the submitted version.

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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EDITED BY
Aneta Monika Gawlik,
Medical University of Silesia, Poland

REVIEWED BY
Masanobu Kawai,
Osaka Women's and Children's Hospital, Japan
Raffaella Rossetti,
Italian Auxological Institute (IRCCS), Italy
Berit Kristrom,
Umeå University, Sweden

*CORRESPONDENCE
John C. Achermann
j.achermann@ucl.ac.uk

†These authors share last authorship

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Analysis of genetic variability in Turner syndrome linked to long-term clinical features

Jenifer P. Suntharalingham[1], Miho Ishida[1], Antoinette Cameron-Pimblett[2], Sinead M. McGlacken-Byrne[1], Federica Buonocore[1], Ignacio del Valle[1], Gaganjit Kaur Madhan[3], Tony Brooks[3], Gerard S. Conway[2†] and John C. Achermann[1*†]

[1]Genetics & Genomic Medicine Research and Teaching Department, UCL Great Ormond Street Institute of Child Health, University College London, London, United Kingdom, [2]Institute for Women's Health, University College London, London, United Kingdom, [3]UCL Genomics, UCL Zayed Centre for Research into Rare Disease in Children, UCL Great Ormond Street Institute of Child Health, University College London, London, United Kingdom

Background: Women with Turner syndrome (TS) (45,X and related karyotypes) have an increased prevalence of conditions such as diabetes mellitus, obesity, hypothyroidism, autoimmunity, hypertension, and congenital cardiovascular anomalies (CCA). Whilst the risk of developing these co-morbidities may be partly related to haploinsufficiency of key genes on the X chromosome, other mechanisms may be involved. Improving our understanding of underlying processes is important to develop personalized approaches to management.

Objective: We investigated whether: 1) global genetic variability differs in women with TS, which might contribute to co-morbidities; 2) common variants in X genes - on the background of haploinsufficiency - are associated with phenotype (a "two-hit" hypothesis); 3) the previously reported association of autosomal *TIMP3* variants with CCA can be replicated.

Methods: Whole exome sequencing was undertaken in leukocyte DNA from 134 adult women with TS and compared to 46,XX controls (n=23), 46,XX women with primary ovarian insufficiency (n=101), and 46,XY controls (n=11). 1) Variability in autosomal and X chromosome genes was analyzed for all individuals; 2) the relation between common X chromosome variants and the long-term phenotypes listed above was investigated in a subgroup of women with monosomy X; 3) *TIMP3* variance was investigated in relation to CCA.

Results: Standard filtering identified 6,457,085 autosomal variants and 126,335 X chromosome variants for the entire cohort, whereas a somatic variant pipeline identified 16,223 autosomal and 477 X chromosome changes. 1) Overall exome variability of autosomal genes was similar in women with TS and control/comparison groups, whereas X chromosome variants were proportionate to the complement of X chromosome material; 2) when adjusted for multiple comparisons, no X chromosome gene/variants were strongly enriched in monosomy X women with key phenotypes compared to monosomy X women without these conditions, although several variants of interest emerged; 3) an association between *TIMP3* 22:32857305:C-T and CCA was found (CCA 13.6%; non-CCA 3.4%, p<0.02).

Conclusions: Women with TS do not have an excess of genetic variability in exome analysis. No obvious X-chromosome variants driving phenotype were found, but several possible genes/variants of interest emerged. A reported association between autosomal *TIMP3* variance and congenital cardiac anomalies was replicated.

KEYWORDS

Turner syndrome, X chromosome, monosomy, diabetes mellitus, hypothyroidism, autoimmunity

1 Introduction

Turner syndrome (TS) affects at least 1:2500 newborn females, where there is partial or complete loss of the second sex chromosome (1–6). The age of presentation in TS varies (2). Some girls with TS are diagnosed *in utero* or soon after birth due to congenital renal anomalies, heart anomalies (coarctation of aorta, bicuspid aortic valve) or lymphedema (2, 5, 6). Others are diagnosed later in childhood due to short stature, recurrent otitis media or congenital heart defects, or during teenage years due to primary amenorrhea or absent puberty (7). Prompt diagnosis and multidisciplinary support can allow for age-appropriate management including growth hormone therapy, pubertal induction and psychological input when needed (8, 9).

In adulthood, women with TS also have a higher prevalence of common conditions such as diabetes mellitus (DM), autoimmunity, hypothyroidism, hypertension, and cardiovascular disease (2, 4, 6, 8, 10). These features often contribute to excess mortality in women with TS when compared to the general population (4, 6, 10–12). Identifying specific genes or pathways contributing to the underlying mechanisms of these conditions is important for developing personalized approaches to treatment for women with TS and for targeting of long-term health surveillance.

The association between karyotype and phenotype in women with TS is still unclear. Karyotypes found in TS include aneuploidies (e.g. monosomy 45,X [40-50%], 45,X/46,XX mosaicism [15-25%], 45,X/46,XY [3-10%], 45,X/47,XXX [3%]), as well as structural X chromosome variants (isochromosome Xq (46,X,i(Xq) or 45,X/46X, i(Xq) mosaicism [10%], 45,X/46,X,r(X) ring mosaicism [7%], or rarer Xp and Xq deletions) (4, 8). Several clinical features have been reported to be more prevalent with specific karyotypes. For example, a 45,X karyotype may have the highest morbidity, whereas women with a 45,X/46,XX mosaic karyotype are less prone to obesity and hypertension and generally have the fewest comorbidities (2, 4). Autoimmune diseases, hearing loss and congenital cardiac features have sometimes been reported to be associated with an isochromosome karyotype, but studies are inconsistent (4, 13–17). Women with ring X karyotypes may have a predisposition towards elevated HbAc1 and alanine transaminase (ALT) (4), indicating a potential increased risk of diabetes and fatty liver disease. Tissue level mosaicism (45,X/46,XX, 45,X/46,XY) has also been proposed to influence phenotype, but strong data for this effect are limited (2, 3, 18).

The mechanism (or mechanisms) by which aneuploidy or structural variance of the X chromosome gives rise to the broad range of phenotypes in TS is still under investigation (2, 19–22).

The most established hypothesis is that TS phenotypes are largely a consequence of haploinsufficiency of genes that are normally biallelically expressed from both X chromosomes in women, and from the X and Y homologues in men. Most crucially this includes genes located in the pseudoautosomal regions (PAR), homologous regions present on the short arm (PAR1) and long arm (PAR2) of both sex chromosomes (X and Y) (2, 23, 24) (Figure 1, *left illustration*). PAR1 genes are typically haploinsufficient in all women with non-mosaic TS, irrespective of karyotype (20, 24). The clearest example of this is haploinsufficiency of the PAR gene *SHOX*, which is linked to short stature in TS (19, 25, 26). However, specific phenotypic effects of haploinsufficiency of other PAR genes is much less well established. Furthermore, haploinsufficiency of genes that escape X inactivation (Xi) may also be important. X chromosome inactivation is a mechanism whereby there is dosage compensation to prevent overexpression of X genes in diploid females. Up to 15% of genes escape X inactivation (21, 23, 27, 28), sometimes in a tissue- or time-specific manner, and are candidates for TS phenotypes as their net expression level would be reduced compared to when two X chromosomes (46,XX) are present.

In addition to haploinsufficiency of PAR gene or genes that escape X-inactivation, several other hypotheses exist for contributory mechanisms to phenotypes in women with TS. One hypothesis is that disruption of genes on the X chromosome has a "knock-on" or "ripple" effect elsewhere in the genome, either on the X chromosome itself or on autosomal loci (22). For example, haploinsufficiency of key genes may affect autosomal transcription (e.g., *ZFY*, *ZBED1*), translation (e.g., *DDX3X*, *EIF1AX*, *RPS4X*), splicing (e.g., *DDX3X*, *AKAP17A*) and DNA methylation/chromatin modification (e.g., *KDM5C*, *KDM6A*, *TBL1X*, *USP9X*), with ZFY having a potential key regulatory role in this regard (2, 22, 27, 29–32). Circular RNA and other global changes may also be implicated (31, 33, 34). Importantly, additional second variants (for example in autosomal genes) have also been proposed to contribute to phenotype when combined with haploinsufficiency of a PAR

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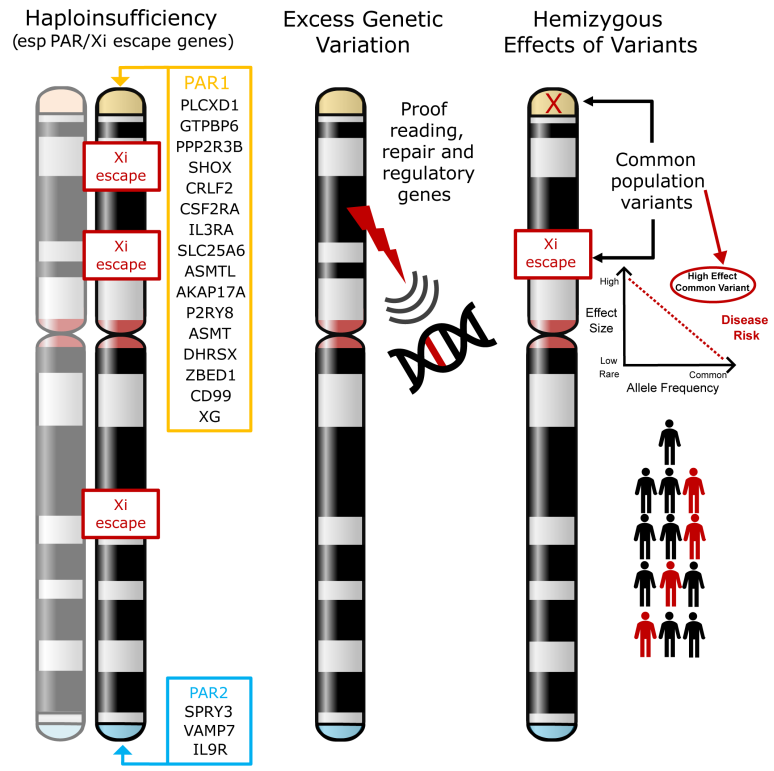

FIGURE 1
Models of potential mechanisms leading to phenotypes in Turner Syndrome. The most established theory involves haploinsufficiency of X chromosome genes (especially in the pseudoautosomal regions (PAR1, PAR2)) or in genes that escape X inactivation (Xi) (*left illustration*). The hypotheses tested in this current work are whether loss of X chromosome genes results in more widespread genomic variability (for example, through loss of a DNA repair or proof-reading gene) (*middle illustration*), or whether in the presence of haploinsufficiency, common hemizygous variants in genes (especially in the PAR region) can act as major modifiers of phenotype (*right illustration*). PAR, pseudoautosomal region; Xi, X inactivation.

gene or a gene that may escape Xi. A reported example of this is where second variants in the autosomal gene *TIMP3* likely combine with disruption of the related X chromosome gene *TIMP1* to increase the risk of cardiovascular anomalies (e.g., bicuspid aortic value) in a "two-hit" mechanism (35, 36).

In this study, we aimed to expand on these concepts to address three hypotheses:

1) Whether disruption of an X chromosome gene with a proofreading or DNA repair function has a wider "ripple" effect on the genome, resulting in increased genetic variability that might contribute to excess morbidity and other features (Figure 1, *center illustration*).
2) Can common hemizygous variants in X chromosome genes (especially in the PAR region or in genes that escape X inactivation) contribute to the risk of associated features in TS (Figure 1, *right illustration*). Having a single copy (haploinsufficiency) of a gene in this region may already infer a risk for a given phenotype. We hypothesize that in this scenario, common population variants in the remaining expressed allele could have an unexpectedly strong influence on phenotype, as this is a unique biological situation where they are present in a hemizygous (monoallelic) state. Thus, the functional influence of common variants could be "exposed" and have quite a marked effect on the risk of developing conditions such as DM and autoimmunity ("X chromosome two-hit hypothesis").
3) If variants in the autosomal gene *TIMP3*, when coupled with loss of *TIMP1* (X chromosome) are associated with an increased risk of congenital cardiac anomalies ("autosome two-hit hypothesis"), as has been reported previously (35, 36).

In order to investigate these hypotheses further, we undertook a large-scale genetic analysis in 134 women with monosomy X and associated karyotypes and investigated genetic variability in relation to control groups and phenotypic features.

2 Materials and methods

2.1 Cohorts and setting

2.1.1 Turner syndrome

The study was conducted as part of the Reproductive Life Course Project (IRAS ID 184846; NRES Committee London-

Chelsea (16/LO/0682)) at University College London Hospitals (UCLH), London, UK. A total of 134 women were recruited from the UCLH Turner Syndrome clinic (2015-2019) overseen by specialist multi-disciplinary professionals. All women provided written, informed consent to take part (median age 35.7 years; range 19.2 to 68 years).

The original diagnosis was made by G-banded karyotype analysis undertaken by routine clinical cytogenetic services. A mosaic screen of at least 30 cultured lymphocytes was typically done. Following recruitment to this study, single nucleotide polymorphism (SNP) array analysis was undertaken on a recent leukocyte-derived DNA sample (see below). Women were excluded if they had evidence of 46,XX or 46,XY mosaicism, or if a Y fragment was identified on original karyotype or on recent SNP array analysis. Women with complex structural variance or complex aneuploidies with a 46,XX or 47,XXX cell line were also excluded.

Within this cohort, three women who were originally reported to have a non-mosaic 46,X,i(Xq) karyotype were subsequently found to have a low-level mosaic (45,X) line on SNP analysis (45,X/46,X,i(Xq)). Three other women had karyotypes that were discordant with the original results, but in these cases historical records were limited. No women had a significant Y line or 46,XX line present. In all situations the most recent SNP array karyotype was used for grouping in this study (Table 1).

Key clinical parameters that were chosen for further analysis in relation to genetic variability were DM/impaired glucose tolerance (IGT), obesity, autoimmune disease, hypothyroidism, hypertension, congenital cardiovascular anomaly, and hearing loss. These conditions were defined as:

1) Diabetes mellitus: A fasting plasma glucose of ≥7.0 mmol/l (126 mg/dL), or ≥11.1 mmol/l (200 mg/dL) after 120 minutes on a standard oral glucose tolerance test (75g oral glucose). Impaired glucose tolerance: A plasma glucose of between 7.8 mmol/l (140 mg/dL) and 11.1 mmol/l (200 mg/dL) after 120 minutes on a standard oral glucose tolerance test (75g oral glucose). For the purposes of analysis, we combined individuals with diabetes and individuals who had IGT under the label "Diabetes".
2) Obesity: A body mass index (BMI) greater than 30 kg/m^2.
3) Autoimmunity: A diagnosis of autoimmune disorders such as celiac disease, inflammatory bowel disease, or antibody positive hypothyroidism.
4) Hypothyroidism: An elevated TSH and long-term treatment with thyroxine replacement. The size of this subgroup allowed for this to be analyzed as a separate entity and may have included women with autoimmune hypothyroidism who were no longer auto-antibody positive.
5) Hypertension: A persistent elevation in blood pressure (140/90 mmHg or higher) and treated with long-term antihypertensive therapy.
6) Congenital Cardiovascular Anomaly (CCA): The presence of a bicuspid aortic valve or coarctation of the aorta or any form of cardiac surgery in childhood. Unfortunately, serial aortic root dimension data in adulthood were not available for analysis.
7) Hearing loss: The use or recommended use of a hearing aid.

TABLE 1 Overview of cohorts studied and related karyotypes.

Cohort	Karyotype	n
Turner syndrome: "Monosomy"	45,X	75
Turner syndrome: "Ring"	45,X/46,X,r(X)	20
Turner syndrome: "Complex"	46,X,del(Xp)/Complex	5
Turner syndrome: "Isochromosome"	46,X,i(Xq) and variants	34
46,XX: Control	46,XX	23
46,XX: Primary Ovarian Insufficiency	46,XX	101
46,XY: Control	46,XY	11

"Isochromosome" used here and elsewhere to include isodicentric Xq (i(Xq)). n, number.

2.1.2 Comparison group: primary ovarian insufficiency

For comparisons of global genetic (exome) variability, data from 101 women with POI were obtained from the Reproductive Life Course Study at University College London Hospitals, London. Those with a known cause of ovarian dysfunction (e.g., abnormal karyotype, iatrogenic POI) were excluded (37). Women recruited to this study provided written informed consent for genetic analysis as part of the Reproductive Life Course Study at University College London Hospitals (ethical approval: NRES Committee London-Chelsea [16LO0682]).

2.1.3 Control group: human random controls

Control DNA samples (46,XX, n=23; 46,XY, n=11) were obtained from Human Random Control DNA Panels (European Collection of Cell Cultures, Public Health England, Sigma-Aldrich).

2.2 DNA extraction from whole blood

Total DNA was extracted and purified from whole blood using a QIAamp Blood Maxi Kit or QIAamp Blood Midi Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Samples were submitted for SNP array and exome sequencing through UCL Genomics.

2.3 Genotyping and mosaicism analysis

SNP array analysis was undertaken using Illumina Global Screening Arrays (v3.0) containing 654,027 markers, following the Infinium HTS Assay Reference Guide (# 15045738 v04) (Illumina, Inc. San Diego, CA, USA). Raw data files were analyzed in Illumina Genome Studio version 2.0 and X chromosome mosaicism calculations were made using methods described by Conlin et al. (38).

2.4 Custom exome sequencing

2.4.1 Exome capture and sequencing

Exome sequencing was performed using a customized Nonacus Exome CG panel (Nonacus, Birmingham, UK) and Nonacus protocol (Protocol Guide v1.2.2) with minor modifications. In brief, 200ng of genomic DNA was used for exome pre-capture library preparation. Library preparations were carried out on a Hamilton StarLet robotic platform (Hamilton Company, Reno, NV, USA) and library qualitative checks were undertaken using Tapestation 4200 (Agilent Technologies, Santa Clara, CA, USA). Sample libraries were sequenced on a NovaSeq6000 Platform using an S4 flow cell (Illumina).

2.4.2 Exome analysis and variant calling

FASTQ files were generated and processed through the bioinformatics pipeline shown in Supplementary Figure 1. Scripts for genome alignments and variant calling were provided by Nonacus. In brief, reads were aligned to the GRCh38 reference sequence using Burrows-Wheeler Aligner (BWA) and grouped by unique molecular identifiers (UMIs) with fgbio (v.0.4). Variant calling was undertaken with Platypus software (v0.8.1) with a default parameter, and multiple nucleotide polymorphisms (MNPs) were split with vcflib (v1.0.0).

Generated VCF files were uploaded to the Qiagen Clinical Insight (QCI) Interpret web-based platform for variant annotation and classification. All exome variants for all samples were exported from QCI as CSV files, these files were then merged and underwent two parallel approaches for standard variant and somatic mosaic variant filtering (Figure 2). For the somatic mosaic analysis, variants were included with at least 40X coverage and with a variant allele fraction of between 0.05 (5%) and 0.25 (25%). This approach was adopted to have enough sensitivity to detect genuine changes, whilst having high enough specificity to avoid "noise" at the lower range and potential heterozygous variants at the upper range. Analysis was undertaken using R version 4.1.1 (39). Variants were subcategorized according to predicted translational impact such as missense, synonymous, frameshift, and stop-gain. This approach provided a quantitative overview of the variety and types of variants in the TS cohort compared to the control/comparison groups described above.

2.4.3 X chromosome variant enrichment analysis and phenotype

To investigate whether genetic variants in X chromosome genes (and especially PAR genes) are associated with phenotype, X chromosome variants from the standard filtering pipeline (Figure 2) were further analyzed in subgroups of women with monosomy X with and without the following conditions: DM/IGT (25 with versus 24 without), obesity (19 with versus 53 without), autoimmunity (24 with versus 28 without), hypothyroidism (32 with versus 43 without), hypertension (16 with versus 36 without), and CCA (for bicuspid aortic valve or coarctation of the aorta) (17 with versus 35 without). Analysis first involved filtering for common variants with the Genome Aggregation Database (gnomAD) allele frequency between 0.1 to 0.9 and quantifying the proportion of the "alternative" allele in the two monosomy X subgroups (condition versus no condition) at gene level (the number of unique individuals having a variant in each gene) and at variant level (the number of individuals with any given variant). The range 0.1 to 0.9 was used as we hypothesized that common population variants could influence phenotype, and

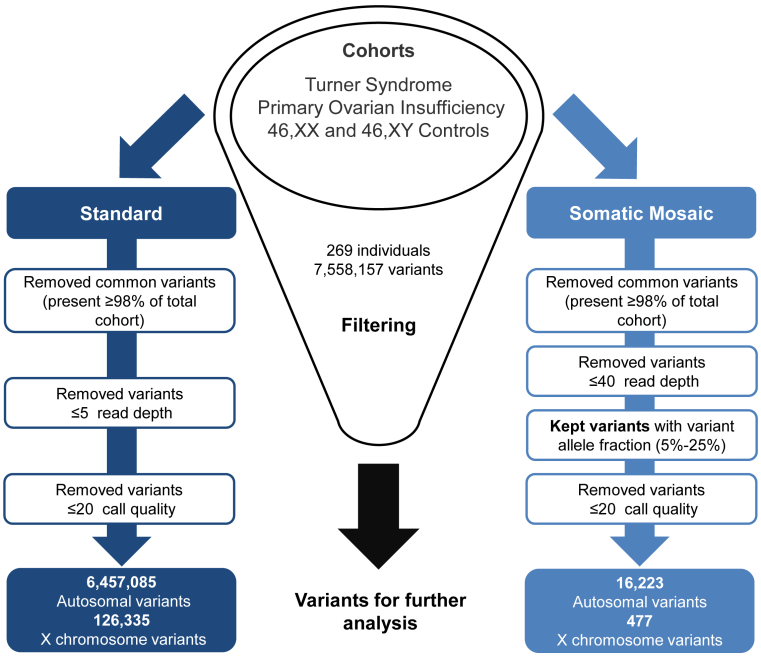

FIGURE 2
Exome data filtering analysis. The two parallel filtering approaches used for standard variant and somatic mosaic variant analysis.

that the proportion of these in condition versus non-condition groups would have to vary around a common population allele frequency. These cut-offs were used to accommodate a normally distributed effect size of 0.35 around a mean population value (see below), which would not be possible at extreme ends of allele frequency. Only the group of TS women with monosomy X were included in the initial study as the presence of a single X chromosome simplified analysis as only hemizygous variants had to be considered. Population control data for any variants of interest were obtained from gnomAD (v3.1.2) (accessed March 2023) (40). Only 46,XY control data were included as the presence of a single X chromosome simplified analysis because only hemizygous variants occur (rather than heterozygous or homozygous combinations). Fisher's exact test and multiple comparison testing using the Bonferroni method was performed in R (39) to assess the significance of any findings. Genes and variants of interest were determined using an "effect size" greater than +0.35 or -0.35 between groups (i.e. the difference in alternative allele frequency in those with a condition compared to those without a condition). This effect size was calculated based on initial power calculations, and confirmed in a *post-hoc* power analysis based on final group size and ratios of those with and without a condition (Supplementary Table 1). Clinical conditions linked to these genes were determined using the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org). Gene expression data for genes of interest were obtained using the consensus summary in the Human Protein Atlas (https://www.proteinatlas.org).

Following the initial analysis of X chromosome variants linked to phenotype in the 45,X monosomy subgroup, a separate analysis of PAR gene variance was undertaken in a wider cohort of women with additional TS-associated karyotypes using a similar design, as PAR1 genes are predicted to be haploinsufficient in all these women.

Analysis of "hearing loss" was also undertaken using the same pipelines (14 with versus 33 without), but these data are presented separately as the groups are smaller and the end points potentially less reliable.

2.5 *TIMP3* variants and congenital cardiovascular anomalies

In order to evaluate the link between *TIMP3* (chromosome 22) variants and CCA, exome sequencing data for variants in this gene were analyzed in relation to cardiac status in 95 women where phenotypic data were available. The minor allele frequency (MAF) of common variants detected was calculated and compared between groups (CCA [n=22] versus non-CCA [n=73]) using Fisher's Exact testing. MAF was also compared to data obtained from gnomAD (v3.1.2) as well as previous reports of *TIMP3* variants in TS cardiac cohorts (35, 36).

2.6 Statistical analyses

2.6.1 Mosaicism proportion

Where data were available, statistical analysis between the original and new percentage mosaicism values was undertaken in GraphPad Prism (version 9.5.5 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com) using the Wilcoxon matched-pairs signed rank test.

2.6.2 Genetic variability and X chromosome variant enrichment analysis

Statistical analysis for genomic variability between TS and control/comparison cohorts was performed using a one-way ANOVA (Kruskal-Wallis) test in GraphPad Prism. For X chromosome variant filtering, and gene and variant level counts, Fisher and Bonferroni adjustment tests were undertaken in R using the tidyverse packages (39, 41). Bonferroni multiple adjustment comparisons were made using a very stringent approach taking into account all genes with data on the X chromosome. Graphical outputs were generated either using GraphPad Prism or using ggplot2 in R (41).

2.6.3 TIMP3 variant enrichment analysis

Statistical analysis for potential enrichments of variants in *TIMP3* related to CCA was performed using Fisher's Exact tests in GraphPad Prism.

3 Results

3.1 Overview of cohort

An overview of the study cohort and sub-groups is shown in Table 1. Monosomy X (45,X) was present in 75/134 (56.0%) women whereas 44% (59/134) of women had related karyotypes. Typical SNP array outputs for different karyotypes are shown in Figures 3A–E, showing haploinsufficiency of PAR1 in all situations. For those women diagnosed with mosaic isodicentric Xq (45,X/46X,i(Xq)) or ring (45,X/46,X,r(X)) karyotypes, the proportion of 45,X cells was often higher on the more recent ("new") SNP array analysis compared to the original karyotype analysis (Wilcoxon matched-pairs signed rank test P value <0.0001) (Figure 3F), although different platforms for assessment were used.

3.2 Genetic variability in women with TS

To investigate whether disruption or changes in the X chromosome in TS affect genetic (exome) variation in general (Figure 1, *center illustration*), total autosomal and X chromosome variants were compared between cohorts as well as the potential translational impact of any changes.

Out of the 7,558,157 variants identified in the study (Figure 2), the mean number of variants *per individual* was approximately 28,000. The standard filtering approach for germline or early somatic events had a combined total of 6,457,085 autosomal variants and 126,335 X chromosome variants. The somatic mosaic filtering approach looked at low variant allele frequency changes between 5-25% and this gave a total of 16,223 autosomal variants and 477 variants on the X chromosome.

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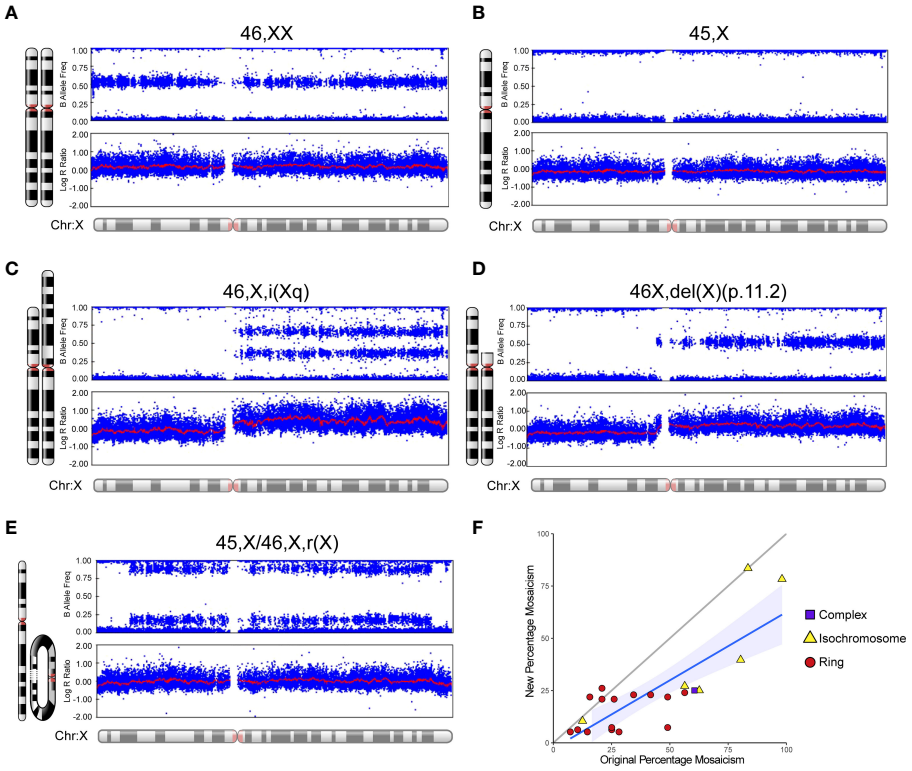

FIGURE 3
Examples of SNP array data for different karyotypes and mosaic changes over time. Selected karyotype examples are shown for: **(A)** 46,XX; **(B)** 45,X ("monosomy" group); **(C)** 46,X,i(Xq) ("isochromosome" group/isodicentric Xq); **(D)** 46,X,del(X)(p.11.2) ("complex" group); and **(E)** 45,X/46,X,r(X) ("ring" group). **(F)** Scatter plot to show the "new" percentage mosaicism obtained from the SNP array compared to the "original" percentage mosaicism at diagnosis via G-banded karyotype. The percentage mosaicism refers to the percentage of the variant (isochromosome, ring) cell line or chromosome. Array data were analyzed and visualized in Illumina Genome Studio v2.0. Plots were generated of the B-allele frequency (the normalized measure of the allelic intensity ratios of two alleles A, B) and the log R ratio (the normalized measure of signal intensity for each SNP marker, as log2 of the ratio between observed and expected for two copies of the genome). The mean value is represented by a red horizontal line. For B allele frequency, the number of bands seen on the plot minus one usually indicates the number of chromosomes at that given locus. B allele frequencies (BAF) of 0.0, 0.5 and 1.0 are expected in a normal sample, representing AA, AB and BB, respectively. For the log R ratio, a signal clustering around zero shows when the region has two copies; higher or lower signal intensities indicate when there are more or less copies in a genomic region, respectively. Where relevant, the percentage of mosaicism was calculated using the pipeline developed by Conlin et al. (38).

3.2.1 Autosomal variants

There were no significant differences between the total number of autosomal variants found in the different TS cohorts compared to the different control/comparison cohorts (Figure 4). No differences were found in different subcategories of variants. These findings suggest there is no excess genetic variation in autosomes in TS women.

3.2.2 X chromosome variants

The total number of X chromosome variants was proportionate to the amount of X chromosome material present in each karyotype (Figure 5). For example, the number of X chromosome variants in the monosomy group (45,X) with a single X was very similar to the 46,XY control group, whereas those with isochromosomes had variant numbers approaching 46,XX women or 46,XX POI comparison cohorts. Any significant differences between groups are shown in Supplementary Figure 2. Overall, these findings suggest there is no excess genetic variation in X chromosomes in TS women.

3.2.3 Somatic mosaic autosomal and X chromosome variants

Somatic mosaic changes in the autosomes showed no significant differences between the TS subgroups and the control/comparison cohorts (Figure 6A). In general, the number of somatic mosaic variants on the X chromosome was low in all groups studied (Figure 6B). Thus, no excess of somatic mosaic variants was observed in leukocyte-derived DNA.

3.3 X chromosome genetic variability and phenotype

To address whether common hemizygous variants in X chromosome genes contribute to associated features in TS (Figure 1, *right illustration*), we initially focused subsequent analysis only on women with monosomy X (45,X) who had detailed phenotypic data available for DM, obesity, autoimmunity, hypothyroidism, hypertension and cardiac surgery for congenital

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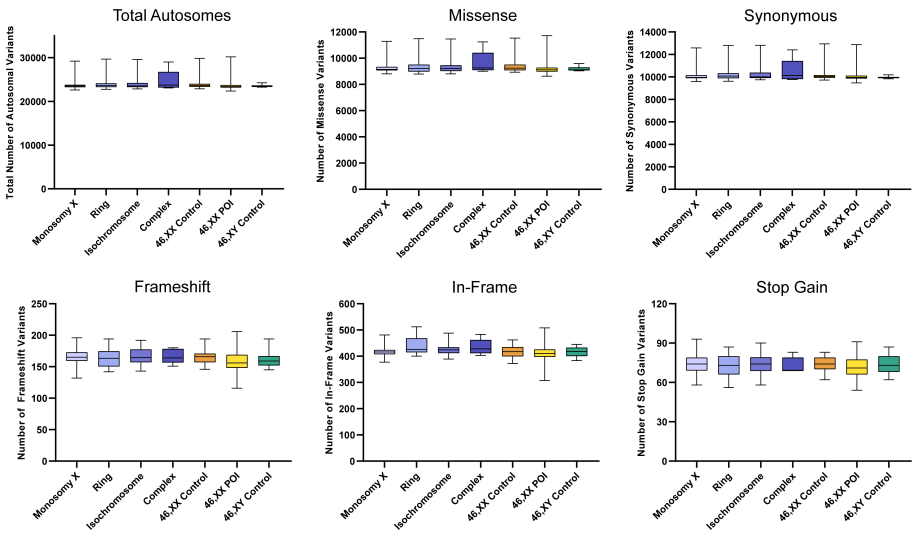

FIGURE 4
Number of autosomal variants in each cohort and predicted translational impact. The total number of variants are shown in the top left panel. The number of variants predicted to cause missense, synonymous, frameshift, in-frame and stop gain changes are shown subsequently from left to right. Data are represented as box plots showing the lower quartiles, upper quartiles and the median with the whiskers showing the range of the data. No statistically significant differences ($p<0.05$) were found between any of the cohorts in each analysis (Kruskal-Wallis one-way analysis of variance). Sequencing parameters (read depth, call quality) for all groups are shown in Supplementary Figure 3A. POI, primary ovarian insufficiency.

heart defects. Only women with monosomy X were included in the initial analysis as they have just a single copy of the X chromosome, avoiding any issues with multi-allelic expressed genes and allowing us to focus on hemizygous variants that could act as important drivers of phenotype especially on the background of haploinsufficiency of PAR genes and of genes that escape Xi. As we hypothesized that *common population variants* could have a major effect in this biological context, cohorts of 45,X women with

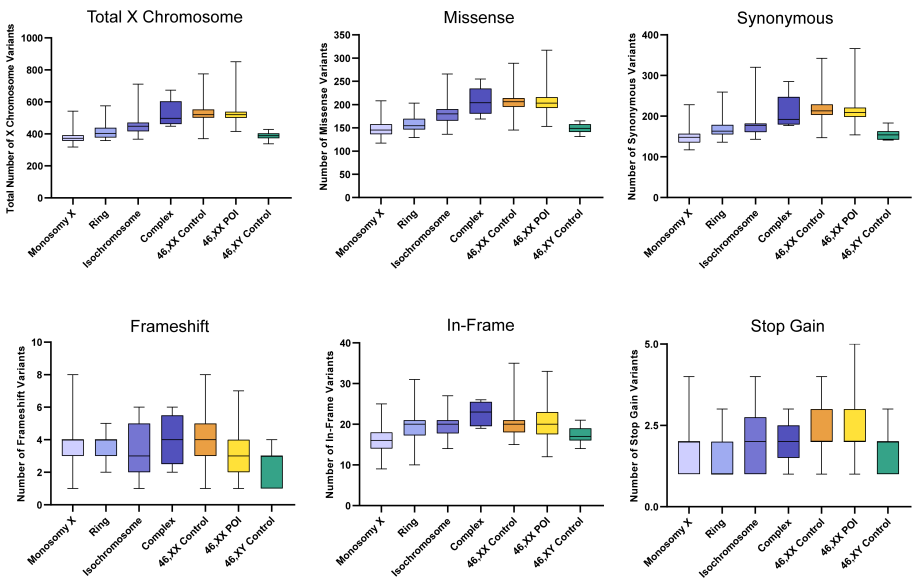

FIGURE 5
Number of X chromosome variants in each cohort and predicted translational impact. The total number of variants are shown in the top left panel. The number of variants predicted to cause missense, synonymous, frameshift, in-frame and stop-gain changes are shown subsequently from left to right. Data are represented as box plots showing the lower quartiles, upper quartiles and the median with the whiskers showing the range of the data. The number of X chromosome variants appeared proportionate to the amount of X chromosome material. There was no excess of variability in monosomy (45,X) compared to 46,XY controls, or in other TS subcategories compared to 46,XX controls. Statistical differences between groups are shown in Supplementary Figure 2. Sequencing parameters (read depth, call quality) for all groups are shown in Supplementary Figure 3B. POI, primary ovarian insufficiency.

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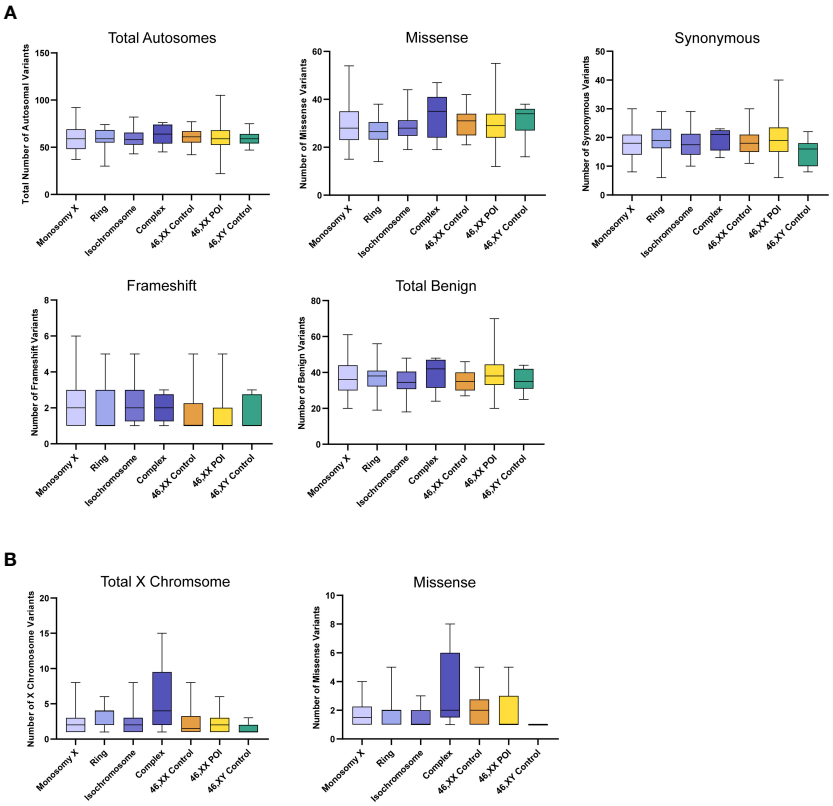

FIGURE 6
Number of autosomal somatic variants in each cohort and predicted translational impact for somatic variants. **(A)** The total number of somatic autosomal variants are shown in the top left panel. The number of somatic variants predicted to cause missense, synonymous, frameshift, and predicted total benign somatic changes are shows subsequently from left to right. Data are represented as box plots showing the lower quartiles, upper quartiles and the median with the whiskers showing the range of the data. No statistically significance differences ($p<0.05$) were found between any of the cohorts in each analysis (Kruskal-Wallis one-way analysis of variance) **(B)** The total number of somatic X chromosome variants are shown in the left panel and missense variants are shown on the right. Sequencing parameters (read depth, call quality) for all groups are shown in Supplementary Figure 4. POI, primary ovarian insufficiency.

and without a given phenotype (e.g., diabetes versus non-diabetes) were directly compared for enrichment of X chromosome genetic variants that could act as "risk" or "protective" alleles in this situation.

Data for gene level analysis of all X chromosome genes containing variants for each major phenotypic condition are shown in Figure 7. These are presented as scatterplots of the proportion of gene variants in 45,X women with a condition against the proportion in those without a condition. PAR genes are shown in red. Similar data for variant level analysis are shown in Figure 8.

Differences between the proportions of variants in genes in those with and without a condition ("effect size") are shown in Table 2 and Supplementary Table 2. A positive effect (and odds ratio >1.0) would be expected with a potential "risk" gene (i.e., higher in those with a condition), and a negative effect (odds ratio <1.0) would be expected with a potential "protective" gene (i.e., higher in those without a condition). Data are shown in Table 2 for genes where effect size was greater than +0.35 or -0.35. Although these genes all showed significant enrichment on individual burden testing (Fisher's test), none of them were significant when adjusted for multiple testing of X chromosome genes (>600) (Bonferroni correction).

Despite the lack of significance following multiple corrections (for more than 600 genes), further review of the expression, biological role and clinical associations of these genes was undertaken (Table 2). For DM, potential "risk" genes were *MIR4769* and *F8*, whereas "protective" genes were *DOCK11* and *STEEP1*. In the obesity group, *LANCL3*, a hypothalamic expressed gene associated with carbohydrate metabolism (42) and *RPL36A–HNRNPH2* were potential risk genes. *FAM47A* and *TFE3* were potential risk genes for hypertension, whereas *IRS4*, an insulin signaling-pathway gene associated with central hypothyroidism (43), potentially linked with cardiac defects. *CHM* was potentially protective for DM and hypertension. Most of these gene level changes were the result of just one or two single nucleotide variants in a gene, most of which were individually not likely to have major functional effects based on function prediction algorithms (SIFT, PolyPhen), splice analysis or Combined Annotation Dependent Depletion (CADD) scores (Supplementary Table 2).

No PAR genes were enriched in the analysis although p.Pro500Ala (X-1601004:C-G) in *AKAP17A*, linked to autoimmunity and showed non-adjusted significance in the variant level analysis (variant allele frequency 0.75 in autoimmune and 0.39 in non-autoimmune; effect size 0.36; gnomAD males 0.54; p-value

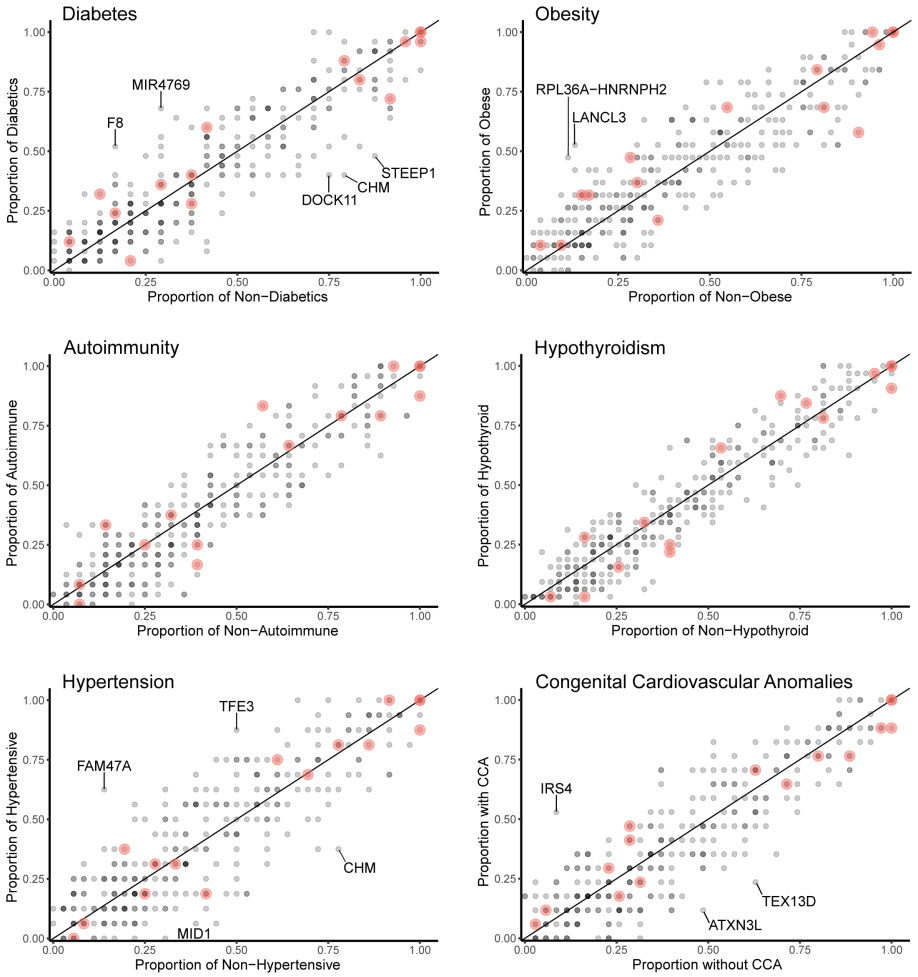

FIGURE 7
Scatterplots (gene level) of the proportion of X chromosome gene variants in 45,X women with a condition against the proportion of variants in the same gene in those women without the condition. The number of data points at any given coordinate is shown by the intensity of the circle. Red circles indicate genes that are located in the pseudoautosomal regions. Genes that have an effect size greater than 0.35 are labeled. Sequencing parameters (read depth, call quality) for groups are shown in Supplementary Figure 5.

<0.01, adj.p-value 1.0) (Figure 8; Supplementary Table 2). Extending the analysis of just PAR genes to a larger group of women with other karyotypes (Supplementary Figure 6) did not strengthen association of this variant with autoimmunity (AI n=42, non-AI n=53) (variant allele frequency 27/42 = 0.64 in AI and 31/53 = 0.58 in non-AI, effect size 0.06, p-value 0.18, adj.p-value 1). Analysis of PAR gene variants in relation to phenotype for a larger group of women with different TS-associated karyotypes is shown in Supplementary Materials (Supplementary Figures 6–8; Supplementary Tables 3, 4). Hearing loss data are shown in Supplementary Tables 5, 6 and Supplementary Figure 9.

3.4 *TIMP3* variants and congenital cardiovascular anomalies

Focused analysis of variants in *TIMP3* in relation to cardiac anomalies (CCA n=22, non-CCA n=73) revealed a significant enrichment of the 22:32857305:C-T (rs11547635) variant in the CCA group (MAF CCA 0.14 versus MAF non-CCA 0.03, p-value <0.02) (Table 3). This variant is predicted to be synonymous (p.S83S) with a MAF of 0.09 in gnomAD, but has been reported previously in association with CCA in TS in two datasets (Table 3). No effect on splicing was predicted using several algorithms (https://spliceailookup.broadinstitute.org/). A proposed combined effect of this *TIMP3* variant with *TIMP1* copy number (1.0) for cardiac risk (35) could not be explored further as we excluded women with 46,XX lines (and higher *TIMP1* copy number) from the cohort. Notably, there was a statistically higher proportion of women with monosomy X in the CCA group, and lower proportion of those with ring chromosomes (see Supplementary Figure 6 and legend).

Recently, a variant in the candidate cardiac risk gene *CRELD1* (c.9943412, G>A) has been reported to be associated with CCA in women with TS (44). We could not replicate this finding in our cohort (p-value 0.13), potentially due to our smaller sample size, and low allele frequency of this variant.

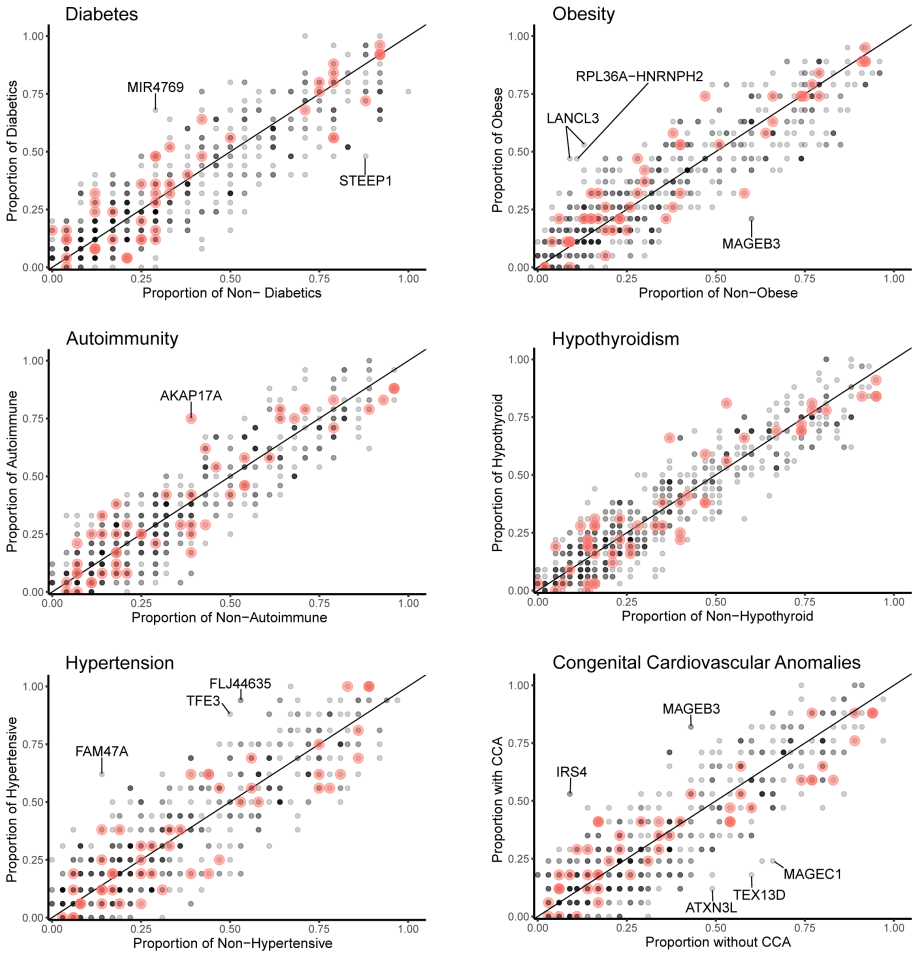

FIGURE 8
Scatterplots (variant level) of the proportion of X chromosome gene variants in 45,X women with a condition against the proportion of variants in the same gene in those women without the condition. The number of data points at any given coordinate is shown by the intensity of the circle. Red circles indicate genes that are located in the pseudoautosomal regions. Genes that have an effect size greater than 0.35 are labeled. Sequencing parameters (read depth, call quality) for groups are shown in Supplementary Figure 5.

4 Discussion

TS is an important condition with many long-term associated features but the underlying mechanism (or mechanisms) leading to these comorbidities is still not clear. Often this is assumed to be haploinsufficiency of PAR genes on the X chromosome, but other subtle mechanisms related to X gene dosage or autosomal/X-chromosome "ripple effects" may be important (2). By using large-scale high-throughput exome sequencing of a cohort of 134 adult Turner women with a range of long-term associated conditions, we have been able to start to address some of these questions.

Before engaging in in-depth genomic analysis, our first goal was to assess the karyotypes of this cohort of women using a SNP array approach, rather than the traditional G-banded karyotyping and 30 cell mosaic screen. Using this platform, a current karyotype was determined for all participants. Three women (3/134, 2.2%) originally reported to have non-mosaic isochromosomes (46,X, i(Xq)) were found to have a low-level mosaic 45,X line present on array (45,X/46X,i(Xq)). Three additional women had other changes in karyotype, but original records were limited, or small numbers of cells screened. Overall, SNP array proved to be a useful approach to assessing karyotype for this cohort and there was concordance with original recorded karyotype in more than 95% individuals.

In the SNP array dataset, mosaicism levels for ring and isochromosomes were calculated using the approach reported by Conlin et al. (38). As expected, ring chromosomes were present in a low percentage as they are only viable together with a 45,X mosaic line, whereas isochromosome proportions were more variable and generally higher. When the "original" mosaic percentage of these ring and isochromosome cell lines reported by G-banded karyotype/mosaic screen was compared to the "new" array-derived mosaicism percentage in the same individual, a lower proportion of ring and isochromosome cell lines was seen, and higher proportion of 45,X line. This observation may represent a clonal selection advantage for the 45,X line with time especially in the hematopoietic system, and dynamic changes over time have been reported in forms of revertant mosaicism (45) and even related to loss of the Y chromosome with age (46). Alternatively, these findings may reflect the different methodologies used; historic

TABLE 2 Proportion of X chromosome genes harboring variants in 45,X women with a condition against the proportion of the same gene harboring variants in those women without the condition.

Gene	Condition	Alt. individuals, with condition	Alt. individuals, without condition	Effect size	OR (95% CI)	Fisher Exact test (p value)	Bonferroni corrected (p.adj)	OMIM	HPA gene expression
FAM47A	Hypertension	0.63	0.14	0.49	9.73 (2.2-52)	0.0007	0.23	–	Testis
IRS4	Congenital cardiovascular anomaly	0.53	0.09	0.44	11.26 (2.2-80)	0.0008	0.26	Central hypothyroid-ism	Hypothalamus, pituitary, ovary
LANCL3	Obesity	0.53	0.13	0.39	0.14 (0.04-0.5)	0.001	0.38	–	Hypothalamus, cerebellum, thalamus
MIR4769	Diabetes	0.68	0.29	0.39	4.97 (1.3-21)	0.01	1.00	–	–
TFE3	Hypertension	0.88	0.50	0.38	6.76 (1.3-70)	0.01	1.00	XL-LD + pigmentary disorder	Non-specific
RPL36A-HNRNPH2	Obesity	0.47	0.11	0.36	0.15 (0.03-0.6)	0.002	0.66	XL-LD (*HNRNPH2*)	Retina, ovary, breast, bone marrow
F8	Diabetes	0.52	0.17	0.35	5.22 (1.2-27)	0.02	1.00	Hemophilia A	Heart, tongue, adipose
DOCK11	Diabetes	0.40	0.75	-0.35	0.23 (0.05-0.9)	0.02	1.00	–	Adipose, bone marrow, macrophages
ATXN3L	Congenital cardiovascular anomaly	0.12	0.49	-0.37	0.15 (0.01-0.8)	0.01	1.00	–	Testis (spermato-genesis)
MID1	Hypertension	0.06	0.44	-0.38	0.09 (0.002-0.7)	0.01	1.00	Opitz GBBB syndrome	Cerebellum, colon, heart
CHM	Diabetes	0.40	0.79	-0.39	0.18 (0.04-0.7)	0.01	1.00	Choroido-remia	Non-specific
TEX13D	Congenital cardiovascular anomaly	0.24	0.63	-0.39	0.19 (0.04-0.8)	0.02	1.00	–	Testis (spermato-genesis)
STEEP1	Diabetes	0.48	0.88	-0.40	0.14 (0.02-0.6)	0.01	1.00	XL-LD	Non-specific
CHM	Hypertension	0.38	0.78	-0.40	0.18 (0.04-0.7)	0.01	1.00	Choroido-remia	Non-specific

Only women with monosomy X (45,X) and well-defined phenotypic data were included in this analysis. Gene level data are shown. Data are shown where effect size is 0.35 or above. A positive effect size for a condition denotes a potential "risk" allele, whereas a negative effect size denotes a potential "protective" allele. Numbers in the groups analyzed are: diabetes (DM/IGT) n=25, non-diabetes n=24; obese n=19, non-obese=53; hypertension n=16, non-hypertension, n=36; congenital cardiovascular anomaly (CCA) n=17, non-CCA n=35. Bonferroni corrections were made for all genes on the X chromosome where variants were identified (>600 genes). CI, confidence interval; HPA, human protein atlas; OMIM, Online Mendelian inheritance in Man; OR, odds ratio; XL-LD, X-linked learning difficulty.

mosaic screens were undertaken on relatively small numbers of cells, whereas SNP array derives a mosaicism level based on signal intensity. Unfortunately, we were not able to repeat traditional karyotype mosaic screens on this cohort.

Having defined karyotypes in the cohorts, targeted exome sequencing was used to test the hypothesis of whether changes in the X chromosome with TS are associated with differences in global genomic variability either across the autosomes or in the X chromosome itself. It has been hypothesized that haploinsufficiency of key X chromosome genes could have "ripple effects" affecting transcription, translation, splicing or methylation/chromatin across the genome (2, 21–23, 27, 29–34, 47, 48). We extended this concept further to investigate whether there are global differences in genetic variability in TS, potentially as a consequence of loss of a DNA proofreading or repair gene on the X chromosome (Figure 1). With the reduction in cost and ease of throughput for exome sequencing,

TABLE 3 Common variants in *TIMP3* in relation to congenital cardiac anomalies (CCA).

TIMP3 Variant	Protein	gnomAD MAF	CCA MAF (alleles) (n=22)	Non-CCA MAF (alleles) (n=73)	Fishers Exact test (p-value)	Previously reported CCA MAF	Previously reported Non-CCA MAF
22:32857293: T-C	p.H83H	0.61	0.57 (25/44)	0.51 (75/146)	0.61	0.50 (Corbitt et al., 2018) (35) 0.44 (Corbitt et al., 2019) (36)	0.50 (Corbitt et al., 2018) (35) 0.51 (Corbitt et al., 2019) (36)
22:32857305: C-T*	p.S87S	0.09	0.14 (6/44)	0.03 (5/146)	0.02	0.14 (Corbitt et al., 2018) (35) 0.12 (Corbitt et al., 2019) (36)	0.06 (Corbitt et al., 2018) (35) 0.04 (Corbitt et al., 2019) (36)

gnomAD version (v3.1), GRCh38; *also known as rs11547635. Congenital cardiac anomaly (CCA) group included 22 individuals and non-CCA group included 73 individuals. MAF, minor allele frequency.

we looked at exome variability in the entire TS cohort and subgroups, and compared the data to 46,XX controls, 46,XY controls and additional comparison cohort of 46,XX women with POI. Using this approach, no significant differences in autosomal genomic variability were found between these groups and X chromosome genomic variability tracked very clearly with the amount of X chromosome material present. A filtering approach to detect lower-level potential somatic mosaic events was developed, as somatic variability in DNA derived from rapidly replicating blood cell lineages is an ideal system to assess this. However, no significant changes between the groups were seen. These data show that global genetic (exome) variability in TS is likely to be unaffected.

In the past 35 years, investigations of associations between genetic variability and phenotype in TS initially focused on parent of origin effects of the X chromosome, based on the potential existence of imprinted X chromosome genes (Supplementary Table 7). Other studies considered the influence of mostly non-coding single nucleotide variants on either specific features of TS, or more general features (e.g., bone mineral density (*VDR*, *ESR1*); response to growth hormone treatment (*GHRd3*)) (Supplementary Table 7). Data for coding sequence changes, especially for X chromosome genes, are very limited.

Here, we hypothesized that, whilst haploinsufficiency of key X chromosome genes is often thought to be an important underlying pathogenic event in TS, monosomy X is a unique biological situation where common genetic variability in X chromosome genes could be "uncovered" by the unexpected loss of the second sex chromosome, and these variants could act as strong "drivers" of phenotype. Women with TS can have a broad range of conditions such as diabetes mellitus, obesity, autoimmune disease and hypothyroidism and hypertension, as well as developmental features such as congenital heart defects. Research efforts to try to understand the biological basis of some of these conditions are very important in order to develop new and personalized treatments for the TS community. We therefore investigated whether any gene level or variant level changes in any gene on the X chromosome were significantly enriched in monosomy X women with a specific condition compared to those without it ("risk" allele), or conversely whether changes could be found more often in women without a given condition compared to those with it ("protective" allele).

Using this approach, several potentially enriched genes and variants were found, but the effect size was limited (all less than 0.5 between groups) and statistical differences did not withstand multiple comparison testing. Furthermore, biological correlations of proposed gene function with phenotype were not obvious, except in the case of obesity and *LANCL3*. This gene has been linked to carbohydrate metabolism/high fat diet induced obesity in rats, is expressed in the hypothalamus and has been shown to escape X inactivation in TS, so further investigation may be warranted (32, 42). The only PAR gene that emerged was a variant in *AKAP17A* (X:1601004:C-G) associated with autoimmunity. This gene has been implicated in inflammatory bowel disease (49) but this variant was predicted to be benign.

Given a previously reported association between the 22:32857305:C-T (rs11547635) variant in *TIMP3* and congenital cardiovascular anomalies in two cohorts of women with TS, we undertook a specific analysis of *TIMP3* and CCA in our study group. *TIMP3* encodes Tissue Inhibitor of Metalloprotease 3, an extracellular matrix protein involved in angiogenesis and cardiac remodeling (50, 51). The *TIMP3* gene is located on chromosome 22. It is hypothesized that loss of *TIMP1* (on the X chromosome) coupled with variations in *TIMP3* can predispose to CCA in women with TS, in an autosomal "two-hit" hypothesis (2, 35, 36). Very recently, the rs11547635 variant (22:32857305:C-T) in TIMP3 has also been associated with aortic regurgitation in a longer term follow up study of Turner women (52). Notably, we were able to replicate the enrichment of this 22:32857305:C-T variant with CCA in our cohort (Table 3). Indeed, MAF data were very similar to previous reports (35, 36). This finding is important not only for independently reproducing other studies, but it also demonstrates that rare variants may influence phenotype in a two-hit manner, which is the basis of our X chromosome gene hypothesis. Furthermore, the MAF for this *TIMP3* variant is relatively low (CCA 0.14 versus non-CCA 0.03) and effect size small (0.11). Our X chromosome variant analysis was far more stringent, focusing on an effect size cut off at +/- 0.35 and adjusting for multiple comparisons for all X genes. Given the *TIMP3* data, several of the variants we detected in the X chromosome may be biologically significant. This could be addressed in the future in larger cohorts, combining our datasets with additional cohorts.

This study has several limitations. Firstly, clinical associated phenotypes can change with time, so an individual currently classed as non-diabetic may develop diabetes later in life. Indeed, some phenotypes may be influenced by other factors such as family history, have multifactorial origins (such as hypertension, obesity or combined autoimmune (Type 1) and metabolic effects (Type 2) in

diabetes mellitus), or be inter-dependent (e.g., DM/BMI; hypothyroidism/autoimmunity). In order to improve phenotype accuracy, gold standard approaches and detailed re-evaluations were undertaken, such as oral glucose tolerance testing to capture women with undiagnosed diabetes as well as those on established treatment. Only women with robust phenotypic data were included for analysis. Secondly, variant numbers can be influenced by sequencing quality (quality, depth) and batch effects. We compensated for this by processing all samples at the same time and using robotic approaches to undertake library preparations. Third, the numbers of individuals in the phenotype groups for X chromosome variant analysis were relatively small and the power of burden testing when adjusted for multiple comparisons would have been stronger if more individuals had been included. Our hypothesis was that *common* population variants in X chromosome genes are exposed and contribute to phenotype. However, the power to detect statistical differences is dependent on sample number, and potentially important changes with a smaller effect size may have been missed. As discussed above, the potential influence of *TIMP3* on congenital cardiac phenotypes was replicated, even for a variant of low allele frequency and small effect size. Fourth, some studies have suggested that tissue mosaicism for a covert 46,XX or 46,XY cell line may influence phenotype (2). In our study, any individuals with evidence of a 46, XX or 46,XY line in the blood were excluded, and two independent samples (karyotype, SNP array) were analyzed usually many years apart, so – whilst tissue specific mosaicism cannot be excluded – we feel it is unlikely. Finally, we did not undertake analysis of DNA methylation effects across the genome, or transcriptional/translational networks. This would require specific methylation assays or RNA studies and would still be limited to analysis of leukocyte profiles unless other tissues of interest were available.

Despite these limitations, this is a major hypothesis-based study using SNP array and exome sequencing in one of the largest populations of women with TS studied at a detailed genetic level to date. This work highlights how rapidly advancing technologies can increasingly be applied at scale to address key questions in well-characterized clinical cohorts, with the aim of developing more specific or personalized approaches to treatment for associated conditions in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://doi.org/10.17605/OSF.IO/AJBEP, Open Science Framework (53).

Ethics statement

The studies involving humans were approved by NRES Committee London-Chelsea (16/LO/0682). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JS, GC and JA conceptualized the study. JS, AP-C, SM-B, MI, ID, FB, TB, GK, GC and JA undertook data curation. MI and JS undertook formal data analysis. GC and JA were involved in funding acquisition. AP-C, GC and JA undertook investigation, diagnosis, and management. GC and JA oversaw project administration and supervision. MI and ID undertook validation. JS and FB were responsible for data visualization. JS, FB and JA wrote the original draft with input from GC. All authors were involved in reviewing and editing the final manuscript. GC and JA had full access to all data in the study and had final responsibility for the decision to submit for publication. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1227164/full#supplementary-material

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EDITED BY
Malgorzata Ewa Wiecek,
Medical University of Silesia, Poland

REVIEWED BY
Ozgur Oktem,
Koç University, Türkiye
Giuliano Marchetti Bedoschi,
University of Sao Paulo, Brazil

*CORRESPONDENCE
Eleonora Porcu
eleonora.porcu@unibo.it

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Reproductive health in Turner's syndrome: from puberty to pregnancy

Eleonora Porcu[1*], Linda Cipriani[2] and Giuseppe Damiano[2]

[1]University of Bologna, Bologna, Italy, [2]Infertility and IVF Unit, IRCCS Azienda Ospedaliero-Universitaria of Bologna, Bologna, Italy

Turner syndrome (TS) is a genetic pathology that affects about 1/2500 newborn females. Turner's syndrome is characterized by highly variable genetic anomalies that consist in a partial or complete deletion of the X sexual chromosome; it can be present as a monosomy or as a mosaicism with two o three different cellular lines. 50% of the patients with Turner's syndrome has a 45 XO karyotype while the remaining cases have karyotypes with mosaicism or X isochromosome or with partial or whole Y chromosome. This pathology is characterized by multiple anomalies that involve physical and cognitive development and in particular endocrine, cardiovascular, reproductive, auditive and visual systems. Integrity of the X chromosome in essential for fertility. In TS is accelerated germ cells apoptosis. About 30% of TS girls have some pubertal development, 10-20% undergo menarche and 2-8% go through spontaneous pregnancy. Women with TS should be informed about the risk of premature menopause and should be referred, if possible, to a specialist evaluation with a doctor expert in assisted reproductive techniques. In adolescents and in adults, Premature Ovarian Insufficiency (POI) can be evaluated clinically and biochemically with the classic combination of amenorrhea and elevated FSH concentrations (hypergonadotropic hypogonadism). However, in postpubertal adolescents and adult women, reproductive hormones may remain within the normal range before POI is clinically evident, despite significant depletion of the ovarian reserve. Today, reproductive medicine offers the opportunity of fertility preservation in women with premature ovarian insufficiency (POI). Two techniques have been suggested such as ovarian cortex cryopreservation and oocytes cryopreservation.

KEYWORDS

Turner syndrome, pubertal development, fertility preservation, mosaicism, oocyte cryopreservation

Introduction

The objective of this manuscript is to review the current knowledge on Turner's syndrome and reproductive health.

Turner's syndrome (TS) is a genetic pathology that affects about one of 2,500 newborn girls (1).

Turner's syndrome is characterized by highly variable genetic anomalies that consist of a partial or complete deletion of the X sexual chromosome; it can be present as a monosomy or as a mosaicism with two or three different cellular lines.

Of the patients with Turner's syndrome, 50% have a 45 XO karyotype, while the remaining cases have karyotypes with mosaicism or X isochromosome or with partial or whole Y chromosome.

The disease course of premature ovarian insufficiency (POI) differs significantly among the causative genes and the types of mutations. The representative genes whose mutations are significant features in the syndromes and in family history are FOXL2, CLPP, FSHR, and FMR1. Mutations in FOXL2, in the form of BPES type 1, are associated with POI. The fragile X mental retardation gene (FMR1) has been known to be associated with POI. The fully expanded form causes the loss of the RNA-binding FMR1 protein and results in fragile X intellectual disability (2).

This pathology is characterized by multiple anomalies that involve physical and cognitive development, in particular the endocrine, cardiovascular, reproductive, auditory, and visual systems.

Phenotypic characteristics of TS are classic facial appearance, neck webbing, short stature, risk for heart and renal defects, and gonadal dysgenesis with an increased risk of premature ovarian insufficiency (3). The medical care of a girl with TS in a specialized hospital center is complicated due to the complexity of her condition (4).

The integrity of the X chromosome is essential for fertility. In TS, there is accelerated germ cell apoptosis (5, 6). Oocyte apoptosis is accelerated from the early stage of fetal life, and the reduced number of germ cells disturbs primordial follicle development, thereby causing the formation of streak gonads. There are three possible causes of accelerated germ cell loss in 45,X ovaries. First, chromosomal pairing failure due to X chromosomal aneuploidy could induce meiotic arrest. Second, germ cell apoptosis could be caused by impaired coupling between oocytes and granulosa cells. Lastly, ovarian dysfunction in women with TS is partly attributable to the reduced dosage of several genes on the X chromosome (such as BMP15, PGRMC1, and some other genes on the X chromosome) (7). About 30% of TS girls have some pubertal development, 10%–20% undergo menarche, and 2%–8% go through spontaneous pregnancy (8–10).

Women with TS should be informed about the risk of premature menopause and should be referred, if possible, to a specialist evaluation with a doctor expert in assisted reproductive techniques (11).

The combination of amenorrhea and elevated FSH concentrations (hypogonadotropic hypogonadism) can be used to evaluate POI in adolescents and adults (12). However, in postpubertal adolescents and adult women, reproductive hormones may remain within the normal range before POI is clinically evident, despite significant depletion of the ovarian reserve.

Today, reproductive medicine offers the opportunity for fertility preservation in women with POI (13). Two techniques have been suggested, such as ovarian cortex cryopreservation and oocyte cryopreservation (14).

Diagnosis

The prenatal diagnosis of Turner's syndrome can be achieved with invasive methods (amniocentesis, chorionic villous sampling), but some ultrasonography features such as increased nuchal translucency, the presence of cystic hygroma, coarctation of the aorta, left-sided cardiac defects, brachycephaly, renal anomalies, polyhydramnios, oligohydramnios, and intrauterine growth retardation should give rise to the suspicion of this syndrome. Abnormal maternal serum screening (alfa-feto protein, beta HCG, inhibin A, and unconjugated estriol) may also suggest the diagnosis of Turner's syndrome. However, neither ultrasound nor maternal serum screening should be considered diagnostic of Turner's syndrome, and karyotype confirmation should be mandatory.

The degree of mosaicism prenatally detected is not generally predictive of the severity of the Turner's syndrome phenotype. Many of the pregnancies with a diagnosis of Turner's syndrome ended in successful term births.

The American College of Medical Genetics recommends the execution of a standard karyotype at 30 cells in all those born on suspicion of Turner's syndrome. This analysis allows to identify the cases of mosaicism that involve at least 10% of cells (95% CI). In the case of a high suspicion of hidden mosaicism, other metaphases or studies of fluorescent *in situ* hybridization (FISH) can be performed. Also, probing for the Y chromosome should be performed in all these patients. In cases of virilization in patients with Turner's syndrome, the search for a possible Y chromosome is necessary, as is the exclusion of a gonads or adrenal gland's neoplastic pathology. After birth, the diagnosis of Turner's syndrome should be suspected in all patients who present unexplained retard of pubertal maturation, edema of the hands and feet, retronuchal thickening, left-sided cardiac defect, low posterior hairline, low set ears, small mandible, short stature, elevated Follicle-stimulating hormone (FSH) levels, cubitus valgus, typical facies, multiple pigmented nevi, short fourth metacarpal, high arched palate, and chronic otitis media.

An early diagnosis of this disease allows for the identification of cardiac and auditory defects and pubertal development retardation in order to establish a correct therapy to prevent complications (15).

TS is diagnosed with a standard karyotype counting 15–30 cells. If mosaicism is suspected, it may be necessary to increase the number of cells to 100 cells. In total, 50% of TSs will have a 45,X karyotype, and 40% will have a structural abnormality of the second X chromosome (16).

Most TSs with mosaic karyotypes do not have the classic features of TS. The karyotype study should be performed if short stature, delayed puberty, lymphedema, and aortic co-arctation are present in history (15).

Pubertal development

Turner's syndrome is classified among the conditions of POI. The number of germinal cells is normal until 18 weeks of gestation, then the degeneration process begins. From early childhood (2–5

years), increased levels of FSH and LH are measured, and in the adult age, these reach menopausal levels.

TS patients with monosomy X have small, striated gonads and hypogonadotropic hypogonadism, while about 30% of girls with TS with mosaicism have spontaneous puberty, 4% reach menarche, and 1% are fertile (17).

Almost 90% require hormone replacement therapy to ensure progress in puberty, maintain secondary sexual development, and promote bone health (18).

Up to 30% of patients with Turner's syndrome show signs of pubertal development, and 2%–5% present regular menstrual cycles without therapy; 2% of these patients have a spontaneous pregnancy (19). Recently, some ovarian follicles were observed also in 12–19-year-old patients with monosomy 45 XO. Aso (20) investigated the possibility of predicting spontaneous menarche and regular menstrual cycles in 50 patients with Turner's syndrome. The patients were divided into three groups: in the first group, patients with spontaneous menarche before 16 years and regular cycles for at least 18 months; in the second group, patients with spontaneous menarche before 16 years but irregular cycles and secondary amenorrhea; and in the third group, patients without spontaneous breast development before 14 years or with primary amenorrhea at 16 years. The authors analyzed the values of FSH and LH in these patients at 12–13 years. The results confirmed that the patients with a karyotype with mosaicism have more frequent regular cycles. The patients with FSH levels lower than 10 mIU/mL at the age of 12 years presented spontaneous menarche and regular menstrual cycles, confirming the significance of FSH levels as a preventive sign of spontaneous menarche and regular cycles, as well as the absence of spontaneous menarche, hypoplastic ovaries, or reduced development of the uterus (21).

The occurrence of spontaneous pubertal development is directly associated with the presence of the second X chromosome in the karyotype; patients with X monosomy have a much lower incidence of spontaneous puberty than patients with mosaicism (21). Purushothaman (22) confirmed that some karyotypes, including monosomy 45 XO, Xq deletions, and 46 XY mosaicism, are associated with poor fertility potential, while other karyotypes, such as 46 XX mosaicism and terminal Xp deletions, are more frequently related to spontaneous menarche.

However, in a recent study by "The US National Institute of Health," spontaneous pregnancies in women with a 45 XO karyotype, the classic form of Turner's syndrome, were observed, suggesting that there are different alleles involved in the regulation of fertility, placed in different positions on the X chromosome (Table 1) (8).

A Swedish study describes 12% of pregnancies in women with Turner's syndrome (57/482), with a live birth rate of 54% in 124 pregnancies. Approximately 40% (23/57) of pregnancies were spontaneous, 5% (three of 57) were obtained with IVF, 2% (one of 57) were obtained with IUI, and 53% (30/57) with oocyte donation (23).

It is very important to establish the timing of introduction, the type, the dose, and the route of administration of estrogen therapy. Estrogen therapy is initiated around the age of 11–12 years if the gonadotropin level is high and the Anti-Mullerian Hormone (AMH) is low and gradually increased over a 2–3-year period to mimic the physiological increase (24). Therapy begins with half of a 14-mg patch applied weekly or a whole 14- or 25-mg patch for 1 week per month at age 11–12 and escalates every 6–12 months based on response and potential for growth. When estradiol transdermic is not available or compliance is an issue, evidence supports the use of oral micronized estradiol. Delaying estrogen treatment is detrimental to bone development and other aspects of the child's health. Estrogen doses increase at 6-month intervals and can mimic the normal pubertal period (25).

In girls, growth hormone (GH) therapy is recommended; initiation of GH therapy before low-dose estrogen is important for growth (26).

To minimize the risk of irregular bleeding, endometrial hyperplasia, and endometrial cancer, additional oral progestogen

TABLE 1 Background data and pregnancy outcome in women with IS (8).

Characteristic	Own oocytes	Oocyte donation	All TS
Background data			
No. of women	27	30	57
- Spontaneous pregnancy (n)	23		
- IVF (n)	3		
- Insemination (n)	1		
Age, y, median (range)	27 (16–42)	37 (24–44)	32 (16–44)
Marital status, n (%)			
- Married/cohabitant	27 (100)	30 (100)	57 (100)
Socioeconomic status, n (%)			
- Student	5 (18)	9 (30)	14 (24)
- Employed	22 (82)	20 (67)	42 (74)
- Unemployed	0 (0)	1 (3)	1 (2)
Chromosomal constitution, n (%)			
- 45,X	1 (4)	16 (53)	17 (30)
- 45,X/46,XX	25 (92)	2 (7)	27 (47)
- Y	1 (4)	3 (10)	4 (7)
- Iso, ring, trisomy X	0 (0)	9 (30)	9 (16)
Pregnancy outcome			
- No. of pregnancies	82	42	124
- Delivery, n (%) (liveborn rate)	36[a] (44)	31 (74)	67 (54)
- Liveborn children (n)	37	31	68
- Miscarriage, n (%)	37 (45)	11 (26)	48 (39)
- Legal abortion, n (%)	8 (10)	0	8 (7)
- Extrauterine pregnancy, n (%)	1 (1)	0	1 (1)

[a]One set of twins.

therapy (for 10 days/month or continuously) is required after 2 years of estrogen treatment (27).

Turner's syndrome and fertility

Spontaneous pregnancy in TS has an incidence of 2%–7% (28).

Oogenesis and folliculogenesis are compromised in girls with TS; a high rate of abnormal follicles was detected, and follicular atresia was accelerated (29). The presence of follicles depends on karyotype (mosaicism), age, and concentration of FSH and AMH (30) In fact, spontaneous puberty is correlated with high levels of AMH and inhibin B (31).

Spontaneous pregnancies in women with 45,X and mosaic TS are between 2% and 7% (24). The miscarriage rate reported is 31% (32). Spontaneous pregnancies in TS have a high risk of fetal sex chromosome aneuploidy and trisomy 21 and a 3.8% rate of aneuploidy. An increased risk of chromosomal aneuploidy may include premature aging of oocytes (33).

Possible complications of spontaneous pregnancy are aortic dissection due to dilatation of the aorta and cardiac decompensation (34).

Fertility preservation: evaluation for potential fertility in women with TS

The evaluation for potential fertility in girls with TS consists of:

- Evaluation of karyotype
- Evaluation of ovarian reserve (AMH and antral follicles count).

The karyotype of women with TS was correlated with the size of the ovarian primordial follicle pool and spontaneous puberty; in fact, studies have shown that the chromosomal structure of women with TS has an impact on ovarian function (35).

Ovarian reserve capacity varies among women with TS, so it is important to select patients who can preserve their fertility as early as possible. Predictive parameters are serum AMH level, serum FSH level, karyotype analysis, development of spontaneous puberty, and ultrasound assessment of antral follicle count (AFC). AMH concentrations are higher in patients with spontaneous puberty and mosaic karyotype.

Women with TS should receive an early diagnosis, an assessment of ovarian reserve, and, in cases of residual ovarian function, options for fertility preservation (36).

Fertility preservation can be obtained by four different methods: oocyte donation, oocyte cryopreservation, embryo cryopreservation, and heterologous transplantation of ovarian tissue. The embryo cryopreservation after ovarian stimulation is the only treatment encoded by the ethics committee American Society for Reproductive Medicine (ASRM) to preserve fertility in patients with Turner's syndrome, but this method cannot be applied in pre-pubertal patients. In these patients, the only possible methods are oocyte and ovarian tissue cryopreservation. The purpose of ovarian tissue cryopreservation is the recovery of primordial follicles in the ovarian cortex before the end of the atretic process in order to culture them *in vitro* (37, 38). Atresia and damage can affect primordial and primary follicles during freezing, and preovulatory antral follicles do not usually survive the procedure of ovarian tissue cryopreservation, whose clinical efficiency should be confirmed (35).

An accurate selection of Turner's syndrome patients who can be submitted to treatment for fertility preservation is very important. The discussion about the opportunity to do these treatments in patients with karyotypes without mosaicism is still open. Hreinsson (35) suggested that patients aged 12 and 13 should be subjected to ovarian tissue cryopreservation in order to obtain a relevant number of primordial follicles; 90% of the patients with XO karyotype had already had FSH levels above 40 mIU/mL in pre-pubertal age. The ideal age to perform oocyte or ovarian tissue cryopreservation is not yet established, but some authors suggest that the age of 12–14 years is more suitable (39).

Another study (38) suggests that ovarian tissue cryopreservation should be performed before puberty, as at the beginning of puberty, the number of follicles may already have been reduced. Overall, the biopsy is an invasive method that should be reserved only for selected patients; at the beginning, the evaluation of ovarian reserve should be done through noninvasive methods such as plasmatic screening of FSH, AMH, and inhibin B. The extent and rate of decline of the plasmatic level of AMH, the reduction of inhibin B, and the increase of FSH are associated with ovarian failure. In particular, AMH is a predictive marker of ovarian reserve, but the sensibility and specificity of this marker should be assessed by further studies (22). A study by Hagen (30) showed a close association between levels of AMH and ovarian reserve. Turner's syndrome patients with X monosomy or with the absence of spontaneous pubertal development and premature ovarian failure had already very low levels of AMH before 25 years (AMH < 2–7 pmol/L). On the contrary, patients with mosaicism or with preserved ovarian function had normal levels of AMH.

Another treatment to preserve fertility in these patients is heterologous transplantation of ovarian tissue. Mhatre and Mhatre (40) described the first case of transplantation of ovarian tissue from the mother to a 15-year-old daughter with Turner's syndrome. The menstrual cycles have returned for 12 months, but the effects of immunosuppressive therapy and ovarian function in the following years should be assessed through additional studies.

Lau et al. (41) recently described the application of oocyte cryopreservation in patients with Turner's syndrome by analyzing the reproductive state of 28 patients with Turner's syndrome; 46% of the patients had a partial or complete deletion of the X chromosome, 32% had mosaicism, and 21% had X isochromosome or X ring chromosome.

The age of diagnosis was variable; 21% of cases were diagnosed at prenatal or neonatal age, 21% between 6 months and 10 years, and 57% between 11 and 20 years. Six patients (21%) had spontaneous pubertal development: five of these had mosaicism, whereas one with monosomy X had reached the stage of development Tanner 2. Thus, 14% (all with mosaicism karyotype) had a spontaneous menarche. The ultrasound exam showed in seven of 21 patients regular uterine and ovarian morphology; only

one of these patients had a karyotype with X monosomy. The levels of FSH were examined in 21 patients and were associated with karyotype. Approximately 10 of 11 patients (91%) with karyotype 45 XO had levels of FSH greater than 40 IU/mL, whereas two of four patients with X isochromosome or X ring chromosome had levels of FSH lower than 40 IU/mL. The mean plasma concentration of FSH in the group with mosaicism karyotype resulted significantly lower than in the group with karyotype with monosomy X or X ring chromosome (25.9 IU/L ± 10.4 IU/L vs. 80.7 IU/L ± 8.0 IU/L and 53.9 IU/L ± 19.2 IU/L, $p < 0.05$). The increase in FSH levels directly correlated with increasing age in the patients with karyotype 45 XO: the increase in FSH levels above 40 IU/L appeared at the age of about 16 years. Based on the criteria listed above, four patients (14%) were suitable for fertility preservation. The features of these patients and their reproductive history are shown in Tables 2, 3.

One of the four selected patients (patient 4) has undergone ovarian stimulation with a GRH agonist and recombinant FSH at 450 IU for 5 days and then 600 IU for the other 5 days. Two oocytes at the stage of metaphase II were recovered at the oocyte retrieval and were vitrified (41). Moreover, El-Shawarby (39) treated a patient with mosaic Turner's syndrome who was 22 years old and obtained eight oocytes that were vitrified for future use. These results confirm previous observations of Abir (38): the preservation of fertility should be suggested to patients with mosaicism or X isochromosome. However, the discussion about the patients with X monosomy is still open.

Similarly, further evidence is needed to identify the appropriate age for oocyte cryopreservation.

Oocyte cryopreservation

This technique is no longer considered experimental (42); therefore, oocyte preservation is now one of the best choices to preserve fertility in cancer patients (43–47). In recent years, this technique has been used for various medical, social, ethical, and legal indications.

Oocyte cryopreservation has several safety reports, and more than 3,000 live births have been achieved with no evidence of an increase in infantile abnormalities (48).

With regard to efficiency, fertilization of cryopreserved oocytes, pregnancy, and delivery outcomes are like those of fresh *in vitro* fertilization (IVF) (44, 48).

Oocyte cryopreservation is a safe and effective technique to protect fertility, even in women with mosaic TS (49). This technique has been used successfully in a small number of women with TS, and the number of mature oocytes retrieved in a single cycle is very small. Therefore, the procedure must often be repeated to finally obtain more than 10 mature oocytes. The patients' oocytes have been found morphologically and chromosomally normal (50).

The first live birth using cryopreservation of own oocytes was recently published (51).

In total, 31 patients with TS received fertility counseling at the Infertility and IVF Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy, between 2000 and 2022. Karyotype, spontaneous menarche, and menstrual rhythm were evaluated during the counseling. The ovarian reserve was determined by measuring serum AMH, FSH, and ultrasound AFC. Seven patients (mean age 23.2 years ± 3.9 years) underwent ultrasound-guided oocyte retrieval after controlled ovarian stimulation. The mean value of AMH was 0.6 ng/mL ± 0.4 ng/mL, the mean value of FSH was 12.1 IU/mL ± 3.6 IU/mL, and the mean antral follicles count was 3.4 ± 2.1. Gonadotropins associated with gonadotropin-releasing hormone (GnRH) agonist (leuprolide acetate) or antagonist (cetrorelix acetate) were used to perform ovarian stimulation. Ovulation was triggered with recombinant hCG. Ovarian stimulation was monitored with seriated estradiol blood tests and pelvic ultrasounds. When follicles reached a diameter of 16–17 mm and the estradiol serum levels were considered appropriate, ovulation was triggered with hCG 36 h prior to transvaginal ultrasound-guided oocyte retrieval. The mean number of oocytes retrieved was 2.7 ± 1.3, and the mean number of mature (MII) oocytes stored was 2.2 ± 0.7 per patient.

The selection of patients suitable for cryopreservation can be done through the evaluation of predictive factors for the ovarian follicles' presence, as hypothesized by Borgström (52). In total, 57 patients with Turner's syndrome, aged between 8 and 19.8 years, underwent laparoscopic ovarian biopsy. In 15 patients (26%), some follicles in the analyzed ovarian tissue were recovered (86% with mosaicism, 10.7% with X monosomy), whereas eight of 13 patients

TABLE 2 Characteristics of the four patients with Turner's syndrome who are potentially eligible for fertility preservation (41).

Patient No.	Age at diagnosis (years)	Karyotype (%)	FSH (IU/L)	Age at FSH measurement (years)	Ultrasonographic findings	Spontaneous puberty (Tanner stage)	Age of spontaneous menarche
1	16	45,X (74/46), XX (247), XXX (24)	NA	NA	1 ovary and uterus seen	5	13
2	14	45.X (98)/47, XXX (2)	7.7	14	1 ovary and uterus seen	4	13
3	10	45.X (6¥46), XX (94)	7.1	10	Both ovaries and uterus seen	5	11
4	9	45,X (98)47, XXX (2)	6.6	15	Both ovaries and uterus seen	5	14

NA, non available.

TABLE 3 Current reproductive status and fertility outcome of four potential fertility preservation candidates (41).

Patient No.	Current age (years)	Current reproductive status and fertility outcome
1	31	Spontaneous pregnancy at the age of 24 years; hormone replacement therapy for 6 years
2	31	Hormone replacement therapy for 15 years
3	23	Normal menses ranging 30–40 days
4	18	Normal menses; ovarian stimulation and oocyte cryopreservation at the age of 16

(62%) with spontaneous menarche and 11 of 19 (58%) with spontaneous pubertal development had some follicles. The higher percentage of follicles was in female patients aged between 12 and 16 years. The patients with some ovarian follicles had more often normal values of FSH and low levels of AMH.

Therefore, spontaneous pubertal development, mosaicism, and normal hormone levels are significantly associated with a higher probability of having ovarian follicles; consequently, they can be used as screening factors to identify patients suitable for ovarian biopsy. Recently, Huang (53) proposed to combine ovarian tissue cryopreservation and oocyte cryopreservation to preserve the fertility in Turner's syndrome. He described a case of a 16-year-old patient with Turner's syndrome (karyotype 45,X(20%)/46 XX (80%)) submitted to oocyte retrieval of 11 immature oocytes from the ovarian cortex; eight of these oocytes were vitrified after *in vitro* maturation (maturation rate: 73%) (53).

Oocyte cryopreservation is also a feasible technique for fertility preservation in selected postpubertal female children at risk for premature ovarian failure due to accelerated follicle loss in Turner's syndrome. However, further studies would be needed to test the results of oocyte cryopreservation in young girls (54).

Ovarian tissue cryopreservation

This procedure can be performed at any age without the need for spontaneous puberty or gonadotropin stimulation (55). The TS procedure is still experimental; no live births have been reported (56).

Oocyte donation

Oocyte donation is another method to preserve fertility in Turner's syndrome.

Oocyte donation is most frequently used in TS (33). The clinical pregnancy rate of oocyte donation is 30%–46%, with a low percentage of miscarriages (23). Compared to conventional IVF, pregnancies achieved with donated oocytes are associated with a higher incidence of gestational hypertension and preeclampsia (57).

The study by Press (58) showed a similar pregnancy rate for cycles between patients with Turner's syndrome and patients with idiopathic premature ovarian insufficiency in cycles of oocyte donation. Khastgir (59) confirmed the efficacy of this method and obtained a 41.2% pregnancy rate for cycles and a 17% implantation rate for embryos in patients with Turner's syndrome through oocyte donation. Moreover, the study demonstrated a positive predictive value of the endometrial thickness on the day of embryo transfer: there is a higher pregnancy rate for the cycle when the endometrium is thicker than 6.5 mm.

Pregnancy in Turner's syndrome

The application of methods to preserve fertility in Turner's syndrome should be associated with suitable counseling about the risks of pregnancy in these patients. Maternal and fetal complications are increased in TS pregnancies. The possible risks are diabetes, placental insufficiency, metabolic disease, preeclampsia, and intrauterine growth restriction (60). The risk of fetal loss and congenital malformations is increased both in spontaneous pregnancies and in pregnancies obtained through oocyte donation. In a review of Tarani (61), including 160 spontaneous pregnancies in patients with Turner's syndrome, the incidence of fetal loss, chromosomal abnormalities, congenital malformations, and perinatal death was respectively 29%, 20%, and 7%.

Birkeback (62) analyzed the reproductive state of 412 women with Turner's syndrome by consulting the dates of the Danish register; 33 of these women (only one with 45 XO karyotypes, 27 with mosaicism, and five with structural anomalies of the second X chromosome) have given birth to 64 children. The karyotype of 25 children out of 64 was analyzed, and in six cases, a chromosomal anomaly was diagnosed (24%). Other studies show a higher incidence of trisomy 21 (4% vs. 0.4% in the general population) and Turner's syndrome (15% vs. 0.5% in the general population) in patients with Turner's syndrome (63–65). The increased incidence of chromosomal anomalies in these fetuses could be one of the possible causes of the increased abortion rate; other favoring factors are uterine malformations, reduced uterine development, reduced uterine vascular perfusion, reduced endometrial receptivity, and the presence of autoantibodies that correlate with the higher incidence of autoimmune pathologies in the Turner's syndrome patients. Further studies (59) documented a high incidence of miscarriages in patients with Tuner's syndrome who underwent cycles of oocyte donation. This finding confirms that the major incidence of uterine anomalies in these patients represents an important cause of miscarriage. The age of the recipients in cycles of oocyte donation did not seem to influence the success rates.

Bakalov (66) evaluated the association between uterine development in Turner's syndrome and hormone therapy. The normal uterine development observed in Turner's syndrome patients with spontaneous pubertal development would exude the existence of an intrinsic uterine defect that previous studies had suggested (67). The uterine development in Turner's syndrome seems rather to be related to the administration of hormone replacement therapy (HRT), as was demonstrated in 86 women

with Turner's syndrome, aged 18–45, who underwent an ultrasound examination of the uterus. About 1/4 (24.4%) of the women had a normal uterine development, while most (44.2%) had a small uterus and 1/3 (31.4%) had an immature uterus. The patients who took HRT had a significantly larger uterus than the patients who used oral contraception or without therapy. Duration and typology of HRT influenced uterine development; treatments including estradiol showed the highest efficacy.

The age of first exposure to estrogens, the stature and weight of the patients, and the previous intake of growth hormone do not seem to be related to uterine measures, but previous studies recommended initiation of estrogen therapy at the age of 12–15 years.

Karyotype was not associated with uterine dimensions, unlike suggested in previous studies (66, 68) that showed a correlation between normal uterine development and mosaicism (Table 4).

The available dates for the pregnancies of Turner's syndrome patients are still limited. A retrospective study by Bodri (69) analyzed the outcomes of the pregnancies of 21 patients with Turner's syndrome who underwent 30 cycles of oocyte donation between 2001 and 2004. Among the 17 pregnancies obtained, 12 were clinic, with a high rate of biochemical miscarriage (29% vs. 12.9% in the general population). The implantation and pregnancy rate were respectively 22% (15 of 68) and 30% (nine of 30). The premature birth rate was 50%, and intrauterine growth retardation was found in 55.5% of fetuses. Hypertension was diagnosed in five of eight pregnancies, and there were three cases of preeclampsia. The increased incidence of hypertensive disease in this group of patients, observed also in previous studies (69, 70), is not related to increased maternal age nor the high incidence of multiple pregnancies as in the general population who undergo oocyte donation.

TABLE 4 Variables influencing uterine volume (66).

Continuous indenendent variables			
	R^2	*F* value	*p*-value
Age	0.24	5.08	0.027
Height	0.008	0.68	0.411
Weight	0.017	1.46	0.230
Body surface area	0.016	1.40	0.240
Age at estrogen exposure	0.022	1.85	0.177
Years of estrogen exposure	0.161	15.94	0.0001
Nominal independent variables			
	Level	Volume (mL; mean ± SD)	*p*-value
History of GH use	Yes (n = 26)	18.3 ± 12.0	0.066
	No (n = 60)	22.8 ± 12.3	
Current HRT	Yes (n = 69)	23.0 ± 12.6	0.0019
	No (n = 17)	15.0 ± 8.6	
Spontaneous menarche	Yes (n = 13)	30.0 ± 16.9	0.014
	No (n = 73)	19.9 ± 10.7	
Type of estrogen	E2 (n = 10)	28.0 ± 10.0	E2 vs. none, p = 0.0015
	CE (n = 28)	25.0 ± 14.6	E2 vs. OC, p = 0.045
	OC (n = 27)	19.7 ± 11.0	CE vs. none, p = 0.0032
	None (n = 20)	15.4 ± 8.6	

E2, estradiol; CE, conjugated estrogens; OC, oral contraceptives.

All the patients underwent cesarean sections because of preeclampsia (two cases) and fetopelvic disproportion (the remaining). Fetopelvic disproportion has been described as the main indication for cesarean section in different studies (8, 71).

These data suggest the opportunity for careful monitoring of the pregnancies obtained in Turner's syndrome and the necessity to reduce the multiple-pregnancy rate that is associated with an increased rate of hypertensive disease. Mortality for cardiovascular complications, in particular aortic dissection, is threefold increased in women with TS compared to the general population. The risk of death from aortic dissection in TS is two of 1,000 (27).

The risk of maternal death in a pregnancy obtained through oocyte donation is about 2%; seven cases of dissection of the aorta during the pregnancy were reported in the literature. The hyperdynamic and hypervolemic vascular state associated with the pregnancy seems to increase the risk of dissection of the aorta. Moreover, gravidical hyperestrogenism can change the structural integrity of the aorta and make it more susceptible to damage. The risk of aortic dissection is increased in the early weeks of the pregnancy and in the third trimester.

Boissonas (72) reported a case of a patient with Turner's syndrome (45 X0–46 XY karyotype) who had a pregnancy from oocyte donation. The patient did a cardiac screening before the pregnancy that resulted in normal but had dissection of the aorta at 38 weeks of gestation after the diagnosis of the bicuspid aortic valve at 16 weeks of gestation. ASRM recommends a cardiac screening in all patients with Turner's syndrome who want a pregnancy (73). The screening should include echocardiography, ECG, and magnetic resonance. The detection of a severe cardiac anomaly should represent a contraindication to assisted reproduction in this group of patients. In patients with Turner's syndrome who undergo ART, a single embryo transfer should be performed in order to avoid the hemodynamic overload associated with multiple pregnancies (15). Recently, the French College of Obstetricians and Gynecologists (FOG) brought together a committee that included the French Societies of Obstetrics and Gynecology, Cardiology, Cardiac Surgery and Vascular Surgery, Anesthesia, Endocrinology, the Study Group on Oocyte Donation, and the Biochemical Agency, with the aim of establishing guidelines about the management of the patients with Turner's syndrome before and during the pregnancy.

The recommendations include a list of the exams required before the pregnancy, information for the patients, indications

about monitoring the pregnancy and kind of delivery, and postnatal follow-up (74).

It is essential to perform a multidisciplinary evaluation before pregnancy with a team including maternal–fetal medicine specialists and cardiologists to evaluate the possible risks (7, 75, 76).

Conclusions

In TS, the reproductive consequences are primary amenorrhea and premature ovarian insufficiency. Estrogen replacement therapy should be started around the age of 12 to reduce the morbidities related to hormone deficiency. After menarche, the option of oocyte cryopreservation should be offered to patients with mosaic Turner's syndrome. Pregnancy is a high risk in TS; management of pregnancy with a multidisciplinary specialist team should be implemented in order to reduce complications for mothers and infants.

Author contributions

EP: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. GD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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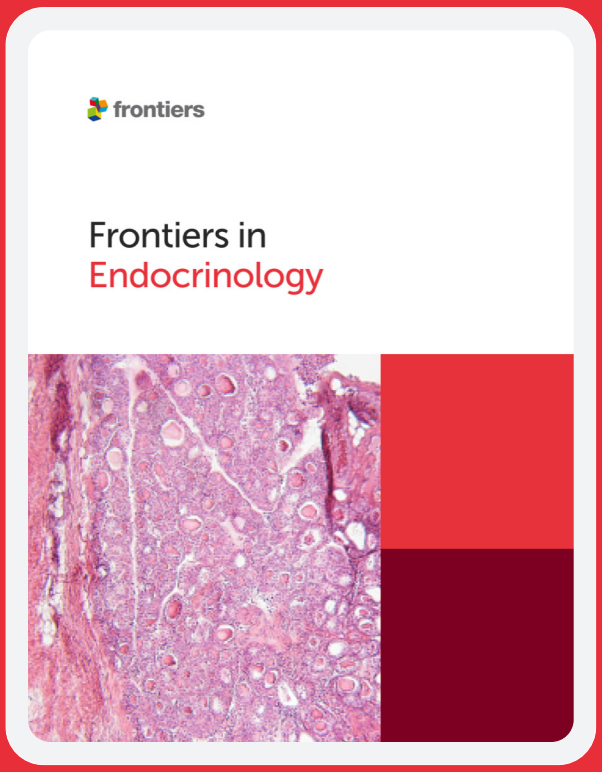

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