HOT TOPICS OF DEBATE ON TURNER SYNDROME: GROWTH, PUBERTY, CARDIOVASCULAR RISKS, FERTILITY AND PSYCHOSOCIAL DEVELOPMENT

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HOT TOPICS OF DEBATE ON TURNER SYNDROME: GROWTH, PUBERTY, CARDIOVASCULAR RISKS, FERTILITY AND PSYCHOSOCIAL DEVELOPMENT

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Turner syndrome (TS) is a relatively common chromosomal disorder affecting approximately 1 in 2000 live female births. Short stature is the most common clinical presentation of the syndrome; it is observed in 80 % to 100 % of the girls with TS. Growth is stunted to a variable degree at different phases of childhood and adolescence in TS. Although there is consensus on treating growth failure as early as possible, there is ongoing research to ameliorate final height in TS girls. The co-existence of primary ovarian failure in girls with TS further complicates the management of short stature. It remains to be determined what is the best age to begin treatment for pubertal induction and/or maintenance of puberty; the best compound, dose, or protocol to induce puberty. TS patients have increased mortality and morbidity due to cardiovascular (CV) complications and multiple risk factors for ischaemic heart disease, including hypertension, insulin resistance and dyslipidaemia. The CV phenotype can be better assessed through identification of surrogate risk markers and the relationship of these markers with TS - associated traits. There are very few comprehensive studies on the dynamics of arterial tree and the associations of these dynamics with influential factors in young TS patients. Deregulated signalling within the GH-IGF1 axis may extend beyond the realm of physical stature in TS, resulting in effects on the CV system and other organ systems. Although GH deficiency is associated with CV risk, and excess of GH is also associated with increased CV risk. Because TS patients receive supraphysiological doses of GH, there may be increased risks for CV complications, although retrospective studies failed to document such an association. There are still other unknown issues in the area of TS and CV issues such as the definitions of "abnormal" aortic diameters, high blood pressure. There is scarce data regarding how we can prevent catastrophic CV events in girls and women with TS. There is a call for prospective studies regarding the pathogenesis, diagnosis and prevention of CV disease in TS.TS patients usually have psychological problems related to self-confidence and self-respect due to the high burden of health-related issues they have to cope with at different stages of life. There remains controversy on how to support them to accept their differences and empower them to take an active role in their care. This e-book intends to provide insight on hot topics of debate in TS. The e-book is subdivided into small sections to describe the

content of the articles such as growth, puberty, cardiovascular issues, metabolic issues, autoimmunity, cognitive and psychosocial issues to facilitate reading. We hope it will serve as a reference tool for clinicians and researchers who are involved in the diagnosis and management of females with TS.

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EDITORIAL
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Editorial: Hot Topics of Debate on Turner Syndrome: Growth, Puberty, Cardiovascular Risks, Fertility and Psychosocial Development

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Keywords: Turner syndrome, growth, puberty, psychosocial, cardiovascular risk

Editorial on the Research Topic

Hot Topics of Debate on Turner Syndrome: Growth, Puberty, Cardiovascular Risks, Fertility and Psychosocial Development

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Turner syndrome (TS) is the most common female sex chromosome disorder with an incidence of 1 in 2,000 to 1 in 2,500 live female births (1). Individuals may be diagnosed at different stages of life beginning from *in utero* till adulthood, abnormal maternal screening, or fetal abnormalities, in infancy through the presence of lymphedema, in childhood as a result of growth failure, in adolescence as a result of short stature with pubertal delay, and in adulthood as a result of premature ovarian failure (2). The key to the care of this population includes proactive screening for co-existing medical conditions, including imaging for cardiac and renal anomalies, and monitoring for obesity and hypertension, developmental/psychoeducational abnormalities, hearing loss, autoimmune diseases, and short stature. Ovarian dysfunction and infertility should be anticipated (2).

The purpose of this Research Topic is to gather together research and review papers, which may serve to highlight the diverse challenges in the care of females with TS with the expectation that this will allow more critical appraisal of existing studies, identify critical research gaps, and pave the path for future studies.

In terms of addressing issues regarding growth and puberty in females with TS, Gawlik et al. document successful induction and progression of puberty by transdermal estrogen in girls with TS at a mean age of 15.1 year over a mean follow-up period of 2.4 year. They also report adequate increase in uterine size without compromising stature, irrespective of the karyotype status. Therefore, the fixed-dose transdermal estrogen regimen suggested by the authors seems to be effective in females with delayed diagnosis of TS. Regarding the growth and pubertal timing in females with TS, Woelfle et al. present a comprehensive report based on KIGS® (Pfizer International Growth Database including 7,219 females with TS between 1987 and 2012), and they demonstrate evidence of positive secular trends on age at onset of puberty and on final height akin to that reported on the normal population.

These finding may be indicative of earlier diagnosis and thus, of earlier start of GH and estrogen treatments than in the past. Because KIGS® data exclusively include GH-treated females with TS, whether these trends also apply to growth hormone (GH)-naïve counterparts is currently unknown. However, the doubling in prevalence in spontaneous puberty does suggest that environment-related trends may also apply to females with TS.

In addition to induction of puberty, the bone-health related advantages of estrogen replacement in TS have been longitudinally evaluated by Li et al. using a regional estrogen replacement protocol in China, and the authors report the positive impact of estrogen on bone mineral density and muscle strength despite a relatively short term follow-up period.

It is well-established that females with TS are at increased risk of excess adiposity and its related complications. The tempo of the derangement in metabolic health profile and the associated key factors have not been adequately studied. To this end, Lebenthal et al. document abnormal metabolic profiles in young prepubertal girls with TS, which confirms the presence of risk factors inherent to TS itself. The time-related increase in metabolic derangements with an increase in prevalence of overweight/obesity status also confirm that non-TS related factors as in the general population are also operative in TS. While the more prominent clustering of metabolic anomalies in females with 45,XO karyotype may suggest closer follow-up and earlier intervention in this group, factors associated with a more dismal metabolic outcome in 45,XO females await further studies. In a theme parallel with this latter article, Sun et al. review derangements in pancreatic β- cell function and their reflections on glucose metabolism in TS. The β- cell failure in TS may be due to Xp hapotype gene deficiency and to overexpression of some genes of Xq; this is also an area that also awaits further studies. Although females with TS are well-established to have increased autoimmunity, its association with dysglycemia is currently unestablished. In their review, Sun et al. also indicate that the theoretical adverse effect of GH therapy on glycemic regulation in this non-GH deficient population has not been proven, possibly owing to increase in lean mass with GH treatment.

In a preliminary report on autoimmunity in TS, Gawlik et al. find no significant difference between females with TS and healthy controls regarding regulatory T cell percents, but in a subgroup analysis between anti- thyroid peroxidase antibody positive and negative females with TS, a trend toward iXq karyotype with reduced percents of helper T cells was observed. These findings call for further studies to reach hard end-point conclusions on the mechanisms of autoimmunity in TS.

Thoracic aortic disease, be it congenital or acquired, is a major determinant of morbidity and mortality in TS [reviewed in Mortensen et al. (3)]. Cardiovascular risk assessment in TS, particularly for aortic dissection, unfortunately has remained inadequate, which is due to a limited understanding of the pathophysiology of thoracic aortic disease in TS. Cardiovascular magnetic resonance (CVMR) is the gold standard for non-invasive assessment of thoracic aortic disease. Obara-Moszynska et al. confirm the superiority of CVMR over echocardiography in identifying anomalies such as dilatation of the aorta, pericardial fluid, and functional impairment of ventricles in a young females with TS. Aortic dissection has been reported as early as 4 year in TS (4). However, the availability of CVMR is limited in many developing countries, and it requires general anesthesia in patients under 6 years of age. These shortcomings of CVMR indicate the need to find potential markers to diagnose aortic pathology in TS. To this end, Mainkurve and O'Gorman reviewed the potential role of natriuretic peptides and osteoprogerin for aortic pathology in TS. While some associations of these markers have been found with aortic disease, their predictive value remains to be determined.

While the endocrine- related issues in TS are addressed in many studies, studies evaluating psychosocial problems in TS are scant [reviewed in Culen et al. (5)]. Referring to a former study of theirs (6) and the current study, Anaki et al. document that dysfunction in social tasks in TS is most likely due to spatial-visual factors, and that the capacity of females with TS to understand the emotional and cognitive status of others is similar to healthy controls.

Rovet and Van Vliet examine a subgroup of females with TS from the Canadian GH trial regarding potential psychosocial benefits of of GH treatment. Studies have shown that short children have been affected by juvenilization, teasing, bullying, victimization, loss of independence/ overprotection, and exclusion [reviewed in Lipman and McCurry (7)]. However, this conclusion has not been strongly confirmed by Rovet and Van Vliet, who document modest effect of GH treatment on psychosocial functioning in females with TS. In this study, Rovet and Van Vliet included a respectable number of patients with a relatively lower rate of follow-up data loss compared to other studies (8). Therefore, the authors conclude that it is important not to overemphasize the benefits of GH treatment on heightism since final height in many GH treated females with TS remains suboptimal.

In conclusion, we hope that this Research Topic will serve as a point of reference and source of inspiration for researchers and clinicians interested in addressing controversial issues related to the care of females with TS.

AUTHOR CONTRIBUTIONS

AU drafted the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All co-authors revised the manuscript for important intellectual content, and approved the final version to be published.

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ORIGINAL RESEARCH
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Secular Trends on Birth Parameters, Growth, and Pubertal Timing in Girls with Turner Syndrome

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Background: Whether children with chromosomal disorders of growth and puberty are affected by secular trends (STs) as observed in the general population remains unanswered, but this question has relevance for expectations of spontaneous development and treatment responses.

Objectives: The aim of the study was to evaluate STs in birth parameters, growth, and pubertal development in girls with Turner syndrome (TS).

Study design: Retrospective analysis of KIGS data (Pfizer International Growth Database). We included all TS patients who entered KIGS between 1987 and 2012 and were born from 1975 to 2004, who were prepubertal and growth treatment naïve at first entry (total number: 7,219). Pretreatment height and ages at the start of treatment were compared across 5-year birth year groups, with subgroup analyses stratified by induced or spontaneous puberty start.

Results: We observed significant STs across the birth year groups for birth weight [+0.18 SD score (SDS), $p < 0.001$], pretreatment height at mean age 8 years (+0.73 SDS, $p < 0.001$), height at the start of growth hormone (GH) therapy (+0.38 SDS, $p < 0.001$) and start of puberty (+0.42 SDS, $p < 0.001$). Spontaneous puberty onset increased from 15 to 30% ($p < 0.001$). Mean age at the start of GH treatment decreased from 10.8 to 7.4 years (–3.4 years; $p < 0.001$), and substantial declines were seen in ages at onset of spontaneous and induced puberty (–2.0 years; $p < 0.001$) and menarche (–2.1 years; $p < 0.001$).

Conclusion: Environmental changes leading to increased height and earlier and also more common, spontaneous puberty are applicable in TS as in normal girls. In addition, greater awareness for TS may underlie trends to earlier start of GH therapy and induction of puberty at a more physiological age.

Keywords: Turner syndrome, height, growth, birth weight, birth length, puberty, secular trend

Abbreviations: BL, birth length; BMI, body mass index; BW, birth weight; GH, growth hormone; ST, secular trend; TS, Turner syndrome.

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INTRODUCTION

Secular trends (STs) in birth parameters (1–3), growth (4, 5), and timing of puberty (6–8) are observed in normal populations in various settings. Changes in nutrition, better access to health care, and other environmental factors have been implicated as causative factors for these changes (4). Whether STs that affect normal populations also modulate growth and puberty of children with genetic or chromosomal disturbances that inherently affect growth and puberty remains unanswered.

Turner syndrome (TS) is caused by structural abnormalities in or complete loss of an X chromosome. It affects approximately 1 in 2,500 live-born female girls. The clinical phenotype of TS varies substantially, but in the majority of subjects includes short stature and ovarian failure, leading to hypogonadism and infertility (9).

In subjects with TS, haploinsufficiency of the SHOX gene has been proposed as an important cause of the growth phenotype in TS, since patients with heterozygous mutations in SHOX exhibit Leri-Weill syndrome, a bone dysplasia associated with short stature (10), whereas homozygous mutations with loss of both SHOX gene copies lead to a rare severe osteodysplasia (11). However, haploinsufficiency for the SHOX gene does not fully explain the growth phenotype and its variation in TS subjects. Probably, other factors such as estrogen deficiency (12), loss of additional X-chromosomal genes, or more general aneuploidy effects might be implicated in TS-associated short stature (13).

Although the majority of girls with TS have normal birth parameters, the frequency of TS in newborns with low birth weight (BW) and length is higher than expected (14, 15). This has been explained in part by loss or altered expression of X-chromosomal genes that are involved in fetal growth (16). Data analyzing the evolution of birth parameters in a sufficiently sized TS cohort over time to identify a ST in BW and length are lacking.

Girls with TS frequently exhibit delayed or absent pubertal development due to early ovarian failure. The exact molecular mechanisms leading to ovarian dysfunction in TS remain obscure. In a minority of patients (5–20%), puberty starts spontaneously and may even lead to spontaneous menarche in few subjects (17, 18). This seems to occur more frequently in subjects with a higher degree of mosaicism. As for auxological parameters, data on the presence or absence of an ST on spontaneous or induced puberty in TS are not available.

OBJECTIVES

To assess STs on birth parameters, spontaneous growth and pubertal development in patients with TS and to evaluate whether clinical management of girls with TS has changed over time.

Patients

The patients studied had received recombinant growth hormone (GH, Genotropin®, Pfizer Inc.) as part of the pharmacoepidemiologic survey known as KIGS® (Pfizer International Growth Database). KIGS was established in 1987 as a worldwide observational registry to monitor outcomes and safety of Genotropin (somatropin, Pfizer Inc., New York, NY, USA) treatment in children with short stature. The KIGS survey was conducted in accordance with the Declaration of Helsinki (19).

As of June 2012, TS patients who entered the KIGS registry between 1987 and 2012 were included (total number: 7,219). Only patients who were at the prepubertal stage and naïve to any growth treatment at first entry were included. Birth years ranged from 1975 to 2004. The diagnosis of TS was made according to standard clinical practice and was confirmed by karyotype by the treating physicians. Patients' characteristics are depicted in **Table 1**.

Aims and Hypotheses

The main objective of this study was to assess STs on birth parameters, spontaneous growth, and pubertal development in patients with TS and to evaluate whether clinical management of girls with TS had changed over time. We had the following hypotheses:

- There is a positive ST for birth parameters.
- There is a positive ST for height before initiation of GH treatment.
- There is a positive ST for onset of puberty in the total TS cohort.

In order to address the outlined objectives, we analyzed data from three subcohorts derived from the KIGS database.

To assess STs, KIGS data on BW, birth length (BL), height SD score (SDS) at 8 years of age (chronological age 7.0–9.0 years) before initiation of any treatment, height SDS, and age at the start of GH therapy and at the start of puberty were analyzed in time intervals which were defined by year of birth (before 1980, 1980–1984, 1985–1989; 1990–1994; 1995–1999; and 2000–2004).

Methods

Cohort 1a was used to assess trends both in pretreatment height and in the age at the start of GH therapy. Inclusion required sufficient pretreatment data without exposure to therapies affecting growth (GH, oxandrolone, and sex steroids). To assess trends in

TABLE 1 | Patients characteristics (total cohort).

Variables	*N*	Median	P10	P90	Mean	SD
Birth weight SD score (SDS)	6,372	−1.05	−2.52	0.39	−1.05	1.19
Birth length SDS	4,500	−0.87	−2.52	0.82	−0.85	1.41
Midparental Height SDS	6,571	−0.49	−2.01	1.00	−0.50	1.17
Height SDS at age 8 years (Prader)	1,091	−2.19	−3.23	−1.21	−2.20	0.81
Height SDS at age 8 years (Ranke)	1,091	0.13	−1.15	1.34	0.12	1.02
Age at the start of growth hormone (GH) tx	7,219	9.60	4.41	13.97	9.35	3.63
Height SDS at the start of GH tx (Prader)	7,219	−3.22	−4.55	−2.03	−3.26	1.06
Height SDS at the start of GH tx (Ranke)	7,132	0.19	−1.17	1.54	0.19	1.12
Weight SDS	7,219	−1.44	−3.06	0.30	−1.43	1.34
Body mass index SDS	7,218	0.31	−1.09	1.87	0.34	1.17
GH dose (mg/kg/week)	7,219	0.31	0.18	0.38	0.30	0.09

pretreatment height, TS girls born before 1980 were included for analyses regarding BL/BW and pretreatment height at 8 years (unless they started recombinant GH before 1985). To analyze trends in age at the start of GH therapy, TS girls born before 1980 were excluded (as the approval of TS as an indication for GH therapy occurred beyond this birth cohort and this group, by definition, was relatively old at the start of GH). For this analysis, we also excluded the last group born between 2000 and 2004 as their maximum age was only 11 years old in 2012 when KIGS data collection ended. Data included in statistical analysis of trends are shaded gray in **Tables 2** and **3**.

We divided data in three categories:

(a) Displayed and tested (presented as shaded data in **Tables 2** and **3**).
(b) Displayed but not tested (Ht and age), since these data are relevant where sufficient data are available but are likely prone to bias. Thus, data are displayed as they are still informative but are not tested.
(c) Not displayed and not tested (puberty), since only insufficient data are available.

Since age at the start of GH treatment changed over time, we additionally compared subgroups from each cohort who had pretreatment measurements at a comparable age of 8 years (between 7.0 and 9.0 years; cohort 1b). Mean exact age at this measurement did not differ across the birth year groups.

Cohort 2 was used to assess trends in puberty timing. It included only those TS subjects with data during the age period when puberty was expected to occur. Therefore, we excluded the last group born between 2000 and 2004 as their maximum age was only 11 years old in 2012 when KIGS data collection ended.

Auxological Methods

Height was converted to SDS using both the height reference for healthy children of Prader (20) and the reference for TS of Ranke et al. (21). To calculate weight SDS, the normal population reference of Freeman et al. was used (22). To calculate body mass index SDS, the normal population reference of Cole was used (23). BW and BL for gestational age SDS were calculated using the reference of Niklasson et al. (24). The midparental height SDS was calculated as follows: (father's height SDS + mother's height SDS)/1.61 (25).

Definitions

The onset of puberty was defined by the visit at which either spontaneous breast development (Tanner stage > B1) was first observed or the date at which estrogen replacement therapy was initiated. The assessment of the qualitative and quantitative aspects of estrogen replacement was done by the treating physicians. Furthermore, available data were stratified into whether pubertal development started spontaneously or was pharmacologically induced. The group with spontaneous start of puberty included patients with spontaneous progression of puberty until menarche as well as those who later required sex steroid substitution before menarche.

Statistical Analysis

Statistical analyses [descriptive data analysis, calculation of SDS, and analysis of variance (ANOVA)] were carried out using SAS software (SAS Version 9.2, SAS Institute, Cary NC, USA). ANOVA models, *F*-tests, were applied to determine if there are any statistical mean differences between the groups based on year of birth. A p-value < 0.05 was considered to indicate statistical significance.

TABLE 2 | Secular trends on birth parameters and growth (mean ± SD).

Birth year	Before 1980	1980–1984	1985–1989	1990–1994	1995–1999	2000–2004	*p*-Value
At birth							
N = [birth weight(BW)/birth length (BL)]	769/492	1163/777	1552/1123	1409/992	948/688	531/428	
BW SD score (SDS)	−1.18 ± 1.18	−1.11 ± 1.20	−1.04 ± 1.20	−1.00 ± 1.18	−0.99 ± 1.17	−1.00 ± 1.16	<0.001
BL SDS	−0.97 ± 1.42	−0.83 ± 1.38	−0.84 ± 1.41	−0.78 ± 1.38	−0.78 ± 1.53	−1.00 ± 1.37	<0.001
Midparental height SDS	−0.82 ± 1.17	−0.65 ± 1.17	−0.44 ± 1.14	−0.44 ± 1.16	−0.37 ± 1.17	−0.29 ± 1.16	<0.001
At GHT start (cohort #1a)							
N =	855	1306	1729	1621	1107	601	
Age (years)	12.68 ± 2.01	10.85 ± 2.93	9.70 ± 3.33	8.83 ± 3.44	7.43 ± 3.24	5.26 ± 2.39	<0.001
Height SDS (Prader)	−3.72 ± 1.05	−3.42 ± 1.02	−3.28 ± 0.95	−3.15 ± 0.98	−3.04 ± 1.22	−2.97 ± 1.13	<0.001
Height SDS (Ranke)	−0.05 ± 01.12	0.10 ± 1.10	0.21 ± 1.05	0.25 ± 1.07	0.30 ± 1.29	0.32 ± 1.08	<0.001
Δ Height − MPH SDS	−2.90 ± 1.11	−2.77 ± 1.18	−2.79 ± 1.16	−2.68 ± 1.19	−2.65 ± 1.37	−2.68 ± 1.23	<0.01
Weight SDS	−1.59 ± 1.31	−1.47 ± 1.32	−1.35 ± 1.30	−1.33 ± 1.36	−1.40 ± 1.40	−1.65 ± 1.38	NS
Body mass index (BMI) SDS	0.42 ± 1.08	0.37 ± 1.13	0.41 ± 1.16	0.39 ± 1.21	0.28 ± 1.17	−0.04 ± 1.21	NS
At age 8 years (cohort #1b)							
N =	18	226	287	280	178	102	
Height SDS (Prader)	−2.69 ± 0.79	−2.41 ± 0.79	−2.22 ± 0.77	−2.14 ± 0.78	−2.08 ± 0.86	−1.96 ± 0.87	<0.001
Height SDS (Ranke)	−0.44 ± 1.01	0.13 ± 1.00	0.10 ± 0.96	0.18 ± 0.98	0.27 ± 1.07	0.39 ± 1.09	<0.001
Δ Height − MPH SDS	−2.86 ± 1.06	−2.83 ± 1.11	−2.79 ± 1.16	−2.81 ± 1.14	−2.71 ± 1.12	−2.55 ± 1.32	NS
Weight SDS	−1.41 ± 1.32	−1.49 ± 1.15	−1.35 ± 1.20	−1.28 ± 1.24	−1.14 ± 1.25	−1.20 ± 1.15	NS
BMI SDS	0.63 ± 1.07	0.35 ± 0.97	0.35 ± 1.06	0.37 ± 1.13	0.48 ± 1.08	0.34 ± 0.95	NS

TABLE 3 | Secular trends on pubertal timing (mean ± SD).

Birth year	Before 1980	1980–1984	1985–1989	1990–1994	1995–1999	2000–2004	*p*-Value
At puberty start (cohort #2; all patients)							
N = [birth weight(BW)/birth length (BL)]	401	536	652	487	136		
Age	14.39 ± 2.09	13.44 ± 2.02	13.37 ± 1.62	13.00 ± 1.81	12.42 ± 1.29		<0.001
Height SD score (SDS) (Prader)	−2.34 ± 0.98	−1.86 ± 1.04	−1.57 ± 0.92	−1.44 ± 0.90	−1.25 ± 0.99		<0.001
Height SDS (Ranke)	0.68 ± 1.21	1.28 ± 1.29	1.62 ± 1.19	1.81 ± 1.16	2.06 ± 1.30		<0.001
Δ Height − MPH SDS	−1.72 ± 1.09	−1.39 ± 1.17	−1.15 ± 1.14	−1.07 ± 1.12	−0.95 ± 1.02		<0.001
Weight SDS	−1.48 ± 1.32	−1.04 ± 1.40	−0.72 ± 1.27	−0.57 ± 1.32	−0.37 ± 1.19		<0.001
Body mass index (BMI) SDS	0.30 ± 1.08	0.38 ± 1.14	0.54 ± 1.14	0.60 ± 1.15	0.65 ± 1.02		<0.001
Age at menarche	16.05 ± 1.78	15.07 ± 1.79	14.82 ± 1.64	14.27 ± 1.85	13.86 ± 1.24		<0.001
Duration B2 to M1 (years)	1.82 ± 1.57	1.79 ± 1.35	1.93 ± 1.40	1.68 ± 1.68	1.97 ± 2.27		NS
Spontaneous puberty (%)	15	17	22	27	30		<0.001
Karyotype 45, *X* (%)	52	52	48	46	59		NS
At puberty start (cohort #2; patients with induced puberty)							
N =	340	445	509	323	95		
Age	14.38 ± 2.05	13.57 ± 1.94	13.66 ± 1.52	13.17 ± 1.46	12.68 ± 1.12		<0.001
Height SDS (Prader)	−2.34 ± 1.01	−1.82 ± 0.99	−1.52 ± 0.95	−1.36 ± 0.86	−1.10 ± 0.95		<0.001
Height SDS (Ranke)	0.69 ± 1.24	1.31 ± 1.24	1.69 ± 1.22	1.87 ± 1.16	2.24 ± 1.26		<0.001
Δ Height − MPH SDS	−1.80 ± 1.06	−1.37 ± 1.10	−1.18 ± 1.12	−1.06 ± 1.09	−0.92 ± 1.02		<0.001
Weight SDS	−1.44 ± 1.32	−1.07 ± 1.37	−0.72 ± 1.30	−0.57 ± 1.27	−0.35 ± 1.19		<0.001
BMI SDS	0.35 ± 1.05	0.35 ± 1.13	0.55 ± 1.14	0.61 ± 1.10	0.62 ± 1.02		0.003
Age at menarche	16.18 ± 1.71	15.33 ± 1.78	15.23 ± 1.55	14.61 ± 1.76	14.34 ± 0.87		<0.001
Duration B2 to M1 (years)	1.75 ± 1.53	1.76 ± 1.34	1.97 ± 1.52	1.52 ± 1.90	2.27 ± 2.65		NS
At puberty start (cohort #2; patients with spontaneous puberty)							
N =	61	91	143	119	41		
Age	14.45 ± 2.32	12.79 ± 2.31	12.37 ± 1.58	11.63 ± 1.64	11.80 ± 1.46		<0.001
Height SDS (Prader)	−2.36 ± 0.81	−2.04 ± 1.27	−1.73 ± 0.77	−1.47 ± 0.93	−1.59 ± 1.03		<0.001
Height SDS (Ranke)	0.63 ± 1.00	1.11 ± 1.48	1.40 ± 1.04	1.78 ± 1.20	1.63 ± 1.33		<0.001
Δ Height − MPH SDS	−1.29 ± 1.14	−1.47 ± 1.48	−1.06 ± 1.21	−0.93 ± 1.18	−1.04 ± 1.06		0.01
Weight SDS	−1.71 ± 1.29	−0.92 ± 1.54	−0.73 ± 1.18	−0.37 ± 1.34	−0.42 ± 1.19		0.008
BMI SDS	−0.00 ± 1.22	0.49 ± 1.22	0.50 ± 1.17	0.68 ± 1.24	0.72 ± 1.03		NS
Age at menarche	15.43 ± 2.04	13.76 ± 1.20	13.55 ± 1.23	13.10 ± 1.47	12.77 ± 1.31		<0.001
Duration B2 to M1 (years)	2.19 ± 1.75	1.89 ± 1.38	1.81 ± 0.98	2.05 ± 1.23	1.36 ± 1.08		NS

RESULTS

Birth Parameters, Auxological Development, and GH Treatment

Distribution of BW and BL of all TS patients in whom birth parameters were available ($n = 6372$) are described in **Table 1**. Throughout the birth year cohorts "before 1980" until "1990–1994", we observed a small ST for BW SDS with subsequent stabilization, with an increase of 0.18 SD over time, corresponding to about 157–180 g (depending on the gestational age; **Table 2**). In addition, a positive ST was observed in midparental height SDS.

Height SDS at the start of GH therapy at the standardized age of 8 years (range between 7.0 and 9.0 years, see Methods) showed a positive ST between before 1980 and 2000–2004, for both Prader and Ranke height SDS statistics (**Table 2**) (**Figure 1** for Ranke height SDS results). In addition, positive STs for height SDS (Prader and Ranke height SDS statistics) could also be observed both at the start of GH therapy (**Table 2**) and at the start of puberty (thelarche) (**Table 3**). Comparable to the ST in height SDS at 8 years of age, an ST in midparental height was also observed (+0.5 SD).

Age at the start of GH therapy declined substantially, from 10.8 years in the birth cohort 1980–1984 to 7.4 years in the birth cohort from 1995 to 1999. As described in Section "Methods", the even more extreme mean ages at the start of GH in the before 1980 and 2000–2004 cohorts are likely artifactual, due to selection biases.

Pubertal Development

Age at the start of puberty (whether induced or spontaneous) declined from 14.4 years in the before 1980 birth cohort to 12.4 years in the 1995–1999 cohort ($p < 0.001$). When stratified by spontaneous or induced puberty, STs toward an earlier start of puberty were evident in both subgroups, and the proportion with spontaneous puberty onset increased from 15% in those born before 1980 to 30% in those born 1995–1999 (**Table 3**).

To determine whether age at spontaneous puberty and age at the start of GH in patients with TS are associated, we performed a correlation analysis, revealing a highly significant correlation between age at the start of GH treatment and age at pubertal onset [correlation coefficient 0.65 (p-value < 0.0001)], which explained 32% of the variability in spontaneous puberty with age at GH start [intercept = 10.3 years ($p < 0.0001$), slope = 0.31 ($p < 0.0001$)].

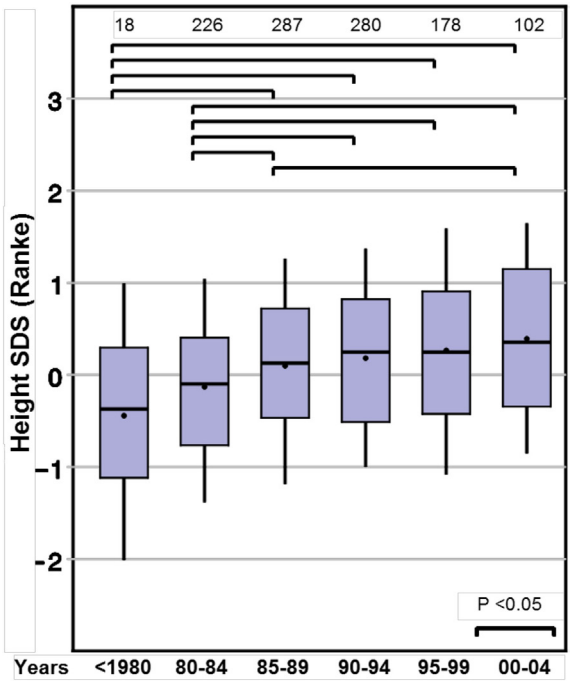

FIGURE 1 | Secular trends on height in 8-year-old girls with Turner syndrome, height SD score (Ranke).

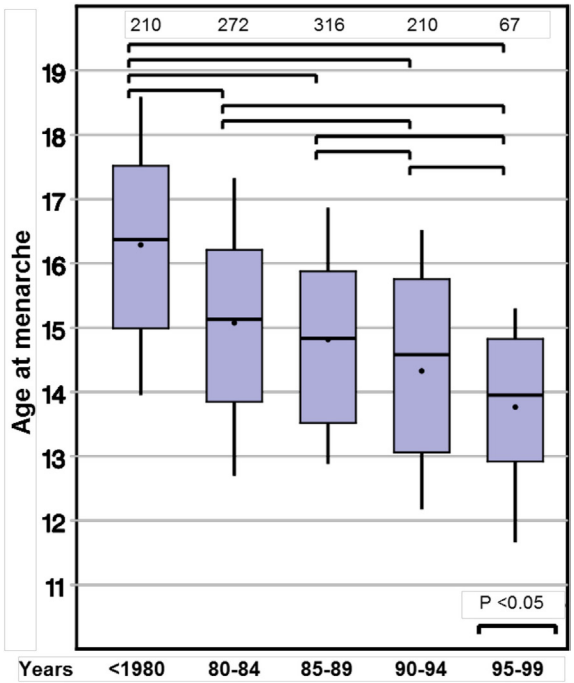

FIGURE 2 | Secular trends on age at menarche in girls with Turner syndrome.

Age at menarche also declined substantially from 16.0 to 13.9 years ($p < 0.001$). Again, this observation remained significant in the two subgroups with either spontaneous or induced puberty (**Table 3**; **Figure 2**).

DISCUSSION

To the best of our knowledge, this is the first study analyzing whether TS girls display the same STs for auxological and pubertal development as those observed in the normal population. We found a highly significant ST for height at 8 years of age (+0.7 SDS). In addition, STs for height were present at the start of GH treatment and at the start of puberty. However, for these latter time points, the mean age differed greatly between the birth year groups, limiting direct comparisons. At the age of 8 years—before either the start of sex steroids or GH treatment may have influenced growth—the TS girls born after 2004 were more than half an SD taller compared to those born before 1980. Reported STs in height in normal populations differ between countries. Eastern and developing countries still show marked positive STs (26, 27), while conversely Western countries show only small (28) or even negative STs in growth (29). In contrast, our large group of TS girls included a wide variety of ethnicities, yet the positive ST in height SDS at the age of 8 years was observed for all, irrespective from their country of residence (data not shown). The remarkable positive ST of more than half an SDS, which corresponds to about 3 cm gain in height even without any endocrine treatment, surpasses contemporary estimates in the normal population. A height gain of 0.5 cm/decade or less would be expected for the corresponding birth year cohorts in most Western countries (30). Thus, the TS girls in this study born between 1975 and 2004 exhibited the same degree of ST on height at age 8 years as was observed one generation earlier in their parents.

It is important to notice that this positive ST in height could result in a delay of diagnosis because TS patients at the age of eight are nowadays nearly in the normal range or "less short." However, our data show a constant shift toward an earlier start in GH treatment (reflecting probably the age of diagnosis) between the before 1980 and the post-2000 birth cohorts. Thus, although still a significant number of TS subjects seem to be diagnosed late, the awareness of TS seems to have improved despite less apparent phenotypical features.

We found a small positive ST for BW (+0.18 SD), but only a minor variation in BL. These findings are in line with data on healthy term infants who show a ST only for BW but not for BL. Comparison of recent growth curves and birth parameters (1, 24, 31) with historical data (2) showed a higher average BW for infants born at term. In contrast to that, BL remained constant in almost all the industrialized Western countries, and no change in this variable has been detected over the last 40 years. Higher maternal body weight and pregnancy weight gain (32) in recent years may be a reason for the observed gain in BW. Since both maternal and paternal genes contribute to infants' birth parameters, the observed ST in midparental height might contribute to the observed changes in birth parameters. Furthermore, the ST in maternal height might be associated with alterations of the intrauterine environment, which again could be linked to the observed ST in birth parameters.

In recent years, epidemiological data from USA (33) and Denmark (8) showed STs toward earlier start of puberty in girls. We therefore were interested whether the same trend could be detected in TS patients in whom spontaneous puberty can be observed in about one third. We found a comparable decline in the age at spontaneous thelarche of about 2 years between those born before 1980 to those born in 2000–2004. As the time interval between visits was usually 6 months, the correct age at thelarche was likely earlier than that which we recorded. However, this limitation applied similarly for each of the five birth year groups with no expectation of bias.

The reasons behind the earlier spontaneous thelarche in TS subjects are unclear. We initially hypothesized that a lower threshold to karyotyping and a broader access to modern genetic diagnostics might have led to an increased proportion of patients with mosaicism, thereby explaining the doubling in the prevalence of spontaneous puberty and the decline in age at thelarche. However, as depicted in **Table 3**, the prevalence of patients with monosomy X did not change across the birth year groups. As in healthy girls, one can speculate that the increase in weight SDS of about one SD in the TS girls with spontaneous thelarche might have influenced the age at thelarche. However, in other settings the increase in weight did not fully explain the ST on puberty in healthy Danish girls (8, 34). Aksglaede and coworkers suggested that factors other than weight, such as changes in living conditions, nutrition during fetal development and childhood, and the wide distribution of endocrine disrupting chemicals (EDCs) (8, 35) might provoke the observed secular change. Probably, TS girls are exposed to the same changes in environmental conditions. Therefore, if EDCs are really causally related to the reported pubertal changes, these might also be related to the even more pronounced decrease in thelarche in TS girls.

As already reported in patients with idiopathic GH deficiency, age at puberty start correlated with age at the start of GH treatment in our study (36). Although the highly significant correlation between age at commencement of GH treatment and onset of spontaneous puberty is only an association and not proof of a causal relation, one can speculate that exposure to elevated GH concentrations might affect gonadotroph function, either through a direct or indirect effect (e.g., mediated by IGF-I). In this context, several *in vitro* studies have demonstrated that IGF-I is able to directly stimulate gonadotropin synthesis and secretion (37), so that a GH-induced increase in circulating IGF-I levels might contribute to the observed decline in age at the start of puberty. However, in a previous study in Italian TS patients, neither age at start nor prevalence of spontaneous puberty differed between GH-treated patients and a small non-GH treated control group who received androgen treatment (18). Furthermore, in our study, age at the start of GH treatment explains only 32% of the observed variability in spontaneous puberty, indicating that additional factors are probably involved in the physiology of earlier age at start and increased prevalence of spontaneous puberty.

The age at pharmacological induction of puberty decreased comparably to the age at spontaneous start of puberty. This phenomenon might be explained in particular by two factors: first, the decrease in age at the start of GH therapy (and probably age at diagnosis) might allow puberty induction at a more physiological age range. Second, the awareness of physicians for the psychosocial and physical sequelae of delayed puberty induction might have improved over time. In this context, one study has reported an even more improved height outcome for the early use of very low-dose estrogens in TS girls (38), and another study reported no significant influence of early start of low-dose estrogens on height development in TS girls (39). Together with the negative impact on bone health due to late estrogen exposure, these data argue strongly against a delay and in favor of an earlier starting age of puberty induction.

Although a ST in thelarche is found in several populations, all recent epidemiological studies (40) showed that the timing of menarche remained mostly unchanged with a consecutively longer interval between thelarche to menarche. In this study, we observed a significant reduction in age at menarche, both in TS girls with spontaneous as well as in those induced puberty. Whereas in TS girls with induced puberty probably the same explanations as for earlier age at thelarche might lead to the decrease in age at menarche, the reasons for the decrease in age at menarche in TS girls with spontaneous puberty remain unclear. Since the group with spontaneous start of puberty included subjects with spontaneous start but later requirement of sex steroid substitution before menarche, we speculate that the earlier menarche in this group is related to earlier sex steroid replacement therapy, comparable to the earlier start of pharmacologically induced puberty.

A major strength of this study is the unprecedented large study sample of girls with TS, allowing a comparison of growth and pubertal development over time in still sufficiently sized birth year cohorts. However, data from post-marketing studies such as KIGS have some important shortcomings. Therefore, the database contains no data from untreated TS subjects, which would allow determining whether the STs at birth, age 8 years or at the start of GH treatment translate to differences in adult height. Furthermore, several authors have speculated on the influence of socioeconomic factors as causative factors for the STs on height, which are not available in the database. Interobserver differences in a multicenter database in determining height and pubertal status are a potential weakness, but are probably balanced out by the large cohort size.

In summary, we find that trends toward increased childhood height and earlier pubertal onset operate not only in normal populations, but also in TS subjects, who also showed a doubling in the prevalence in spontaneous puberty onset between before 1980 to 1995–1999. In addition to these environment-related trends, awareness for TS seems to have improved, leading to earlier ages at the start of GH and pharmacological induction of puberty.

ETHICS STATEMENT

The patients studied had received recombinant GH (Genotropin®, Pfizer Inc.) as part of the pharmacoepidemiologic survey known

as KIGS® (Pfizer International Growth Database). KIGS was established in 1987 as a worldwide observational registry to monitor outcomes and safety of Genotropin (somatropin, Pfizer Inc., New York, NY, USA) treatment in children with short stature. The KIGS survey was conducted in accordance with the Declaration of Helsinki.

AUTHOR'S NOTE

KIGS is sponsored by Pfizer Inc.

AUTHOR CONTRIBUTIONS

FA, BG, AL, and JW developed the study design. FA, CC-H, BG, KO, AL, and JW discussed the findings. JW and BG wrote the first draft of the paper. All the authors contributed to draft revisions and approved the final manuscript.

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Growth Hormone Supplementation and Psychosocial Functioning to Adult Height in Turner Syndrome: A Questionnaire Study of Participants in the Canadian Randomized Trial

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Despite the long-held belief that growth hormone supplementation provides psychosocial benefits to patients with Turner syndrome (TS), this assumption has never been rigorously tested in a randomized control trial. As a sub-study of the Canadian growth-hormone trial, parent-, and patient-completed standardized questionnaires were used to compare 70 girls with TS who received injections (GH group) and 61 similarly followed untreated TS controls (C) on multiple facets of psychosocial functioning. Questionnaires were given (i) at baseline (session 1, mean age = 10.4 y), (ii) before estrogen therapy for puberty induction (session 2, mean age = 13.0 y), (iii) after 1 year of estrogen therapy (session 3, mean age = 14.4 y), and (iv) when growth stopped (session 4, mean age = 16.3 y). Groups were compared for multiple facets of psychosocial function within social, behavioral, self-esteem, and academic domains. Results were also correlated with indices of adult height. We found no global (i.e., across-session) group differences on any scales or subscales of the four domains. In both GH and C groups, age-related improvements were seen for social problems, externalizing behavior problems, and school functioning and age-related declines for social competence and social relations. Both parents and patients claimed GH received less teasing than C but C had more friends than GH. Results from analyses conducted within individual sessions showed that while GH at early sessions claimed to be more popular, more socially engaged, better adapted, and to have higher self-esteem than C, C was reported to be less anxious, depressed, and withdrawn than GH at adult height. The correlation analyses revealed different effects of adult height and height gain on outcome for the two groups. In GH, both height parameters were correlated with multiple parent- and/or self-reported indices from the four psychosocial domains, whereas in C, only adult height and two indices (viz., total self-concept and school functioning), were correlated. The observed modest gains in psychosocial functioning for patients with TS treated with GH highlight the need for alternative approaches to assist them in coping with the challenges of their condition.

Keywords: Turner syndrome, psychosocial functioning, growth hormone, self-concept, behavior problems

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INTRODUCTION

Shortness relative to genetic height potential is a universal characteristic of Turner syndrome (TS). Due to the relative resistance of the growth plates to growth hormone (GH) action in patients with TS, they typically averaged ∼20 cm below their target adult height (1). Consequently, when GH was being extracted in limited quantities from the pituitaries of human cadavers, their growth acceleration was negligible and, so, they were not considered eligible for this treatment (2). With the advent of biosynthetic GH, however, they could now receive supra-physiological doses of GH. Thus, they were expected to show pronounced growth acceleration and increased adult height. Also underlying this expectation was the assumption that their faster growth and taller adult height from this therapy would lead to improved psychosocial adaptation (3).

To date, only two randomized controlled trials (RCT) to adult height have been published on patients with TS, one in Canada comparing GH injections with no injections (4) and one in the U.S. comparing GH and placebo injections (5). Both trials, which followed patients closely until they reached adult height, reported treatment-related height gains of 7.2 and 5.0 cm, respectively. In the only psychosocial report on these patients to date, GH therapy was not seen to affect health-related quality of life (HRQoL) in a small subset of patients from the Canadian RCT tested at age 20 (6). However, the full extent of psychosocial benefits for the larger sample of patients during GH treatment or on reaching adult height is not known.

Described presently are the findings from the majority of participants in the Canadian RCT, who also took part in a concurrent longitudinal study of their psychosocial functioning during the GH trial. Within this sub-study, GH-treated and untreated control (C) patients, and their parents, completed standardized questionnaires at four set intervals from initial randomization until growth cessation. Groups were compared on a large number of endpoints representing four key domains of psychosocial function, namely social, behavioral, self-esteem, and academic characteristics. In addition, correlations were performed between height indices and outcome at trial completion. To our knowledge, comparable data have not been published in the US trial or elsewhere. Therefore, despite data completion for this sub-study more than a decade ago, it still remains the only one to continuously compare multiple facets of psychosocial functioning in GH-supplemented patients *and untreated TS control patients* through to adult height. As such, we believe our findings are unique and still relevant.

MATERIALS AND METHODS

Design and Procedures

Between February 1989 and May 1994, the main study enrolled 154 patients with TS ranging from 7 to 13 years of age. All were prepubertal at study entry. Eligibility criteria for this study were: (a) height below the 10th percentile for chronological age and (b) an annualized height velocity of < 6.0 cm/y [see (4) for additional eligibility criteria and exclusion criteria]. Initially, the patients were stratified into three subgroups based on height relative to chronological age and, then within subgroups, randomized to either a GH-treatment or a no-GH control condition. Treatment involved recombinant human GH (Humatrope Eli Lilly Canada Inc., Toronto, Canada) by subcutaneous injection (dose = 0.3 mg/kg/week) six times weekly. Injections were continued for ∼6 years until an annualized height velocity of < 2 cm/y and bone age of 14 y or greater were attained. In addition, all patients with primary ovarian failure (the majority of cases) were given standardized oral estradiol therapy at ∼age 13 y; the handful of patients near to or above age 13 at study entry, received this 1 year after commencing GH treatment. The protocol for estradiol therapy involved 0.0025 mg oral ethinyl estradiol daily for the first year, 0.005 mg the next year, and cyclic estrogen and progesterone replacement thereafter.

In the sub-study, which took place between February 1989 and December 2002, nurse practitioners from 13 pediatric endocrine clinics across Canada (7) gave families packets of questionnaires printed in English or French. These were provided at four preset intervals: initiation of the trial or "session 1"; just prior to estrogen therapy or "session 2"; after 1 year of estrogen therapy or "session 3"; and when growth stopped or "session 4." Completed questionnaires were returned via mail to The Hospital for Sick Children (SickKids), where research assistants blinded to treatment status scored the tests, maintained the database, and conducted preliminary data analyses. The final analyses were conducted more recently by JR.

All procedures were carried out in accordance with the guidelines of the ethics review committees of each participating institution (**Supplementary Table 1**), which provided approval for both the main study and this sub-study. The SickKids Research Ethics Board provided additional approval for sub-study data coordination and analyses at this facility. All parents gave informed consent while patients gave informed assent or consent. The main trial was registered with ClinicalTrials.gov Identifier NC700791113.

Participants

One hundred and thirty-one of the original 154 patients took part in the sub-study (**Figure 1**). Primary reason for not joining was unwillingness to be involved. Shown in **Supplementary Table 1** are the patient numbers from each participating endocrine clinic. Three children switched sites during the trial due to family relocation.

Of the 131 sub-study participants, 70 belonged to the GH group and 61 to the C group representing 92 and 70% of main-study groups, respectively. One hundred and twenty-two patients (67 GH, 55 C) began at baseline (session 1) while a further nine (3 GH, 6 C), from a site whose PI was initially unwilling to participate, entered at session 2. Sessions 2 and 3 had 111 (67 GH, 44 C) and 82 (53 GH, 29 C) patients, respectively. The differential drop-out for C vs. GH between sessions 2 and 3 may be partially explained by a physician offering treatment independent of the trial to his C patients. Seventy-eight participants (48 GH, 30 C) completed the sub-study at session 4, representing 77 and 70% of GH and C main-study completers and 72 and 54% of participants who began the sub-study, respectively. Completers did not

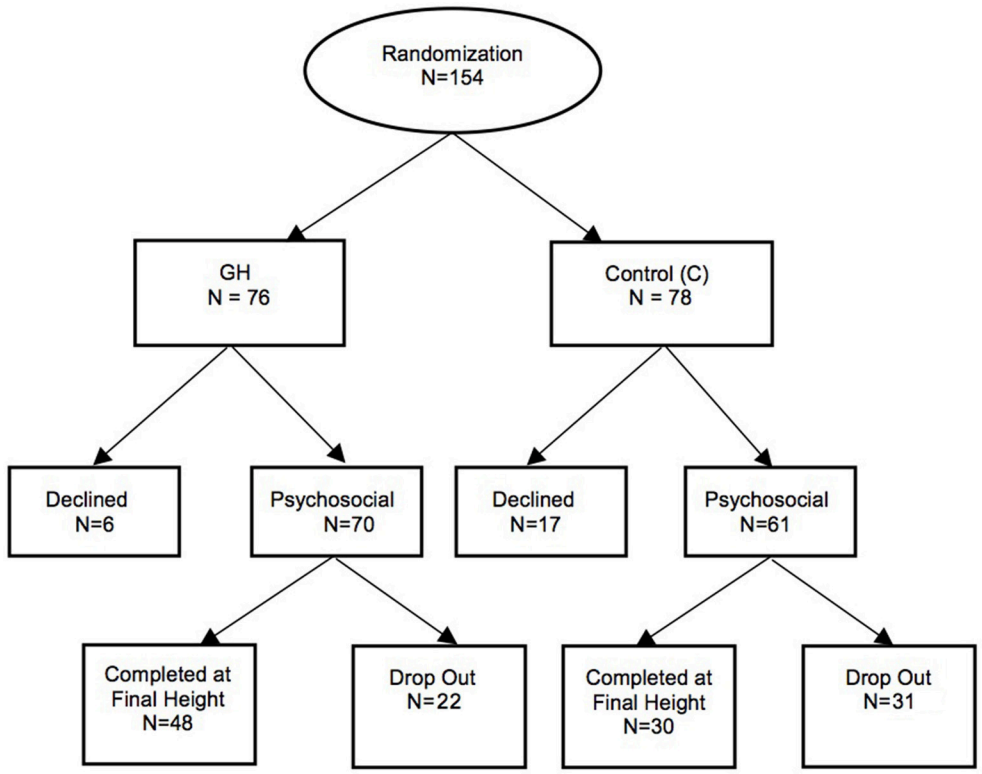

FIGURE 1 | Outline of participation in the psychosocial sub-study.

differ from drop-outs in terms of baseline sociodemographic characteristics (data not shown).

Tests and Measures

Parents initially filled out a brief demographic questionnaire seeking information on marital status and education/occupation used to derive a 5-point index of socioeconomic status (SES) (1 = high status) (8); they also completed the Child Behavior Checklist (CBCL) (9) at sessions 1 to 4. Patients completed the Youth Self-Report (YSR) (10) at sessions 2 to 4 and the Piers-Harris Children's Self-Concept Scale (PHCSCS) (11) at all four sessions.

The CBCL is a widely used standardized questionnaire based on parent report that assesses behavior problems in 4–16-year olds. It contains a series of open-ended questions that are used to derive the following four social-competence (SC) scales: Total Social Competence, Activities, Social Relations, and School. The CBCL also provides a list of 113 factual statements such as "acts too young for age" and "feels worthless or inferior." Parents use a three-point scale (1 = not true; 2 = somewhat or sometimes true; 3 = very true or often true) to rate their daughters on these items. Computerized scoring of the items yields: a Total Behavior Problems (BP) index and scores for Internalizing and Externalizing Problems broad-band scales and the eight narrow-band scales of Withdrawn, Somatic-complaints, Anxious/Depressed, Social, Thought, Attention, Delinquency, and Aggression problems. Internalizing and Externalizing Problems scales are derived from a subset of narrow-band subscales that does not include Social, Thought, or Attention Problems. For SC indices, a higher positive score signifies better functioning and for BP, more behavior problems. Although results were originally generated as T-scores (mean = 50; SD = 10) based on normative test data, we converted them to SD units (mean = 0; SD = 1) as per (12). Somatic Complaints results are not reported presently.

In addition, we separately recorded scores from three of the CBCL items, namely "number of friends" and "time with friends" from the SC component and "gets teased a lot" (item #38) from the BP component. The first two were based on a four-point scale (4 = most favorable rating) and the third, a 3-point scale (see above). As part of the School subscale, parents also rated their daughter's reading and math abilities via a 4-point scale (4 = very good) and indicated her grade at school and if she was in a special class, had failed a grade, or had academic problems.

The YSR is a self-report instrument with a similar structure and scoring system as the CBCL but in the version we provided, lacked information on academics. Scores from two individual items were additionally recorded: "I get teased a lot" (#38) and "I feel lonely" (#12). Because the YSR is only first administered at age 11, this questionnaire was not provided until session 2.

The PHCSCS is a self-report questionnaire consisting of 80 statements such as "I am a good person" or "I am popular with boys." Patients indicated if statements were true or false of themselves. Scoring yielded a Total Self Concept index plus scores for six subscales: Behavioral Adjustment, Intellectual &

School Status, Physical Appearance, Freedom from Anxiety, Popularity, and Happiness/Satisfaction. All PHCSCS results were reported as percentiles based on test norms with higher scores signifying more favorable self-esteem.

For present purposes, results from the three questionnaires were examined within four domains of psychosocial functioning, namely social abilities, behavior problems, self-esteem, and academics. Each domain was derived from the relevant subscales or items of the various questionnaires. Social functioning was based on (a) CBCL and YSR Total Social Competence, Activities, Social Relations, and Social Problems scales, (b) the PHCSCS Popularity index, and (c) selected CBCL and YSR items. Likewise, the behavior-problem domain was represented by (a) CBCL and YSR Total and Internalizing and Externalizing Problems scores, (b) selected CBCL and YSR narrow-band scores (viz., Withdrawn, Anxious/Depressed, Thought Problems, Attention Problems, Delinquency, and Aggression), and (c) Behavioral Adjustment and Freedom from Anxiety scores from the PHCSCS. Self-esteem was based on the four remaining PHCSCS scales, namely Total Self Concept, Intelligence/School, Physical Appearance, and Happiness/Satisfaction. Academic functioning was based on the CBCL School scale and Reading and Math scores.

Data Analysis and Statistics

Groups were compared at baseline and subsequent sessions using an intent-to-treat analysis. Missing baseline data from the nine patients first entering the psychosocial study at session 2 were imputed using the mean scores of the child's height-for-age stratification subgroup; note, these patients did provide baseline height data. For the other missing data from session 2, scores were imputed using a next-observation-carried-backward approach and for missing data from sessions 3 and 4, a last-observation-carried-forward approach based on (13). If a subject had data from sessions 2 and 4 but not session 3, the mean of her session 2 and 4 scores was used.

All data were analyzed using SPSSv24 (14). *t*- and χ^2 tests served to compare groups for demographics and height. For CBCL and PHCSCS questionnaires, post-baseline data were analyzed using mixed-model repeated-measures analyses of covariance (ANCOVA) with Group as the between-subjects factor, Session as the repeated factor, and baseline (i.e., session 1) scores as the covariate. Since the YSR was not administered until session 2 (see above), results for this questionnaire were analyzed by repeated-measures analyses of variance (ANOVA) with Group as the between-subjects factor and Session as the repeated measure. In order to identify whether groups also differed at individual sessions, multivariate analyses of variance (MANOVA) were performed within sessions for all measures belonging to a domain. A power analysis indicated that with 70 and 61 participants per group (average = 65) and with an α of 0.05 and β of 80%, we could detect moderate effect sizes (d = ∼0.45) (15).

To evaluate the impact of adult height and height gain on psychosocial indices, we performed for GH and C groups separately, two series of Pearson correlations between adult height indices and measures of outcome at the final session. Separate series of correlations were conducted for each of the four psychosocial domains. For these analyses, only data from sub-study completers (i.e., no imputed values) were used.

For between-group comparisons, the *p*-value was set at 0.05 using a two-tailed test with the Bonferonni *p*-correction applied if a test had multiple subscales within a domain (e.g., *p*-values for two narrow-band Externalizing subscales were divided by 2). A similar correction approach was applied to the correlations, but a one-tailed test was instead used, given the assumption of better outcome following greater growth (3).

RESULTS

Demographics

For the combined sample, ages reported as mean ± SD at the four sessions were: 10.4 ± 1.6 y at session 1; 13.0 ± 1.0 y at session 2; 14.4 ± 0.6 y at session 3; and 16.3 ± 1.0 y at session 4. Corresponding school grades were 4.7, 7.4, 8.8, and 10.5, respectively. **Table 1** presenting the groups' baseline characteristics shows GH and C did not differ in age, grade, SES, percent English speaking, grade failure, special education, academic problems or experiencing divorce, separation, or death.

Height Data

Table 2 presents the height data of the sub-study participants. Target height, while included in the 2005 paper (4), was not used in current analyses of height response or correlations with outcome data.

Current results are similar to those for the full sample in the main study (4). Groups were both very short initially (mean baseline height below −3 SD) and similar to each other. Subsequently, however, GH was taller than C ($p < 0.05$ session 2; $p < 0.001$ sessions 3 and 4) as well as differed significantly in height gain ($p < 0.001$). GH, between sessions 1 and 4, gained 27.7 ± 9.4 cm, representing a unit change on the NCHS scale of 1.0 SD, whereas C gained only 16.2 ± 7.3 cm representing a unit change of −0.2 SD. For both groups combined, a greater height gain was correlated with a younger age at study entry ($p < 0.001$) while for GH only, taller adult height and younger age at study entry were also correlated ($p < 0.01$).

Since all height indices were significantly correlated ($p < 0.001$) within and across sessions, we chose two height parameters for further analyses in order to reduce the number of

TABLE 1 | Demographic characteristics at baseline.

	GH[a]	Control[a]
Age in years[b]	10.3 (1.7)	10.5 (1.5)
Grade at School[b]	4.6 (1.7)	4.8 (1.4)
SES Class[b]	2.6 (0.9)	2.5 (0.8)
% English Speaking	70.6	75.4
% Failed a grade	20.0	19.3
% In special class	17.5	14.0
% Academic problems	38.3	33.3
% Experiencing divorce etc.	13.6	23.2

[a] Baseline sample based on 64 GCH and 50 C cases at session 1; [b] Results are expressed as mean (SD).

correlations with outcome: adult height in NCHS SD units and height gain (cm) between sessions 1 and 4.

Social Functioning

Table 3 presents the post-baseline scores for all social functioning indices while **Supplementary Tables 2** and **3** contain the baseline scores from CBCL and PHCSCS questionnaires, respectively. **Table 3** also shows the statistical results from MANCOVAs on CBCL and PHCSCS tests and MANOVA on the YSR. **Table 4** provides the findings from the correlational analyses computed between height and outcome indices.

According to parents, GH and C both scored quite poorly on CBCL Total Social Competence (range = 0.7–1.0 SD units below population norms) and Social Problems indices (∼1.0 SD units above population norms). Although significant Group effects were not seen for any of these indices, significant Session effects were observed reflecting age-related declines in Total Social Competence ($p < 0.01$) and Social Relations ($p < 0.01$) and an age-related improvement Social Problems ($p < 0.001$). Also, for Social Problems, a trend-level Group X Session interaction ($p < 0.10$) reflected the somewhat greater improvement over time by C than GH. There were no group differences for any of the baseline social-functioning indices.

For the patient-completed YSR and PHCSCS questionnaires, results indicated no omnibus (i.e., across-session) Group effects on any of the scales or subscales. However, groups showed different patterns of change as indicated by significant Group X Session interactions for YSR Activities ($p < 0.05$), YSR Social Relations ($p < 0.01$), and PHCSCS Popularity ($p < 0.05$), and a trend-level interaction ($p < 0.10$) for YSR Total Social Competence. **Figure 2** depicting the results for YSR Activities and Social Relations scales and **Figure 3**, for PHCSCS Popularity, show that GH initially outscored C but that C later improved scoring comparably to or beyond GH at adult height. Results from analyses based on within-session results indicated GH at session 2 claimed more social engagement and popularity than

TABLE 2 | Mean (SD) height data[a].

	Session 1		Session 2		Session 3		Session 4	
	GH	C	GH	C	GH	C	GH	C
Height (cm)	119.5 (18.5)	120.1 (8.3)	**127.0 (8.4)**	**123.9 (8.4)**	**138.3 (7.1)**	**130.7 (6.0)**	**147.1 (6.4)**	**136.5 (9.2)**
SD (TS norms)	−0.10 (0.9)	−0.17 (0.8)	**0.59 (1.0)**	**−0.15 (0.9)**	**1.32 (1.2)**	**0.60 (1.0)**	**0.60 (0.9)**	**−0.10 (0.9)**
SD (NCHS)	−3.21 (0.8)	−3.28 (0.8)	**−2.80 (1.0)**	**−3.49 (0.8)**	**−2.80 (1.0)**	**−3.89 (0.8)**	**−2.26 (0.9)**	**−3.52 (0.9)**

[a] *Data shown in bold indicate significant group differences.*

TABLE 3 | Mean (SE) scores on social functioning indices at post-baseline sessions[a].

	Session 2		Session 3		Session 4		F-values[b]		
	GH	C	GH	C	GH	C	GP	Session	GP X Session
CBCL									
Total Social Competence[c]	−0.77 (0.10)	−0.83 (0.10)	−0.81 (0.10)	0.74 (0.08)	−0.93 (0.09)	−1.00 (0.07)	0.18	5.63**	1.02
Activities[c]	−0.37 (0.08)	−0.57 (0.08)	−0.43 (0.09)	0.45 (0.08)	−0.55 (0.09)	−0.75 (0.08)	1.10	0.68	2.13
Social Relations[c]	−0.69 (0.09)	−0.82 (0.09)	−0.81 (0.10)	0.73 (0.09)	−0.92 (0.09)	−0.89 (0.08)	0.35	5.23**	0.42
Social Problems[d]	1.07 (0.08)	1.15 (0.10)	0.98 (1.00)	0.86 (0.09)	0.93 (1.00)	0.70 (0.06)	0.53	20.46***	2.58+
Number of friends[e]	2.88 (0.10)	3.03 (0.11)	2.96 (0.09)	3.06 (0.09)	**2.99 (0.08)**	**3.20 (0.07)**	2.50+	20.30***	0.80
Time with friends[e]	2.08 (0.10)	2.05 (0.10)	2.00 (0.09)	2.03 (0.08)	1.93 (0.07)	2.02 (0.06)	0.08	2.41	0.47
Teased[f]	0.70 (0.10)	0.93 (0.10)	0.69 (0.08)	0.80 (0.08)	0.66 (0.07)	0.50 (0.07)	0.13	0.23	5.27**
YSR									
Total Social Competence[c]	−0.60 (0.10)	−0.82 (0.09)	−0.63 (0.11)	0.45 (0.11)	−0.78 (0.12)	−0.72 (0.10)	0.11	2.18	2.59+
Activities[c]	***−0.22 (0.09)***	***−0.62 (0.08)***	−0.44 (0.09)	0.46 (0.09)	−0.49 (0.10)	−0.64 (0.09)	3.37+	2.87+	3.38*
Social Relations[c]	−0.58 (0.08)	−0.75 (0.08)	**−0.70 (0.10)**	**0.37 (0.11)**	−0.82 (0.10)	−0.66 (0.08)	1.08	3.82*	5.65**
Social Problems[d]	0.48 (0.12)	0.51 (0.07)	0.48 (0.09)	0.55 (0.07)	*0.52 (0.10)*	*0.77 (0.09)*	1.83	1.46	0.95
Teased[f]	0.64 (0.10)	0.78 (0.07)	***0.48 (0.09)***	***0.93 (0.09)***	*0.49 (0.09)*	*0.72 (0.09)*	8.05**	1.66	3.39*
Lonely[f]	0.32 (0.07)	0.42 (0.07)	0.34 (0.08)	0.37 (0.10)	0.42 (0.07)	0.55 (0.07)	1.47	1.88	0.47
PHCSCS									
Popularity[g]	***57.4 (3.8)***	***42.6 (4.0)***	*57.7 (3.4)*	*48.3 (3.7)*	53.6 (3.4)	54.2 (2.6)	20.74+	17.33***	30.23*

[a] *See **Supplementary Tables 2** and **3** for CBCL and PHCSCS Session 1 results;* [b] *MANCOVA for CBCL and PHCSCS with Session 1 results as covariate; MANOVA for YSR;* [c] *Expressed in SD units with negative scores indicating suboptimal social functioning;* [d] *Expressed in SD units with positive scores signifying more problems;* [e] *Scored on a 4-point scale (1 = "none"; 2 = "1"; 3 = "2 or 3"; 4 = "4 or more");* [f] *Scored on a 3-point scale (1 = "not true"; 2 = "somewhat or sometimes true"; 3 = "very true or often true");* [g] *Expressed in percentile scores; Results in bold italics indicate significant group difference at p < 0.01 level corrected; results in bold indicate significant group difference at p < 0.05 level corrected; results in italics indicate a trend-level difference at p < 0.10 level; ***p < 0.001; **p < 0.01; *p < 0.05; +indicates a trend-level difference at p < 0.10 level.*

TABLE 4 | Correlations between height parameters and social functioning indices at session 4[a].

	Adult Height (NCHS SD Units)		Δ Height	
	GH	C	GH	C
CBCL				
Total Social Competence	0.394**	0.201	0.252*	0.007
Activities	0.209	0.007	0.161	−0.179
Social Relations	0.363**	0.121	0.252*	0.047
Social Problems	−0.285*	−0.074	−0.140	−0.147
Number of friends	0.041	0.127	0.052	−0.070
Time with friends	0.239*	0.223	0.088	−0.084
Teased	−0.251*	−0.154	−0.098	−0.016
YSR				
Total Social Competence	0.329**	0.257	0.225*	0.104
Activities	0.379**	0.003	0.168	0.004
Social Relations	0.368**	0.254	0.187	0.111
Social Problems	0.018	−0.128	−0.087	−0.216
Teased	−0.065	−0.178	−0.174	−0.061
Lonely	−0.181	−0.152	−0.248*	−0.054
PHCSCS				
Popularity	0.143	0.129	0.271*	0.083

[a]*Results are based on a one-tailed test; **$p < 0.01$, *$p < 0.05$.*

C ($p < 0.01$), whereas parents reported C had better social relations ($p < 0.05$) at session 3. Groups did not differ in their baseline results.

For the individual items, findings revealed C had overall more friends than GH ($p < 0.10$), especially at session 4 ($p < 0.05$), but groups did not differ in time spent with friends or reported loneliness. Regarding being teased, both parents and patients reported high teasing rates for all patients, and these exceeded the rates for patients with psychological problems in the normative reference sample (16). According to parents (CBCL), the Group X Session interaction for teasing was significant ($p < 0.01$) while on self-report (YSR), both Group ($p < 0.01$) and the Group X Session interaction ($p < 0.05$) were significant. **Figure 4** depicting these findings shows that parents (solid lines) claimed GH experienced a constant level of teasing across sessions, whereas C was teased initially more than GH but by session 4, teased less. According to patients (hatched lines), GH experienced consistently less teasing than C, especially at session 3 ($p < 0.01$).

The correlational findings indicated height influenced parent- and/or patient-reported social functioning, but only in GH. For these patients, taller adult height was correlated with higher levels of social competence and better social relations ($p < 0.01$ for both), more social engagement ($p < 0.01$), fewer social problems ($p < 0.05$), more time with friends ($p < 0.05$), and less teasing ($p < 0.05$) while a larger height gain was associated with better self-esteem ($p < 0.05$) and social relations ($p < 0.05$), greater popularity ($p < 0.05$), and less loneliness ($p < 0.05$). There were no significant correlations for C.

Behavior Problems

Table 5 presents the post-baseline BP results while **Supplementary Tables 2** and **3** contain the baseline scores

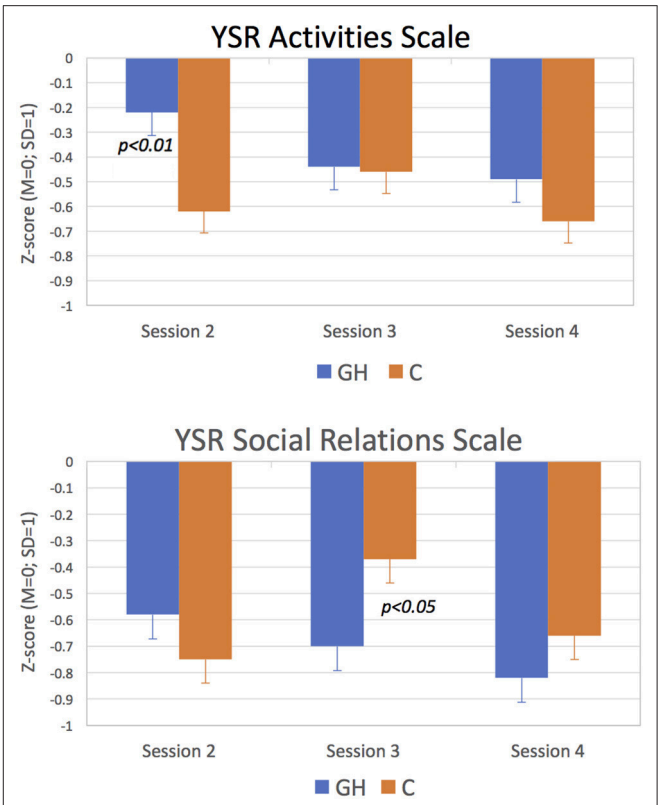

FIGURE 2 | Scores for YSR Activities and Social Relations Scale. GH scored significantly above C on Activities at Session 2 and significantly below C on Social Relations at Session 3 ($p < 0.05$ for both).

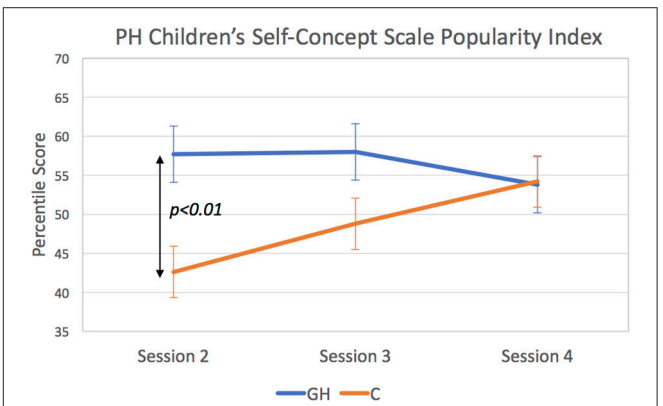

FIGURE 3 | Scores for Piers Harris Children's Self-Concept Scale Popularity Index. GH scored significantly above C at Session 2 ($p < 0.01$).

for CBCL and PHCSCS tests, respectively (YSR not administered at session 1). **Table 6** presents the correlational findings.

For all CBCL and YSR BP indices, no omnibus (i.e., across-session) Group effects were observed. However, most scales indicated significant Session effects reflecting age-related reductions within both groups, particularly between sessions 2 and 3. Significant Group X Session interactions for Withdrawn and Anxious/depressed CBCL scales ($p < 0.05$ for both) indicated

that the groups manifested different patterns of change over time. Specifically, C showed greater improvement than GH on Withdrawn Problems, as well as a steady improvement on the Anxious/depressed scale. GH, by contrast showed little change across sessions. At session 4, C scored significantly below GH on Withdrawn ($p < 0.01$) and Anxious/depressed ($p < 0.05$) problem scales.

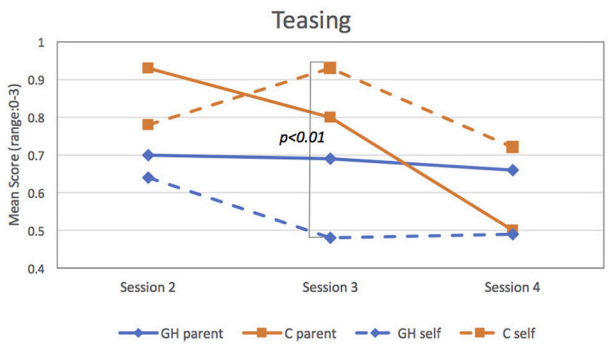

FIGURE 4 | Scores for parents (solid lines) and patients (hatched lines) on "Teasing" item. Patients reported significantly less teasing for GH than C at Session 3 ($p < 0.01$).

For the two PHCSCS BP scales of Behavioral Adaptation and Freedom from Anxiety, findings revealed only significant Session effects ($p < 0.001$ for both indices). For Behavioral Adaptation, results reflected a steady improvement with time; also, at session 3, GH outscored C ($p < 0.05$). Results indicated a steady decline on Freedom from Anxiety for both groups, suggesting increased anxiety with age.

Table 6 shows fewer significant associations for BP than for SC scales (section Social Functioning) and only within the GH group, for whom a larger height gain was associated with lower (i.e., better) YSR Withdrawn and Anxious/depressed scores and higher PHCSCS Freedom from Anxiety scores (**Figure 5**).

Self-Esteem

Table 7 presents the post-baseline results for the four PHCSCS indices evaluating self-esteem, namely Total Self-Concept, Intelligence, Physical Appearance, Happiness/Satisfaction. **Supplementary Table 3** contains the baseline scores for these indices and **Table 8,** the correlation results.

No omnibus (i.e., across session) Group effects were observed for any of the self-esteem indices. However, a significant Group X

TABLE 5 | Mean Post-Baseline (SE) Scores on Behavior Problem Indices[a].

	Session 2		Session 3		Session 4		F-values[b]		
	GH	C	GH	C	GH	C	GP	Session	GP X Session
CBCL[c]									
Total problems	0.62 (0.07)	0.66 (0.07)	0.49 (0.05)	0.49 (0.05)	0.46 (0.05)	0.38 (0.04)	0.58	20.04***	1.29
Internalizing problems	0.53 (0.06)	0.57 (0.06)	0.42 (0.05)	0.46 (0.05)	0.42 (0.05)	0.34 (0.04)	0.15	8.18***	2.14
Externalizing problems	0.43 (0.07)	0.44 (0.08)	0.28 (0.04)	0.32 (0.05)	0.27 (0.05)	0.23 (0.03)	0.21	35.90***	0.67
Withdrawn	0.56 (0.08)	0.56 (0.07)	0.45 (0.06)	0.42 (0.07)	***0.54 (0.06)***	***0.33 (0.07)***	1.00	11.78***	3.73*
Anxious/depressed	0.49 (0.08)	0.60 (0.08)	0.41 (0.04)	0.47 (0.04)	**0.47 (0.07)**	**0.27 (0.07)**	1.03	6.50**	3.62*
Thought problems	0.56 (0.08)	0.54 (0.09)	0.43 (0.06)	0.43 (0.07)	0.31 (0.05)	0.34 (0.05)	0.18	4.59**	0.29
Attention problems	0.89 (0.11)	0.93 (0.11)	0.67 (0.08)	0.59 (0.07)	0.50 (0.07)	0.51 (0.05)	0.28	23.89***	0.64
Delinquency	0.37 (0.07)	0.41 (0.08)	0.22 (0.05)	0.24 (0.05)	0.24 (0.05)	0.18 (0.03)	0.05	15.43***	0.94
Aggression	0.48 (0.08)	0.46 (0.07)	0.34 (0.05)	0.40 (0.07)	0.31 (0.06)	0.27 (0.04)	0.24	30.41***	1.43
YSR[c]									
Total problems	0.22 (0.08)	0.26 (0.03)	0.49 (0.05)	0.48 (0.05)	0.27 (0.05)	0.29 (0.03)	0.13	16.55***	0.07
Internalizing problems	0.23 (0.08)	0.29 (0.03)	0.20 (0.03)	0.24 (0.03)	0.29 (0.05)	0.28 (0.04)	0.34	2.80	0.38
Externalizing problems	0.18 (0.08)	0.15 (0.03)	0.28 (0.05)	0.32 (0.05)	0.14 (0.06)	0.13 (0.02)	0.00	8.03***	0.31
Withdrawn	0.24 (0.10)	0.23 (0.05)	0.29 (0.07)	0.28 (0.07)	*0.39 (0.07)*	*0.34 (0.06)*	0.23	2.85	0.26
Anxious/depressed	0.12 (0.08)	0.21 (0.04)	0.23 (0.05)	0.25 (0.06)	0.29 (0.06)	0.26 (0.05)	0.16	1.36	0.80
Thought problems	0.12 (0.09)	0.26 (0.05)	0.12 (0.03)	0.14 (0.04)	0.14 (0.05)	0.13 (0.02)	0.98	0.72	0.78
Attention problems	0.22 (0.09)	0.27 (0.06)	0.24 (0.05)	0.21 (0.05)	0.34 (0.07)	0.27 (0.04)	0.13	2.21	0.55
Delinquency	0.12 (0.09)	0.11 (0.02)	0.16 (0.04)	0.12 (0.04)	0.15 (0.05)	0.11 (0.02)	0.46	0.24	0.33
Aggression	0.23 (0.09)	0.20 (0.04)	0.17 (0.04)	0.15 (0.04)	0.20 (0.06)	0.15 (0.03)	0.44	0.61	0.10
PHCSCS[d]									
Behavioral adaptation	78.5 (2.7)	71.8 (2.9)	**83.9 (2.0)**	**75.4 (2.7)**	80.4 (2.1)	75.1 (2.4)	2.42	13.53***	0.74
Freedom from anxiety	63.2 (3.3)	57.7 (3.6)	67.9 (3.1)	60.7 (3.2)	58.1 (3.8)	58.9 (2.8)	0.74	11.10***	2.32

*[a] See **Supplementary Tables 1**, **2** for Session 1 results; [b] MANCOVA for CBCL and PHCSCS with Session 1 results as covariate; MANOVA for YSR; [c] Expressed in SD units with higher positive scores reflecting more behavior problems and negative scores indicating very good behavior; [d] Expressed in percentile scores NOTE: Results shown in italics indicate significant group difference at p<0.05 level corrected and in bold italics at p<0.01 level corrected; ***p < 0.001; **p < 0.01; *p < 0.05; [+] indicates a trend at the p<0.10 level.*

Session interaction for Total Self Concept ($p < 0.05$) reflected the increasingly better self-esteem among GH than C. GH also scored significantly above C at session 3 ($p < 0.05$) in Total Self Concept. A trend-level interaction for Physical Appearance ($p < 0.10$) reflected GH's tendency to view themselves as becoming more attractive with age, whereas C viewed themselves as less attractive with age. **Table 8** shows that for GH, height gain was positively correlated with all aspects of self-esteem for GH ($p < 0.01$), but for C, only taller adult height and Total Self Concept were correlated ($p < 0.05$).

Academic Functioning

Although groups did not differ in their academic functioning (**Table 7**), both groups scored higher in reading than math, as is typical of this population (17, 18). Significant Session effects on the School scale ($p < 0.001$) and in Reading ($p < 0.001$) and Math ($p < 0.05$) reflected age-related improvements for both groups. Groups did not differ in grade failure or special-class placement (data not shown). In both groups, taller adult height was significantly ($p < 0.05$) associated with better school performance (**Table 8**).

DISCUSSION

Overview of Current Study and Findings

It is well established that when patients with TS are supplemented with GH, they attain an average adult height of 5–7 cm above untreated patients but still remain short relative to unaffected peers. However, even though GH supplementation has been offered to TS patients now for more than two decades, its full impact on their psychosocial functioning has not been properly evaluated during or at trial completion (19). Given that patients with TS in the industrialized world are almost universally offered this treatment, obtaining a group of such patients to serve as untreated controls is unlikely. Consequently, our study of a wide range of psychosocial functions in patients with TS who received GH supplementation vs. those who did not are timely and fill the existing knowledge void.

Our findings are based on the majority of cases with TS who participated in a Canada-wide trial of GH (4) and were randomized to either a GH-supplementation or no-treatment control group. Both groups, and their parents, completed standardized questionnaires at set intervals until adult height was reached. Comparisons of treated and non-treated patients revealed remarkably few omnibus (i.e., across three post-baseline sessions) differences in their social skills, behavioral problems, self-esteem, or school functions. Nevertheless, they both scored quite poorly relative to the general population on most social-functioning indices and also showed moderately increased behavior problems. Furthermore, for many of the indices, their scores underwent significant changes over time with some characteristics, such as social competence and social relations, showing declines and others, such as behavior problems, improvement. Moreover, findings of significant Group X Session interactions for social problems, perceived popularity, withdrawn and anxious/depressed problems, and self-esteem reflected group differences in their patterns of change over time.

TABLE 6 | Correlations between height parameters and behavior problem results at session 4.

	Adult height (NCHS SD Units)		Δ Height	
	GH	C	GH	C
CBCL				
Total problems	−0.166[a]	−0.184	−0.184	0.100
Internalizing problems	−0.081	−0.146	−0.195	0.087
Externalizing problems	−0.095	−0.129	−0.090	0.205
Withdrawn	−0.067	0.006	−0.181	−0.182
Anxious/depressed	−0.033	0.077	−0.157	−0.079
Thought problems	−0.128	−0.151	−0.164	−0.209
Attention problems	−0.172	−0.010	−0.153	−0.047
Delinquency	−0.010	0.004	−0.067	0.071
Aggression	−0.142	−0.042	−0.091	−0.017
YSR				
Total problems	−0.132	−0.116	−0.190	−0.089
Internalizing problems	−0.067	−0.012	−0.187	−0.110
Externalizing problems	−0.025	−0.214	−0.185	−0.074
Withdrawn	−0.068	−0.149	−0.250*	0.050
Anxious/depressed	−0.075	−0.096	−0.257*	−0.172
Thought problems	−0.137	0.167	−0.131	−0.145
Attention problems	−0.050	−0.046	−0.192	0.008
Delinquency	0.096	0.014	−0.131	−0.116
Aggression	0.125	−0.053	−0.165	−0.112
PHCSCS				
Behavioral adaptation	0.099	0.227	0.164	0.028
Freedom from anxiety	0.232	0.260	0.347**	0.053

[a] *All results are based on a one-tailed test; ***$p < 0.01$; *$p < 0.05$.*

Specifically, GH showed early benefits of therapy but C later caught up to them at the final-height session. When results were analyzed within individual sessions, results showed that just prior to puberty induction (session 2), GH claimed to be more socially engaged and popular than C and 1 year later (session 3), to show better behavioral adaptation and higher self-esteem than C. By contrast, C reported better social relations at session 3 and, according to parents, less anxiety and depression at adult height (session 4). These results, therefore, suggest the early benefits of GH therapy may diminish over time.

Of note, too, were our findings from single items showing C had generally more friends than GH but groups did not differ in time spent with friends or reported loneliness. However, C was teased more than GH, a factor known to contribute to increased depression and poor self-esteem in this population (20, 21). We studied this effect further by performing supplementary regression analyses in which we examined the relative contributions of height vs. teasing on anxiety/depression and self-esteem scores. As shown in **Table 9**, results indicated teasing (likely due to short stature and other physical stigmata) had a worse effect on self-esteem and depression than short stature itself, thus highlighting the need for additional therapies to counteract these adverse effects.

In addition, we found striking group differences in how adult-height indices were related to psychosocial outcome, with far fewer significant correlations for C than GH (2 vs. 21, $\chi^2 =$

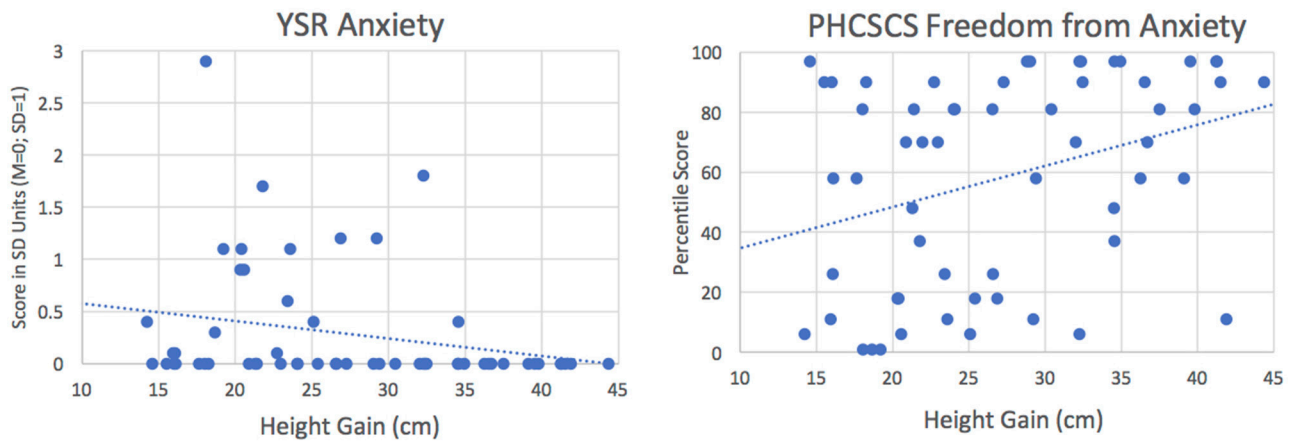

FIGURE 5 | Correlations between height gain and anxiety in GH group. Left: Correlation with YSR Anxiety, $y = 0.75–0.017x$, $r = -0.257$, $p < 0.05$; Right: Correlation with PHCSCS Freedom from Anxiety, $y = 21.1+1.37x$, $r = 0.347$, $p < 0.001$. Results indicate that children showing larger height gains have less anxiety.

TABLE 7 | Mean (SE) post-baseline piers-harris children's self-concept scale and academic scores.

	Session 2		Session 3		Session 4		Group X Session		
	GH	C	GH	C	GH	C	Group	Session	
PHCSCS									
Total self concept[a]	71.0 (3.04)	68.6 (2.7)	**78.2 (2.6)**	**69.1 (2.9)**	72.3 (2.9)	71.0 (2.5)	1.10	9.40***	3.58*
Intellectual and school[a]	68.8 (3.1)	70.3 (3.3)	72.4 (2.7)	70.9 (2.9)	66.3 (3.0)	72.1 (24)	0.14	4.74**	1.96
Physical appearance[a]	55.0 (3.0)	62.5 (3.2)	58.4 (3.1)	54.6 (3.0)	60.0 (2.2)	59.6 (2.2)	0.02	11.09***	2.91+
Happiness and satisfaction[a]	66.5 (2.8)	70.2 (3.0)	74.2 (2.8)	66.4 (3.1)	70.0 (2.9)	68.2 (2.7)	1.69	9.40***	2.27
CBCL									
School[b]	−0.80 (0.07)	−0.70 (0.07)	−0.66 (0.09)	−0.76 (0.091)	−0.44 (0.08)	−0.72 (0.08)	0.93	15.62***	1.43
Reading[c]	3.27 (0.07)	3.35 (0.07)	3.36 (0.07)	3.35 (0.07)	3.27 (0.07)	3.27 (0.07)	0.07	11.95***	0.39
Math[c]	2.73 (0.08)	2.86 (0.08)	2.79 (0.08)	2.89 (0.08)	2.85 (0.08)	2.83 (0.08)	0.72	3.06*	0.81

*[a]Results are expressed as percentile scores; [b]Expressed as z-scores based on normative sample for tests (mean = 0; SD = 1), positive score signifies better outcome; [c]Based on a 4-point scale (4 = very good); Results shown in bold indicate significant group difference at the $p < 0.05$ level; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.*

18.24, $p < 0.001$). In C, only adult height and total self-concept and school functioning were correlated, whereas in GH, a taller adult height and/or greater height gain were associated with better parent- and/or self-reported social competence and social relations, more time spent with friends, higher self-esteem, and better school functioning, as well as less teasing, less loneliness, and fewer withdrawn or anxious/depressed behavior problems. The lattermost findings, which are displayed in **Figure 5**, indicate that the patients who grew more following GH therapy were subsequently less anxious, or conversely, *those who grew minimally had the most anxiety*. Relevantly, since items comprising both CBCL and YSR Anxiety/Depression scales reflect fearful behaviors, sadness, and worrying, as well as suicide contemplation, this may warrant future investigation to determine if those with the least growth are at increased risk.

Findings From Uncontrolled Studies

The findings from this sub-study are at odds with the published literature on GH effects in TS, which is based mostly on uncontrolled trials where adult patients were assessed for psychosocial functioning via HRQoL questionnaires provided several years after competing therapy. For example, a nationwide study of French women with TS treated with GH showed similar HRQoL scores to the general female population. Results, which were unrelated to height (22) or other variables associated with GH treatment (e.g., duration of treatment) (22), were associated with other TS-related problems such as hearing disorders (23). This suggests that health problems and life events *other than short stature* may be contributing to the social impairments in patients with TS. In a Dutch study of women with TS aged ∼20 years, who had participated in either a randomized dose-response trial from an early age or an open frequency-response trial from age 11 (12), results revealed reduced self-confidence and more psychosocial problems relative to a normative sample but no signs of depression (12). These same researchers also observed reduced social and emotional functioning relative to a reference population (24) on self- but not parent-report while scores were positively associated with breast satisfaction, but not height. A study from Belgium (25) of young TS women treated previously with GH and estrogen, who were assessed

TABLE 8 | Correlations between Height Parameters and Self-Esteem and Academic Functioning Indices at Session 4[a].

	Final Height (NCHS SD Units)		Δ Height	
	GH	C	GH	C
SELF-ESTEEM				
Total self-concept	0.208	0.289*	0.362**	−0.037
Intelligence & school[b]	0.227	0.176	0.384**	0.045
Physical appearance[b]	0.200	0.172	0.323**	0.071
Happiness/satisfaction[b]	0.214	0.164	0.374**	−0.022
ACADEMIC/COGNITIVE FUNCTIONING				
CBCL school scale	0.276*	0.316*	0.082	0.148
Reading rating[b]	0.191	−0.104	0.100	0.065
Math rating[b]	0.099	0.029	0.102	0.040

*[a]Results are based on a one-tailed test; [b]Critical p-value divided by 3 for three indices in the category; **p<0.01; *p<0.05 after correction for 3 multiple scales.*

using similar instruments as in our sub-study, reported scores similar to a non-TS reference group at age 18–23 years. However, the treated TS patients had increased attention problems and reduced social acceptance while those with a 45, X karyotype also claimed greater than normal social withdrawal (25). It is important to emphasize that none of the above studies used a similarly followed untreated TS control group.

Two studies published more recently have involved expanded designs and a broader range of tests but are focused mainly on the effects of oxandrolone (O) therapy in TS. A study from Sweden (26) used a case-control design to compare four subgroups of young adult TS women and a population sample of similar aged women for multiple facets of quality-of-life. The TS patients were stratified according to whether or not they received O and/or GH; they were not randomly assigned to their respective conditions. A comparison of the 13 patients who were given only GH (i.e., no O) with the 34 without either GH or O (i.e., no treatment) revealed the GH-only group indicated less social isolation and less pain than the no treatment group. The second study, which was most like ours, used similar tests and a comparable time-frame for comparing groups (27). A total of 133 children with TS from 10 pediatric endocrine clinics across the Netherlands were randomized to three O conditions (placebo, low dose, high dose) with all receiving GH and estrogen; a no-GH comparison group was not studied. The three groups showed similarly elevated rates of internalizing problems and social withdrawal, which as in our study, decreased over the course of the study. Nevertheless, both of these studies are limited because group assignment was not random (26), the number of GH-only patients was small (26), and a no-treatment TS control group was not used (27). Thus, further study of the psychosocial consequences of GH therapy is needed.

Explanations for Current Findings and Sources of Bias

It is not readily clear why the early psychosocial benefits of GH observed presently were not sustained, since the untreated group caught up to (or even surpassed) the treated group once adult height was attained. This finding supports the earlier report on a subset of our cohort who at age 20 indicated no benefits of GH supplementation on HRQoL (6). While some effects may have been eliminated had we used a double-blind placebo-controlled design, the one study with such a design reported no effects of GH supplementation on cognitive or academic functions after one to seven years; however, this study did not, to our knowledge, examine their social abilities (28).

A possible explanation for our findings is the GH group was initially biased to respond more favorably given their greater investment of time and effort with the hope that treatment would lead to favorable social outcomes (29). However, when after injections for at least 6 years and still being short relative to peers (despite slightly increased height), they and their parents may have become disillusioned or more realistic and so less biased in their responses. Another explanation why GH supplementation did not substantially improve psychosocial functioning at adult height may reflect the fact that short stature on its own has little consequence for psychological adaptation. According to Sandberg and Colsman (30), this effect becomes insignificant once other factors such as parents' education and marital status are taken into consideration. It is important to note, however, that when we reanalyzed our data using marital status and SES as covariates in a supplementary analysis (results not shown), most effects not only remained in the GH group, some such as social-relation difficulties became worse and others, such as self-concept and happiness, improved.

Alternatively, the improvements we observed in untreated control patients at adult height may have been real reflecting resignation to their height status and satisfaction with achieving puberty coincident with peers (24); notably, the natural course of TS development and the impact of estrogen therapy are not known (25). However, it is also possible the C cases, who completed our study, represented better functioning individuals than those who dropped out midway through the trial.

It is also not evident why C had more friends than GH throughout the trial, especially at session 4. Although C also had more friends at baseline, the difference from GH in number of friends was not significant at this session. Although it does not seem likely that the GH group was hindered in their friendships by the therapy, this factor needs further investigating.

Our findings that specific internalizing behavioral difficulties were associated with adult height status may reflect parents' misattributing their daughter's troubles to the most obvious possible culprit, namely her short stature, a phenomenon sometimes referred to as a "focusing illusion" (31). Previous findings of psychosocial impairment in patients with short stature may possibly have ignored the fact that their short stature was part of a more serious medical condition. The emotional burdens of these other medical co-morbidities are unfortunately not improved by GH supplementation.

In addition, it should be noted that while GH treatment was not found to be particularly effective in improving psychological well-being from the non-treated state, current findings of higher than normal rates of psychosocial problems among TS patients, as observed by others (32), cannot be ignored. For example, all TS patients showed significantly reduced social competence and social relations scores and had elevated social

TABLE 9 | Summary of simple regression analyses for variables predicting self- or parent-reported anxiety/depression and popularity scores in GH and C.

Variable	GH Group				C Group			
	B	*SE B*	*β*	*t*	*B*	*SE B*	*β*	*t*
ANXIETY/DEPRESSION[a]								
Height gain	−0.102	0.071	−0.181	−0.14	0.104	0.132	0.108	0.79
Adult height	0.071	0.717	0.012	0.10	−1.17	1.070	−0.152	−1.09
Teasing[a]	2.399	0.927	0.304	2.59**	−1.18	1.130	−0.094	−0.69
R^2	0.139				0.034			
F	3.45*				0.615			
ANXIETY/DEPRESSION[b]								
Height gain	−0.101	0.095	−0.136	−1.07	−0.019	0.040	−0.045	−0.48
Adult height	0.579	0.992	0.077	0.58	0.618	0.322	0.181	1.92
Teasing[b]	2.830	1.321	0.264	2.15*	5.103	0.649	0.737	7.86***
R^2	0.083				0.533			
F	1.94				20.94***			
PHCSCS POPULARITY[a]								
Height gain	0.655	0.467	0.180	1.40	−0.190	0.332	−0.069	−0.57
Adult height	1.597	4.465	0.045	0.350	3.605	2.705	0.162	1.33
Teasing[a]	−18.42	5.18	−0.42	−3.56***	−15.43	4.290	−0.433	3.59***
R^2	0.248				0.238			
F	6.06***				5.63**			

[a]*Based on YSR questionnaire;* [b]*Based on CBCL questionnaire;* ***$p < 0.001$; **$p < 0.010$; *$p < 0.05$.

and behavioral problem scores. It is interesting to note, however, that our patients scored somewhat more favorably in terms of BP than those from the Netherlands receiving oxandrolone in combination with GH (27).

Moreover, it is not readily clear why there were so few correlations between height and outcome in the untreated C group. It is possible that this reflects the computation of correlations on absolute height measures and not adjusting height for parents' heights. For example, some shorter parents may have been more accepting of their daughter's short stature than taller parents and this may have contributed to more self-confidence and better social adjustment among those from shorter parents. However, further analysis adjusting for target height was unfortunately not possible because not enough parents were measured for height in our study. In contrast, the large series of significant correlations in the treated group may be that new expectations (and hope) from therapy overrode these initial within-family effects.

The current findings, therefore attest to the possibility of negative consequences from unmet expectations, which need to be addressed by therapies beyond just hormones. Indeed, in a recent review paper on the care of girls and women with TS, Culen et al. (33) claim it is important to provide these patients with state-of-the-art psychosocial therapies after beginning GH treatment. Patients with TS need treatments for coping with the psychosocial challenges of their condition (34), such as counseling (33) and social skills training (35), as well as therapy targeted toward maintaining self-esteem in the face of the negative emotional consequences that accompany the physical and health challenges of this condition (36). Therapies also need to deal with the impact of the teasing, as well as the bullying they may receive (34). It is also important to monitor other TS-related problems such as hearing disorders (22), since minimizing these symptoms may lead to improved quality of life. Given our findings that the TS patients with the poorest GH response were at increased risk of further psychological problems, specific additional resources need to be in place for this subgroup of patients.

Limitations

According to Gardner et al. (37), all studies of GH therapy for short stature (of any cause), including randomized trials, suffer varying degrees of bias. Particular sources of bias include: (i) sequence generation, or how subjects are assigned to different groups despite randomization, (ii) allocation concealment, (iii) blinding, (iv) co-interventions, and (v) selective reporting and data loss. Although our study suffers a few of these shortcomings, it should be pointed out that because randomization was based on stratification for initial height, this source of bias was minimized to a degree. Unfortunately, blinding could not be achieved for children, parents, and medical staff, who all knew the patient's group assignment, since we had a no-injection, not a placebo-injection, control group; however, blinding was maintained for personnel involved in scoring questionnaires and inputting data. We could not control for co-intervention with estradiol, which was given to more than 90% of our sample and since we did not receive information on who were or not additionally treated in this way, we could not control for this in the data analysis. Because puberty induction at a normal age is essential for adult well-being (24), this too may have confounded GH benefits. Furthermore, information was also lacking on treatment adherence and protocol compliance.

Regarding selective reporting, all data collected from the sub-study are presented in this report, except those from a family functioning questionnaire because its unusual scoring method was not adaptable for our data-analytic approach and we did not consider it a valid outcome measure for our objectives. Of note, the rate of data loss for this sub-study was relatively low compared with other psychosocial studies of GH effects (38). Of concern was a factor out of our control, namely the differential but non-significant loss in the control group at the third session due to provision of GH by a private physician. As well, we lacked baseline data on a small set of patients from a clinic where the staff endocrinologist did not initially want to participate but agreed later. It should be noted that we used approved imputation techniques for replacing missing data (13) and were careful to correct for multiple comparisons on instruments providing manifold subscales.

Several methodological limitations of our study also warrant further discussion. The first is we may have lacked sufficient power to find significance with the sample sizes available to us since the original study was powered for detecting height differences between treated and untreated groups. Using existing power tables (15), we found that with ∼65 cases per group, we could at best detect moderate sized effects. When we deployed the established literature on TS and GH to identify what are the effect sizes in these studies (which demonstrated a high degree of variability among themselves), we noted moderate-sized effects for some abilities such as social functions but small effect sizes for others, such as behavioral issues. This signifies the need for larger samples than what were available to us. Also, computation of the power associated with the three trend-level group X session interactions (i.e., CBCL Social Problems, YSR Total Social Competence, PHCSCS Physical Appearance) revealed β-levels of 0.54, 0.51, and 0.56 respectively, indicating our risk of missing true effects was elevated.

A second methodological limitation concerns the tools we used to evaluate psychosocial outcome, which did not directly examine cognitive abilities that may also be sensitive to GH effects (5, 38). Moreover, given the multicenter nature of our study, we had to rely upon questionnaires, which may not have been sensitive enough to evaluate subtle effects arising from GH therapy. Furthermore, several superior social functioning measures became available after our study began. One, for example, the Social Responsiveness Scale, has been shown to strongly discriminate between TS and non-TS controls (32) and was recommended for a TS assessment battery (33). Thirdly, our study was conducted in two languages (i.e., English and French), that may have increased variability. However, it should be noted that all instruments and instructions were professionally translated, no differential loss was noticed between Anglophone vs. Francophone sites, and randomization was equivalent across English and French sites.

Notably, too, we lacked several key pieces of information, which also may have influenced our results. First, we were not provided the information on the patients' karyotype, which too could have influenced both the growth response and psychosocial outcome. Regrettably, too, the heights of parents were not available for determining target height and analyzing effects of the psychosocial intervention. It is noteworthy that in a subsequent observational study at one of the participating sites, parental height did not differ between those choosing GH-therapy vs. no GH (39).

CONCLUSIONS

The equivocal results of the present study reflecting only modest gains in psychosocial functioning among patients with TS treated with GH should not deny them the option of GH supplementation, particularly as more favorable psychosocial outcome on a number of indices was strongly associated with ultimate height or height gain. Furthermore, their increased stature has the potential to improve their abilities to better adapt to their physical environment (e.g., driving, occupational opportunities etc.), which can later lead to improved quality of life (35). It is important to note that when girls with TS and their families from one participating center were presented with the available evidence, the vast majority (78%) chose the option of GH supplementation (39). Since it is possible that starting treatment at an earlier age may lead to a more discernible psychosocial benefit, given our findings of taller height as well as greater height gain in those who started treatment younger, this possibility needs to be explored in future studies of psychosocial outcome.

When counseling these patients and their families, current results suggest that it is important not to overemphasize the benefits of GH supplementation on psychosocial adaptation due to increased height, especially when response to treatment for some patients may be minimal. Above all, expectations should always be kept realistic and all TS-associated health problems should be addressed.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JR spearheaded the psychosocial component under the initial guidance of Dr. Jack Holland. She oversaw all activities including packet preparation and distribution to the many sites and followed their return; she managed the activities of the research assistants in data collection, database scoring, and preliminary statistical analyses; she conducted the final statistical analysis and wrote or co-wrote all earlier versions of the manuscript. GVV gave direction and impetus to manuscript preparation and co-wrote an earlier and the current version of this manuscript. He has been an international spokesperson for this trial, which he entered after its initiation once he immigrated to Canada. He also headed the site at Hôpital St. Justine.

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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.2019.00125/full#supplementary-material

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Late-Onset Puberty Induction by Transdermal Estrogen in Turner Syndrome Girls—A Longitudinal Study

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Objective: Estrogen replacement therapy (ERT) for Turner syndrome (TS) is a widely discussed topic; however, the optimal model of ERT for patients with delayed diagnosis and/or initiation of therapy is still unclear, mainly due to insufficient data. We present the results of a prospective observational single-center study in which the efficacy of late-onset puberty induction by one-regimen transdermal ERT in TS girls was evaluated.

Methods: The analysis encompassed 49 TS girls (63.3% with 45,X) with hypergonadotropic hypogonadism in whom unified transdermal ERT protocol was used for puberty induction (first two months 12.5 μg/24 h, thereafter 25.0 μg/24 h until breakthrough bleeding). Clinical visits for examination and therapy modification took place every 3–6 months. Transabdominal pelvic ultrasound examinations were performed at least twice: at the beginning and at the end of follow-up.

Results: The mean (SD) age at ERT induction was 15.1 (1.3) years. The duration of follow-up was 2.4 (1.1) years. Half of all the patients had at least B2 after 0.57 years, B3 after 1.1 years, B4 after 1.97 years, and menarche after 1.82 years from ERT initiation. With earlier initiation of ERT (≤14 years), B2 ($p = 0.059$) was achieved faster and B4 ($p = 0.018$) significantly slower than with the later start of ERT. Thirty-four (94.4%) patients had at least stage B3 at menarche. The karyotype, initial weight, and body mass index had no impact on puberty tempo during ERT. The uterine volume increased significantly during ERT in all the study group ($p < 0.0001$), and in half of the patients, the increase was at least 12.4-fold. It did not correlate with the duration of treatment ($p = 0.84$) or the dose of estradiol per kilogram ($p = 0.78$), nor did it depend on karyotype ($p = 0.71$) or age at ERT initiation ($p = 0.28$). There were no differences in ΔhSDS during ERT ($p = 0.63$) between the two age groups (ERT ≤14 and >14 years).

Conclusion: The presented easy-to-use fixed-dose regimen for late-onset puberty induction allowed for a satisfactory rate of achieving subsequent puberty stages and did not influence the growth potential.

Keywords: Turner syndrome, puberty induction, menarche, estrogen therapy, transdermal estrogen therapy, puberty, karyotype 45,X

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INTRODUCTION

It is estimated that from 20 to almost 50%, Turner syndrome (TS) girls present some degree of pubertal development, with menarche in approximately 16–20%; however, this occurs nine times less frequently in girls with 45,X than in girls with a mosaic karyotype (1–3). Regular menstrual cycles are observed in 6% of the TS population (1) and only 2–5% achieve spontaneous pregnancy (4). Thus approximately 90% of TS girls and women require or will require estrogen replacement therapy (ERT) to initiate, progress, or maintain pubertal development. It is recommended that TS women should receive estrogen and progestin replacement, known to have long-term effects on puberty, fertility, bone health, metabolism, and psychological functioning (5, 6). The first-choice ERT regimen to initiate and progress pubertal development, and at the same time to mimic physiology and minimize risks, is still being discussed. The latest international guidelines recommend initiating ERT at the age of 12 years in the absence of spontaneous puberty and/or if the follicle-stimulating hormone levels are elevated (2, 7). In order to imitate natural development, an incremental increase in the dose is recommended over a period of 2–3 years until an adult dose has been reached. Although there is no evidence as to the superiority of any one ERT regimen, the transdermal route seems to be the most desirable (7).

The use of transdermal estradiol (E2) facilitates a more physiologic mode of delivery, without first-pass effects in the liver, avoiding unphysiological changes and hormone activity. Transdermal E2 results in faster bone accrual at the spine, increased uterine growth, and greater final height (8, 9). It also seems that transdermal E2 is safer in the context of thrombotic risk: thrombin generation is increased in postmenopausal women using oral estrogens. It could be mediated by the hepatic first-pass metabolism of estrone, the main metabolite of oral E2 (10).

In the absence of products designed specifically for puberty induction, the transdermal method offers the possibility to cut and modify the size of the patch in order to facilitate dose adjustment, although this is not recommended by the producers.

The form of ERT in girls with hypogonadism should mimic the physiology, preserve the growth potential, and, at the same time, minimize the risk of side effects. The 2017 guidelines recommend starting ERT at 11–12 years of age, with a dose increase every 6 months over a period of 2–3 years (7).

The most controversial, mainly due to lack of data, is the ERT model for TS girls with delayed diagnosis and/or the initiation of estrogen treatment. We present the results of a prospective observational single-center study in which the efficacy of late-onset puberty induction by one-regimen transdermal ERT in TS girls was evaluated.

PATIENTS AND METHODS

The study encompassed 62 consecutive TS girls who, between September 1997 and 2017, were treated with transdermal ERT at the Department of Pediatric Endocrinology in Katowice, Poland, using the same study protocol. The data of 13 patients were excluded from the analysis: eight girls were followed up for less than 1 year, three girls with spontaneous puberty presented premature ovarian failure symptoms, one girl initiated ERT in another clinical center, and one had incomplete clinical data. The final analysis encompassed 49 TS girls with hypergonadotropic hypogonadism in whom ERT was used for puberty induction. In all cases, TS was diagnosed based on a cytogenetic analysis using peripheral lymphocytes and was confirmed by karyotyping with routine G-banding according to the recommendations of the American College of Medical Genetics. In 31 girls (63.3%), 45,X karyotype was confirmed. Four girls with karyotype 45,X/46,XY had undergone gonadectomy due to the risk of malignant transformation (GK, TK). Forty-five (91.8%) had been treated with recombinant growth hormone (rGH). No data concerning the duration of rGH were available in four girls, and in one, the growth-promoting therapy was still ongoing.

Throughout the study, all the patients underwent two to four routine visits per year, during which a thorough clinical examination, including pubertal staging according to the method of Tanner (11) and anthropometric measurements, was performed by a single pediatric endocrinologist (AG).

Weight was measured with a precision to 100 g and height with Harpenden stadiometer to 0.1 cm. The body mass index (BMI) was calculated as weight (kg)/squared height (m). Height was expressed as standardized values (hSDS—height standard deviation score) based on the growth chart for healthy Polish girls (12). hSDS was calculated using the following formula: hSDS = child's height − height for 50 pc/0.5 × (height 50 pc − height 3 pc).

In addition, the patients' bone age (BA) was determined based on the X-ray of the non-dominating hand using the Greulich–Pyle Atlas (13).

Transabdominal pelvic ultrasound (US) examinations were performed at least twice, at the beginning and at the end of follow-up, using a 5-MHz convex transducer (Siemens Acuson Antares 5.0, Acuson Sequoia and Acuson 128 XP) (AD-C, KW). The uterine volume was determined using the formula $V = a \times b \times c \times 0.5$ (a—diameter of longitudinal section, b and c—two diameters of transverse section) (14).

ERT Regimen

Hypergonadotropic hypogonadism was diagnosed (2) in all our study patients. A unified protocol of transdermal ERT was applied: for the first 2 months, 12.5 μg of estradiol transdermally per 24 h (half of the patch releasing 25 μg or one-fourth of the patch releasing 50 μg), subsequently 25 μg of estradiol/24 h transdermally until breakthrough bleeding occurred, at which point the therapy was changed to cyclic estrogen–progesterone. The patch was replaced every 3.5 days (twice a week) (5). Compliance and side effects were assessed during every visit.

In order to assess the impact of karyotype and age at ERT initiation on the dynamics of puberty, the patients were arbitrarily divided into subsets with puberty induction of ≤14 or >14 years and with karyotype 45,X or non-45,X.

Statistics and Data Analysis

Statistical analyses were performed with STATISTICA version 13.

Comparisons between two groups were performed with two-sided Student's t-test or Fisher's exact test, as appropriate. Kaplan–

Meier analysis was applied to analyze the time course of breast development and menarche stimulation during treatment. Gehan's Wilcoxon test was used to test the difference between groups in Kaplan–Meier analysis. Data are presented as means and SDs, medians, and ranges, and percentages, and unless stated otherwise, are presented in the text as mean (SD)/(range). *P*-values of <0.05 were considered to be significant.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Medical University of Silesia. Written informed consent was obtained from all patients aged over 16 and from their parents or legal custodians.

RESULTS

Clinical Presentation

The mean (SD)/(range) age at TS diagnosis and/or of the first visit at the study center was 9.8 (4.5)/(0.4–17.6) years old. The age at the induction of transdermal ERT was 15.1 (1.3)/(11.7–17.8) years old. The duration of ERT follow-up was 2.4 (1.1)/(1.0–6.2) years. Before ERT induction, eight (16.3%) girls presented breast development at a stage higher than B1: B2 was observed in six girls, while B3 was observed in two girls. The duration of rGH therapy was 5.1 (2.8)/(1.1–10.9) years.

The clinical data of the 49 girls, also grouped by age and karyotype, are presented in **Tables 1**–**3**.

TABLE 1 | Clinical data of the 49 Turner syndrome patients [45,X—31 (63.3%) and non-45,X—18 (36.7%)].

***n* = 49**	**Mean (SD)/(range)**
Age at ERT start (years)	15.1 (1.3)/(11.7–17.8)
Age of the last visit (years)	17.5 (1.0)/(14.1–19.1)
Weight (kg)	46.7 (8.8)/(30–73.7)
BMI at ERT start (kg/m^2)	20.6 (4.5)/(16.3–30)
BMI at the last visit (kg/m^2)	26.8 (11.4)/(17.8–30.4)
hSDS at ERT start	−2.52 (1.16)/(−0.60 to −5.30)
hSDS at the last visit	−1.93 (0.99)/(0.23 to −5.23)
ΔhSDS	0.59 (0.67)/(−0.80–2.40)
BA at ERT start (years)	12.63 (0.92)/(10.0–14.0)
Uterus volume at ERT start (ml)	1.44 (1.87)/(0.12–8.8)
Uterus volume at the last visit (ml)	10.2 (7.3)/(1.7–40.4)
Increase of uterus volume during ERT (volume at the last visit/volume at ERT start)	19.2 (17.8)/(1.05–58.8)

n, number of patients; ERT, estrogen replacement therapy; BMI, body mass index; hSDS, height standard deviation score; BA, bone age.

Dynamics of Breast Development during ERT

All but three (6.1%) girls presented breast development with progression to at least B3. At the end of follow-up (during the last visit), stages 4 and 5 were observed, respectively, in 25 (52%) and 7 (14.3%) girls. The Kaplan–Meier curves showed that 50% of all the girls had at least B2 after 0.57 years, B3 after 1.1 years, and B4 after 1.97 years of ERT (**Figure 1**). A tendency in patients with earlier ERT initiation (≤14 years) to progress faster to B2 (**Figure 2A**, $p = 0.059$) and significantly slower to B4 (**Figure 2B**, $p = 0.018$) than in patients with late-onset ERT initiation (>14 years) was observed. The karyotype had no impact on the dynamics of achieving consecutive breast development stages during ERT.

No breast tissue response to ERT was observed in three (6.1%) girls, with B1 at the last examination. All three girls belonged to the late-onset therapy group (>14 years), and two had karyotype 45,X. The distribution of B stages at ERT initiation and at the last visit is presented in **Figure 3**.

Menarche during ERT

Menarche was observed in 36 (73.5%) girls during ERT, and it occurred after 1.5 (1.0)/(0.3–4.6) years. Based on the Kaplan–Meier curve, half of the patients were after their first menstruation at 1.82 years from the start of ERT (**Figure 4**). There were no differences in the time to menarche between girls with different karyotypes and with different age at therapy initiation. Most patients, 34 (94.4%), had at least stage B3 of breast development at menarche (B3, B4, and B5 in 17, 15, and 2 patients, respectively).

TABLE 2 | Clinical data of the 49 Turner syndrome patients grouped by age.

Karyotype 45,X; non-45,X *n* (%)	**ERT start ≤14 years (*n* = 10) 8 (80%); 2 (20%)**	**ERT start >14 years (*n* = 39) 23 (59%); 6 (41%)**	***p*-Value**
	Mean (SD)/(range)	**Mean (SD)/(range)**	
Age at the first visit (years)	6.1 (4.4)/(04–13.6)	10.8 (4.1)/(1.1–17.6)	0.002
Age at ERT start (years)	13.3 (0.7)/(11.7–14.0)	15.5 (1.0)/(14.1–17.8)	0.000
Duration of follow-up (years)	3.4 (1.7)/(1.1–6.2)	2.2 (0.8)/(1.0–4.6)	0.048
Weight (kg)	45.1 (9.4)/(31.0–58.5)	47.2 (8.8)/(30.0–73.7)	NS
BMI at ERT start (kg/m^2)	20.9 (3.4)/(16.3–25.9)	21.2 (3.0)/(17.1–30.0)	NS
BMI at the last visit (kg/m^2)	21.5 (3.0)/(17.8–25.4)	23.0 (2.9)/(18.5–30.4)	NS
hSDS at ERT start	−2.36 (1.05)/(−4.58 to −1.00)	−2.56 (1.20)/(−5.30 to −0.60)	NS
hSDS at the last visit	−1.87 (0.98)/(−3.75 to −0.30)	−1.95 (1.00)/(−5.23–0.23)	NS
ΔhSDS	0.50 (0.77)/(−0.80–2.20)	0.61 (0.66)/(−0.75–2.40)	NS
BA at ERT start (years)	12.25 (0.82)/(11.0–14.0)	12.7 (0.9)/(10.0–14.0)	NS
Uterus volume at ERT start (ml)	1.54 (1.56)/(0.12–5.30)	1.41 (1.97)/(0.14–8.80)	NS
Uterus volume at the last visit (ml)	13.8 (14.3)/(1.7–40.4)	9.6 (5.1)/(2.2–22.1)	NS
Increase of uterus volume during ERT (volume at the last visit/volume at ERT start)	14.3 (9.8)/(1.1–31.1)	20.1 (19.0)/(1.05–58.8)	NS

n, number of patients; ERT, estrogen replacement therapy; BMI, body mass index; hSDS, height standard deviation score; BA, bone age; NS, not significant.

TABLE 3 | Clinical data on the 49 Turner syndrome patients grouped by karyotype.

	45,X (n = 31)	Non-45,X (n = 18)	p-Value
	Mean (SD)/(range)	Mean (SD)/(range)	
Age at the first visit (years)	9.4 (5.0)/(0.4–16.7)	10.5 (3.6)/(4.6–17.6)	NS
Age at ERT start (years)	14.8 (1.3)/(11.7–17.3)	15.5 (1.2)/(13.0–17.8)	NS
Duration of follow-up (years)	2.7 (1.2)/(1.0–6.2)	2.0 (0.8)/(1.0–3.8)	0.038
Weight (kg)	47.5 (9.7)/(30.0–73.7)	45.5 (7.2)/(31.0–59.6)	NS
BMI at ERT start (kg/m^2)	21.5 (3.5)/(16.3–30.0)	20.6 (2.3)/(17.4–25.4)	NS
BMI at the last visit (kg/m^2)	22.8 (3.0)/(17.8–30.4)	22.5 (2.9)/(18.6–28.8)	NS
hSDS at ERT start	−2.46 (1.25)/(−5.30 to −0.60)	−2.63 (1.01)/(−4.58 to −1.08)	NS
hSDS at the last visit	−1.82 (1.00)/(0.23 to −5.23)	−2.12 (0.97)/(−4.00 to −0.50)	NS
ΔhSDS	0.64 (0.72)/(−0.80–2.40)	0.51 (0.59)/(−0.75–1.59)	NS
BA at ERT start (years)	12.52 (0.97)/(10.00–14.00)	12.83 (0.80)/(11.5–14.00)	NS
Uterus volume at ERT start (ml)	1.00 (1.17)/(0.12–6.32)	2.33 (2.67)/(0.14–8.80)	NS
Uterus volume at the last visit (ml)	10.4 (8.2)/(1.7–40.4)	10.0 (5.3)/(2.2–21.8)	NS
Increase of uterus volume during ERT (volume at the last visit/volume at ERT start)	18.4 (15.8)/(1–48.7)	20.8 (22.1)/(1.4–58.8)	NS

n, number of patients; ERT, estrogen replacement therapy; BMI, body mass index; hSDS, height standard deviation score; BA, bone age; NS, not significant.

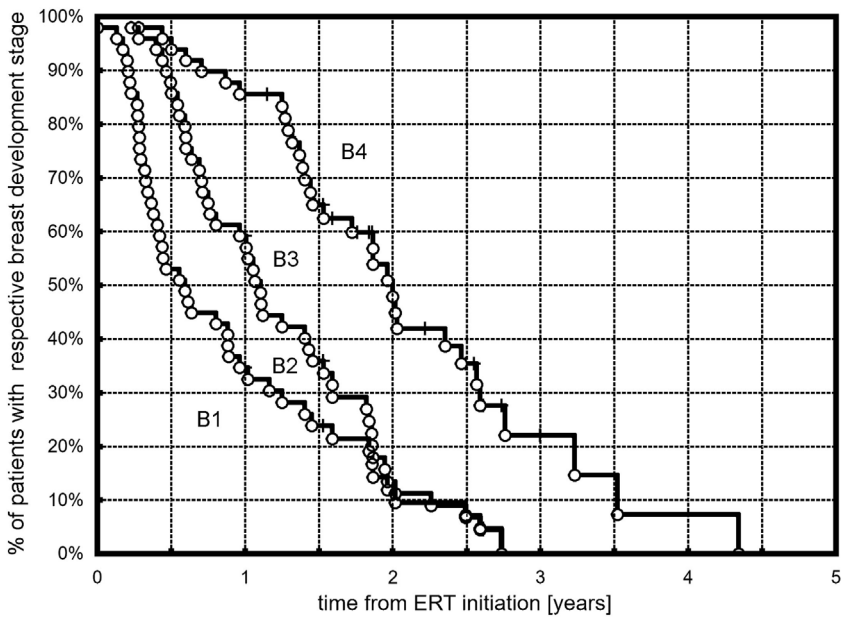

FIGURE 1 | Kaplan–Meier plots showing the time course of B2, B3, and B4 development in the observed TS patients.

The girls' initial weight or their BMI had no impact on the time of menarche.

Dynamics of Uterine Development during ERT

The uterine volume at ERT initiation was comparable in the two age groups (ERT ≤14 and >14 years, $p = 0.84$) and was larger in non-45,X girls than in 45,X ($p = 0.09$). The initial uterine size did not correlate with the girls' weight or hSDS ($p = 0.78$; $p = 0.37$). The uterine volume increased significantly during ERT in all the study group ($p < 0.0001$), in half at least 12.4-fold (**Table 1**). It did not correlate with the duration of treatment ($p = 0.84$) or the dose of estradiol per kilogram of the initial body weight ($p = 0.78$), and it was not dependent on karyotype ($p = 0.71$, **Table 3**) or age at ERT initiation ($p = 0.28$, **Table 2**).

Height

There were no differences in ΔhSDS during ERT ($p = 0.63$, **Table 2**) between the two age groups (ERT ≤14 and >14 years).

Side Effects of ERT

No estrogen-related adverse events were reported.

DISCUSSION

In this paper, we presented the results of a prospective observational study with 49 TS girls in whom the same model of ERT

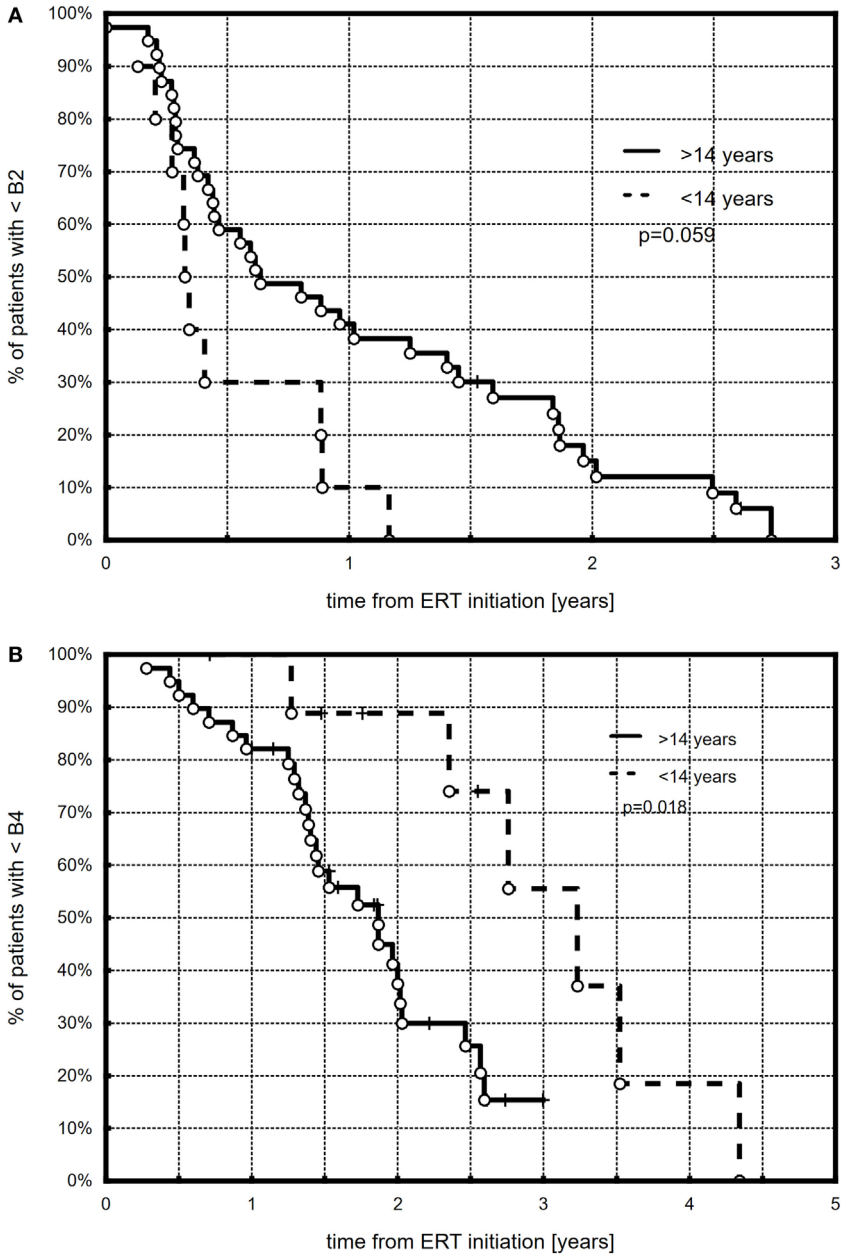

FIGURE 2 | Kaplan–Meier plots showing the comparison between younger (≤14 years) and older (>14 years) patients with respect to B2 **(A)** and B4 **(B)** development.

initiation was used. The mean age at pharmacological puberty initiation in our study group was over 15 years, and only one-fifth of the girls started ERT before the age of 14 years. In view of the recommendations, this is recognized as late-onset puberty induction. The use of a 2- to 3-year model for the induction of puberty in such conditions does not seem to be optimal, especially from the patient's point of view.

Delayed ERT induction in our patients was mostly the result of late TS diagnosis and, consequently, of late onset of rGH therapy; in some cases, it was caused by the patient's and/or her family's reluctance for fear of adverse impact of estrogen on the final height. The age at the first visit in our center for girls with ERT initiation of >14 years old was significantly higher compared to that in girls with ERT before 14 years old. The karyotype, and thus, indirectly, the severity of phenotype presentation, had no impact. In most girls, the BA at the time of ERT initiation was assessed at 13 years of age.

Our observational study focused on the dynamics of puberty advancement during ERT. The applied protocol of transdermal therapy was the authors' original concept (AG) and was not modeled on any previous studies. Our center was one of the first to start transdermal estradiol therapy in TS girls, almost 20 years

ago. We found that half of all the treated girls had at least Tanner stages B2, B3, or B4 after 0.57, 1.1, and 1.97 years of treatment. Girls with ERT initiation of ≤14 years tended to achieve B2 faster and B4 significantly slower than girls with late-onset ERT initiation. At the end of the follow-up, stages 4 and 5 of breast development were observed, respectively, in 52 and 14.3% of the girls. Menarche occurred in more than 70% of the girls. Half of the girls were after their first menstruation at 1.82 years from the start of ERT.

A review of the literature showed studies presenting different schemes of ERT for puberty induction, some related to puberty progression (8, 15–20). Each of these regimens was different and difficult to compare, also due to the different age at ERT initiation. In the study by Nabhan et al., transdermal estradiol therapy in 14-year-old patients was compared to conjugated estrogen. It was characterized by a quite high and fast increase in dose, even in comparison to our protocol. By using this 1-year protocol (first 6 months, 25 μg/day transdermally, thereafter next 6 months, 37.5 μg/day), the breast stage increased more progressively than in our study, and after 1 year, TS patients were of Tanner stage 3 or 4. Breakthrough bleeding occurred in four of their six girls, and it took place earlier than in our study (8).

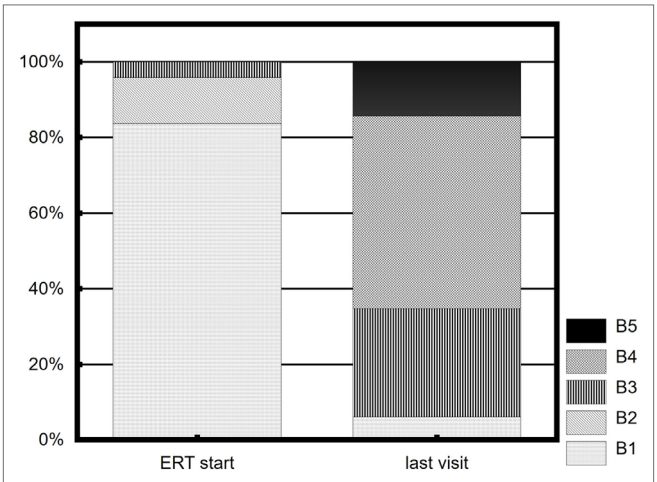

FIGURE 3 | Distribution of breast development stages (B) at ERT initiation and at the last visit.

Other studies were even less comparable with regard to the study protocol and the age at pharmacological puberty induction. Bannink et al. used increasing doses of oral estradiol at the mean age of 12.7 years and observed breast development comparable to normal with a 2-year delay (17). A Dutch study showed that treatment with micronized E2 started at a mean age of 12.7 years facilitated reaching B2 just before 13 years and B4 at a mean age of 14.8 years (16). A 2-year treatment with oral E2 (47 girls, age 13–14), monitored by a Spanish Turner working group showed that using either a fixed or an individualized dose allowed to attain B4 or B5 in 2 years. In the fixed-dose model, a shorter time was needed (2.0 vs. 2.2 years), and a tendency to a higher proportion of girls with a minimum B4 at the study end was observed (65 vs. 42%) (19). Piippo et al. used percutaneous E2 gel for puberty induction in 23 girls of a median age of 13.6 years, with the development of secondary sexual characteristics and uterine growth proceeding gradually, mimicking natural puberty. At the end of the 5-year treatment, all girls reached at least B4. In three patients, spontaneous bleeding occurred after 6 months, and in one after 1.25 years (20).

Despite the different ERT regimens used in the cited studies, the dynamics of breast development was similar to our study:

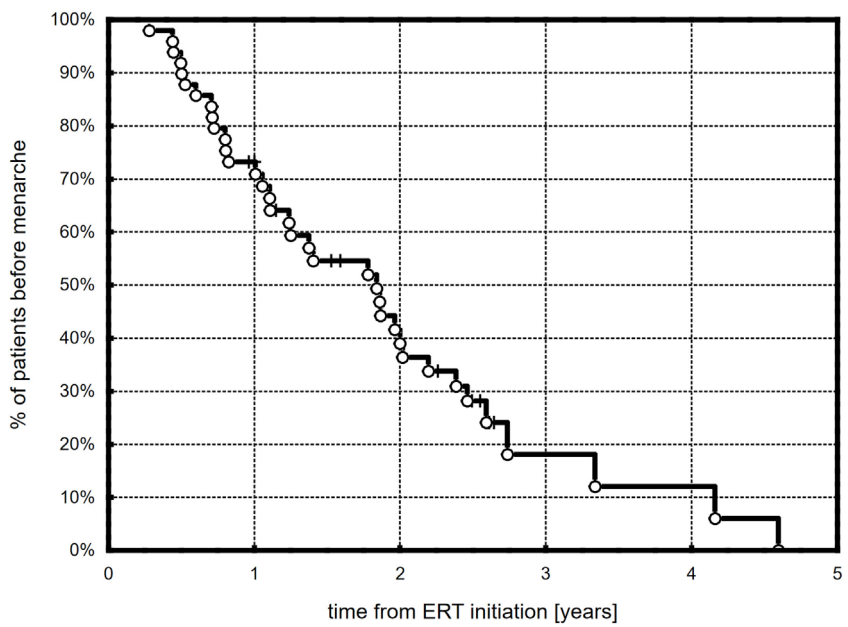

FIGURE 4 | Kaplan–Meier plots showing the time course of menarche in the observed TS patients.

stage B2 during the first months and B4 after approximately 2 years. This is comparable to spontaneous puberty. In some girls, resistance to estradiol therapy was noticed (8). In our study, this occurred in three girls, interestingly, all with late onset of therapy induction.

A unique schedule of ERT was presented by a Swedish group. In the study by Ankarberg-Lindgren et al., with the nocturnal application of transdermal estradiol (0.08–0.12 μg/kg), stage B2 occurred at 3–6 months from the first-night administration of patches in most of the 15 patients with hypogonadism; B3 was observed in seven girls after 3.5–29 months. However, similar to our observation, the authors did not find a correlation between the given dose per kilogram and the rate of progression of breast development (18).

One of the most important issues in the context of ERT is uterine development, both as a marker of therapy effectiveness and as a chance for future *in vitro* fertilization procedures. According to Bakalov and McDonnell, TS women may develop a normal uterus even at a late start of HRT, given the adequate duration of treatment and regardless of karyotype (21, 22). This is in contrast with other studies. Doerr et al. found that only TS women with karyotype 45,X/46,XX had a normal uterine size, whereas approximately 18 and 25% of TS women with karyotype 45,X had a uterine volume and length below −2 SD (23). In a Danish study, the mean uterine volumes by MRI and US in fully matured TS girls were lower than in controls despite appropriate hormonal therapy in TS (24). Transdermal ERT seems to be more effective in uterine size increase compared to conjugated estrogens (8). By using estradiol gel, the uterine volume increased from 5.5 to 31.5 ml with a range of 8.2–82.8 ml (20). Our results were in line with the literature. The initial uterine size did not depend on the girls' weight or height, and girls with 45,X tended to present smaller uterine dimensions. Using fixed transdermal therapy, we observed a marked increase in the uterine volume: at least 12.4-fold in half of our patients. Interestingly, the increase did not correlate with the duration of treatment or the dose of estradiol per kilogram of the initial body weight. Moreover, it did not depend on the age at ERT initiation. Similar to previously published results, the increase in uterine size did not depend on the karyotype (8, 25).

Ideally, ERT should mimic physiology, facilitating normal-pace puberty and promoting growth. This is possible if TS is diagnosed early. However, there are no data to support the specifics of ERT timing and doses in cases of delayed diagnosis and puberty induction. The decision is individual and based on the doctor's experience. In this paper, we presented a model for late-onset puberty induction which resulted in a satisfactory rate of achieving subsequent puberty stages and which did not influence the growth potential. What is important, in the context of compliance, this regimen was easy to use and was well tolerated by the patients.

AUTHOR CONTRIBUTIONS

AG and MH designed the study, analyzed the database, and wrote the manuscript. KS, TG and KS prepared and analyzed the patients' database and wrote the manuscript. AD-C and KW participated as gynecology consultants. EM-T, AA, GK, and TK collaborated in designing the work and drafting the manuscript.

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ORIGINAL RESEARCH
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Effect of Hormone Replacement Therapy on Bone Mineral Density and Body Composition in Chinese Adolescent and Young Adult Turner Syndrome Patients

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A longitudinal observational study was performed comparing BMD and body composition in Turner syndrome girls before and after 1 year of HRT treatment. Whole body BMD, femur neck BMD, total hip BMD, and lean mass were significantly increased, but there was no difference in fat mass, and lumbar spine BMD.

Purpose: Low bone mineral density (BMD) is one of the major health problems in Turner syndrome (TS) patients, and a certain percentage of TS girls are treated with hormone replacement therapy (HRT) to improve their BMD, among other health benefits. While it is generally accepted that HRT improves BMD and body composition in adolescent and young adult TS patients, studies of HRT in Chinese TS patients are limited.

Methods: To investigate the effects of HRT in Chinese TS girls, we performed a longitudinal observational study which compared measurement of BMD and body composition by dual energy X-ray absorptiometry (DXA) using a Lunar DXA densitometer in 20 Chinese adolescent and young adult TS patients (average age = 18) before and after 1 year of HRT treatment.

Results: Whole body BMD (0.85 vs. 0.87 g/cm^2, $P < 0.001$), femur neck BMD (0.6 vs. 0.62 g/cm^2, $P = 0.02$), total hip BMD (0.68 vs. 0.71 g/cm^2, $P = 0.003$) and whole body lean mass (30.39 vs. 31.66 kg, $P = 0.002$) were significantly increased in these patients after 1 year HRT treatment, but there was no difference in whole body fat mass, android:gynoid ratio and lumbar spine BMD.

Conclusions: In summary, our study found that HRT was an effective way to increase whole body BMD, femur neck BMD, total hip BMD and whole body lean mass in Chinese TS girls, with no effect on whole body fat mass, android:gynoid ratio or lumbar spine BMD.

Keywords: hormone replace therapy, bone mineral density, body composition, turner syndrome, China

INTRODUCTION

Turner syndrome (TS), a disease caused by the deletion or structural abnormality of one X chromosome, is characterized by short stature, low hormone, and congenital under development of the uterus and ovaries (1). Although the incidence of TS is between 1/2,500 and 1/3,000 among live-born girls, it is one of the most common chromosomal diseases in females (2). The symptoms of TS are varied, but all TS patients report a higher frequency of medical conditions compared to the normal population (3). For adolescent and young adult patients with TS, bone health and bone mineral density (BMD) are major concerns (4). TS women have a higher frequency of osteopenia/osteoporosis and bone fracture than normal females (5). The reasons for low BMD and increased bone fragility are multifarious, including chronic hormone deficiency (especially estrogen deficiency), X-chromosome abnormalities (especially haploinsufficiency of the SHOX gene), and other environmental factors (such as few/decreased physical activity due to skeletal muscular dysplasia) (2, 4, 6).

Hormone replacement therapy (HRT) is an important strategy for improving BMD in TS patients because chronic estrogen deficiency is one of the major reasons for bone loss in TS patients. However, several studies found that the BMD of TS patients with HRT were still very low (7, 8). One study indicated that lumbar spine BMD was increased after HRT, while other BMD parameters (e.g., hip BMD, forearm BMD and ultra-distal BMD) remained unchanged (9).

HRT should also have a positive effect on preventing body fat mass gain in women with malfunctioning ovaries, because the loss of endogenous estrogen may lead to fat mass gain in those women (10). However, whether HRT can change the body composition in TS patients remains unclear. It has previously been reported that whole body fat mass measured by dual energy X-ray absorptiometry (DXA), body mass index (BMI), and waist-to-hip ratio (WHR) were not changed by cyclical HRT in 9 non-obese TS patients (mean age: 23 years), but whole body lean mass measured by DXA had a tendency to increase ($p = 0.054$) after 1 year HRT treatment (11). Recently, a 5-year prospective randomized controlled clinical trial in Demark found that whole body fat mass did not change in the HRT group, but whole body lean mass increased in the high dose HRT group (Trisekvens with estradiol 2 mg on day 1–22 of the menstrual cycle) of young TS women (mean age: 19.2 years) (12).

To investigate the effects of HRT on BMD and body composition in Chinese adolescent and young adult TS patients, we performed this study by comparing BMD status at baseline with 1 year follow-up after HRT was started in TS patients. BMD and body composition were assessed by DXA which is the diagnostic gold standard tool for the diagnosis of osteopenia/osteoporosis and sarcopenia. To reduce bias caused by other BMD related factors including age, ethnicity, and ovarian function status, we performed this study among TS patients with primary amenorrhea. HRT is initiated at the age of 13 or above for most TS girls, therefore all participants were above 13 years of age and 19 of the 20 participants were over 15 years of age.

Abbreviations: BMD, bone mineral density; TS, Turner syndrome; HRT, Hormone replacement therapy; DXA, dual energy X-ray absorptiometry; BMI, Body mass index; WHR, waist-to-hip ratio; BMC, Bone mineral content; FSH, Follicle stimulating hormone.

METHODS

Participants

A total of 20 adolescent and young adult TS patients (including X0 and mosaicism, diagnosed based on the results of chromosome analysis, for details see **Supplementary Table 1**) with primary amenorrhea participated in the current study. For each subject, cytogenetic analysis was performed on peripheral blood lymphocytes according to standard Giemsa stain G banding technology with 350–450 bands, more than 30 cells were karyotyped per patient (13). Exclusion criteria for our study were other chronic bone diseases which may influence BMD (e.g., osteochondrodysplasia and malignant osteopetrosis), other diseases which may influence BMD (e.g., type 1 or type 2 diabetes, hyperthyroidism, coeliac disease, other thyroid disorders) or treatment with drugs associated with bone metabolism or BMD (e.g., glucocorticoid and growth hormone). Since the karyotype of TS patients is not associated with their BMD status and body composition (14, 15), the present study did not exclude TS patients with mosaicism. The participants were treated and followed by the same physicians in the Divisions of Pediatric Endocrinology and Gynecology Endocrinology of Guangzhou Medical University, Guangzhou Women and Children's Medical Center, China. The baseline clinical data, including age, weight, height, body mass index (BMI), whole body fat mass, whole body lean mass, whole body mass, android:gynoid ratio, whole body BMD, lumbar spine BMD, femur neck BMD, and total hip BMD are shown in **Table 1**. All of the participants were then started on HRT, and measurements repeated at 6 and 12 months post starting treatment. There was only one patient <15 years old who was started with continuous low-dose estradiol valerate therapy (0.5 mg daily for the first 6 months, continued with 1 mg daily for another 6 months; Progynova), the other participants who were over 15 years of age received cyclic HRT (17β-estradiol 2 mg/d for 28 days, adding dydrogesterone 10 mg/day for 14 days on day 14; Fenmatong) (16). Pubertal/sexual maturity was assessed following Marshall-Tanner criteria (17, 18). The study was performed in accordance with institutional guidelines, and written informed consent was obtained from the participants enrolled or the parents of those with a chronological age below 18 years. The study was approved by the ethics committee for human investigation at Guangzhou Women and Children's Medical Center.

BMD Evaluation

Whole body fat mass, whole body lean mass, whole body mass, android:gynoid ratio, and BMD in different sites were measured by dual energy X-ray absorptiometry (DXA) using a Lunar DXA densitometer (Lunar Corporation, Madison WI, U.S.A.). The DXA device is composed of a set of equipment including a super stable X-ray generator, a computer and data

analysis software. The X-rays are emitted by the X-ray generator, which can penetrate the body. Different tissues have different attenuation signals of X-ray due to different thickness and density. After processing by computer software, the content of different tissues is calculated. To date, determination of bone mineral density by DXA is the gold standard for the diagnosis of osteopenia/osteoporosis and sarcopenia (19, 20). In addition, DXA determination of whole body fat mass and whole body lean mass has a high sensitivity and specificity. The low radiation dose of DXA also ensures its safety in adolescents and young adults. The BMD measurements were conducted before HRT therapy, 6 months after HRT therapy and 1 year after HRT therapy.

Statistical Analysis

Basic statistics including means and standard deviation (SD) were computed by SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) for Windows software program version 19.0. Differences between baseline and that of follow-up after HRT were tested by ANOVA for Repeated Measurement Data. Results are presented as a mean±standard deviation (SD). A $P < 0.05$ was considered as the threshold for nominal significance.

RESULTS

The basic clinical data of the 20 TS patients is shown in **Table 1**. Of the participants, the mean age is 18, the mean BMI is 21.3 Kg/m^2, the mean whole body fat mass is 15.59 Kg, the mean whole body lean mass is 30.38 Kg, the mean whole body mass is 45.98 Kg, the mean whole body BMD is 0.85 g/cm^2, the mean lumbar spine BMD is 0.69 g/cm^2, the mean femur neck BMD is 0.60 g/cm^2, and the mean total hip BMD is 0.68 g/cm^2. For reference, data for normal 18 year old girls is shown in **Supplementary Table 1**. The karyotype and Tanner stage of puberty for each of the study participants is shown in **Supplementary Table 2**.

Since there were two follow-up points and some missing data in this study, we used a linear mixed effect model of ANOVA for repeated measurement data. The first step was to analyse the trends of each measurement index in the follow-up period. There was a significant difference in whole body BMD ($P < 0.0001$), whole body BMC (Bone Mineral Content, $P < 0.0001$), whole body bone area ($P = 0.003$), femur neck BMD ($P = 0.01$), total hip BMD ($P = 0.002$), total hip BMC ($P = 0.05$), whole body lean mass ($P = 0.004$), and whole body mass ($P = 0.005$) (**Table 2**). However, there was no significant difference found in lumbar spine BMD, lumbar spine BMC, lumbar spine bone area, femur neck BMC, femur neck bone area, total hip bone area, whole body fat mass, and android:gynoid ratio.

Further analysis showed that after 6 months HRT treatment, whole body BMD ($P = 0.01$), whole body lean mass ($P = 0.004$) and whole body mass ($P = 0.04$) were significantly increased, but there was no significant difference found in the BMD of other sites (**Supplementary Table 3**; **Supplementary Figures 1**, **2**). After 12 months HRT treatment, whole body BMD ($P < 0.001$), whole body BMC ($P < 0.001$), whole body bone area ($P < 0.001$), femur neck BMD ($P = 0.02$), total hip BMD ($P = 0.003$), total hip BMC ($P = 0.04$), whole body lean mass ($P = 0.002$), and whole body mass ($P = 0.007$) were significantly increased, and there was no significant difference found in lumbar spine BMD, lumbar spine BMC, lumbar spine bone area, femur neck bone area and total hip bone area (**Table 3**; **Figures 1**, **2**).

DISCUSSION

In summary, our results suggest that HRT treatment in adolescent and young adult TS patients is generally effective in improving BMD, especially for whole body BMD, hip BMD and femoral neck BMD. However, it must be noted that while there was an improvement for all the patients their BMD was still below that considered normal for females of their age (data not

TABLE 1 | Basic clinical data of adolescent and young adult TS patients.

Variables	Mean (Range)	SD
Age	18.45(16–21)	3.07
Weight (Kg)	44.64(32.5–65)	10.09
Height (cm)	144.89(131.8–163)	8.77
BMI (Kg/m^2)	21.30(16.5–21.4)	4.65
Whole Body BMD (g/cm^2)	0.85(0.77–0.96)	0.07
Lumbar Spine BMD (g/cm^2)	0.69(0.57–0.79)	0.09
Femur Neck BMD (g/cm^2)	0.60(0.44–0.82)	0.1
Total Hip BMD (g/cm^2)	0.68(0.52–0.90)	0.09
Whole Body Fat Mass (Kg)	15.59(8.26–27.16)	5.30
Whole Body Lean Mass (Kg)	30.39(23.85–39.04)	4.55
Whole Body Mass (Kg)	45.98(32.11–66.19)	8.99

BMI, body mass index; BMD, bone mineral density; SD, Standard Deviation.

TABLE 2 | Change of bone mineral status and body composition after 1 year HRT.

Variables	Mean (Range)	SD	*P*-Value
Whole Body BMD	0.86 (0.78–0.94)	0.06	<0.0001
Whole Body BMC	1313.49 (1054.15–1795.19)	181.03	<0.0001
Whole Body Bone Area	1521.58 (1335.39–1800.66)	127.16	0.003
Lumbar Spine BMD	0.73 (0.6–0.86)	0.07	0.1
Lumbar Spine BMC	33.80 (21.85–46.00)	7.38	0.6
Lumbar Spine Bone Area	46.39 (36.22-56.33)	23.63	0.9
Femur Neck BMD	0.62 (0.64–0.86)	0.11	0.01
Femur Neck BMC	2.79 (2.18–3.90)	0.47	0.05
Femur Neck Bone Area	4.53 (3.40–6.04)	0.58	0.8
Total Hip BMD	0.70 (0.55–0.93)	0.10	0.002
Total Hip BMC	19.90 (16.23–26.85)	2.70	0.05
Total Hip Bone Area	28.48 (25.14–34.14)	2.55	0.8
Whole Body Fat Mass	17521.74 (8676.63–29379.22)	5677.88	0.1
Whole Body Lean Mass	31959.37 (24855.73–40769.51)	4424.01	0.0002
Whole Body Mass	49481.11 (33532.36–69764.00)	9350.11	0.005
Height	147.46(138.5–163)	6.03	0.1

BMD, bone mineral density; BMC, bone mineral content; SD, Standard Deviation.

TABLE 3 | Bone mineral status and body composition baseline and after 12 months HRT.

	Baseline ($n = 20$)	12 months ($n = 17$)	*P*-Value
Whole Body Fat Mass (g)	15594.76 ± 5302.42	16874.49 ± 5869.95	0.04
Whole Body Lean Mass (g)	30387.51 ± 4549.32	31657.94 ± 4283.18	0.002
Whole Body Mass (g)	45982.27 ± 8991.47	48532.43 ± 9558.27	0.007
Android:Gynoid Ratio	0.92 ± 0.13	0.93 ± 0.13	0.2
Whole Body BMD (g/cm^2)	0.85 ± 0.07	0.87 ± 0.07	0.0001
Whole Body BMC (g)	1253.86 ± 192.02	1301.21 ± 156.58	0.0001
Whole Body Bone Area (cm^2)	1470.26 ± 131.35	1491.87 ± 108.24	0.0001
Lumbar Spine BMD (g/cm^2)	0.69 ± 0.09	0.73 ± 0.09	0.1
Lumbar Spine BMC (g)	30.66 ± 6.57	32.73 ± 7.09	0.2
Lumbar Spine Area (cm^2)	43.96 ± 5.42	44.42 ± 5.61	0.4
Femur Neck BMD (g/cm^2)	0.6 ± 0.1	0.62 ± 0.11	0.02
Femur Neck BMC (g)	2.62 ± 0.4	2.7 ± 0.39	0.03
Femur Neck Bone Area (cm^2)	4.4 ± 0.63	4.37 ± 0.51	0.5
Total Hip BMD (g/cm^2)	0.68 ± 0.09	0.71 ± 0.1	0.003
Total Hip BMC (g)	18.77 ± 2.49	19.32 ± 2.55	0.04
Total Hip Area (cm^2)	27.71 ± 2.99	27.35 ± 2.78	0.7

BMD, bone mineral density; BMC, bone mineral content.

shown). In contrast to a previous study (9), we did not observe an improvement in lumbar spine BMD which may in part be due to the small sample size of the current study. At the same time, whole body lean mass and whole body mass were significantly enhanced, while the whole body fat mass and android:gynoid ratio did not change.

For TS patients, low BMD and increased risk of bone fractures are two major bone health concerns. Across all age ranges, TS patients have lower BMD compared to age matched normal control girls and women (4, 21, 22). Low BMD during climacterium and senectitude is an important risk factor for bone fractures, therefore it is essential to improve their BMD not only during climacterium and senectitude but also in adolescent and young ages.

In general, HRT was thought to be an important option for improving BMD in TS osteopenia or osteoporotic women because estrogen-deficiency and elevated follicle stimulating hormone (FSH) levels are two of the major reasons for bone loss in TS individuals (4, 23). In those estrogen-deficient and elevated FSH patients, the increasing level of serum estrogen not only increases the number of active osteoblasts but also decreases the high bone turnover rate (24). One previous study demonstrated that low BMD in TS patients was related to high osteoclastogenesis, which in turn was caused by high levels of FSH (23). HRT is an effective way to reduce FSH levels, which may help to improve BMD in TS patients. However, several studies have reported that the BMD of TS patients after HRT are still very low (7, 8).

Some differences in these different studies may be explained by the use of different HRT protocols in different centers. Exogenous E2 can be administered various different routes including transdermally (TD), orally, transvaginally, or by injection. TD administration by-passes the liver, there are no efficacy studies on age at onset and duration of treatment, furthermore, TD is not available in our hospital. Oral estrogens include 17β-estradiol, estradiol valerate and conjugated estrogens (CEE). Previous studies have found no significant differences in fasting insulin concentration, protein turnover, lipolysis, BMI or waist-to-hip ratio between groups with TD vs. oral natural estrogen treatment (25–27). CEE's raise blood pressure and are therefore not recommended. All but three participants were not sexually active, so transvaginal administration was not recommended for TS girls in our study. Administration by injection is not preferred by most patients. Therefore, oral administration was used for the current study. It is recommended that in girls low dose estrogen be given to induce puberty (2–3 years), at which point estrogen should be increased to 2 mg/d and progesterone added. However, GWCMC only has 2 mg 17β-estradiol and because the patients were older than 15 years they were automatically started on cyclic HRT, all the patients subsequently started breakthrough bleeding after treatment was started.

In the present study, we found that whole body BMD, femur neck BMD and total hip BMD were significantly increased after HRT was given among adolescent and young adult TS patients, but there was no difference in lumbar spine BMD. Our results indicated that the mechanism of action of HRT in improving BMD varies depending on the skeletal site being assessed. For whole body BMD, femur neck BMD and total hip BMD, chronic estrogen deficiency may be the major reason for bone loss in these sites. But for lumbar spine BMD, X-chromosome abnormalities, or other genetic factors may be the major reason for bone loss in adolescent and young adult TS patients (14). Our findings also demonstrated that whole body BMD was the earliest site among the enhanced BMD sites, which was increased after 6 months HRT. Femur neck BMD and total hip BMD were significantly increased after 1 year HRT, but total hip bone area did not alter. This finding implies that HRT may increase bone mineral content but not its size. Whole body BMD increased after 6 months HRT and whole body bone area increased after 1 year HRT, which suggests that the increase in bone mineral content precedes an increase in bone size after HRT among adolescent and young adult Turner syndrome patients.

Some studies have also reported an effect of HRT on body composition including fat mass and lean mass in TS patients, although this finding has not been consistent (10–12). The loss of endogenous estrogen may lead to fat mass gain so that TS women were found to have higher fat mass and higher BMI than age matched control women (28). The current study reports that HRT increased whole body lean mass and whole body mass, while the whole body fat mass and android:gynoid ratio were not altered. This finding suggests that HRT has a positive effect on body composition, enhanced lean mass improves muscle

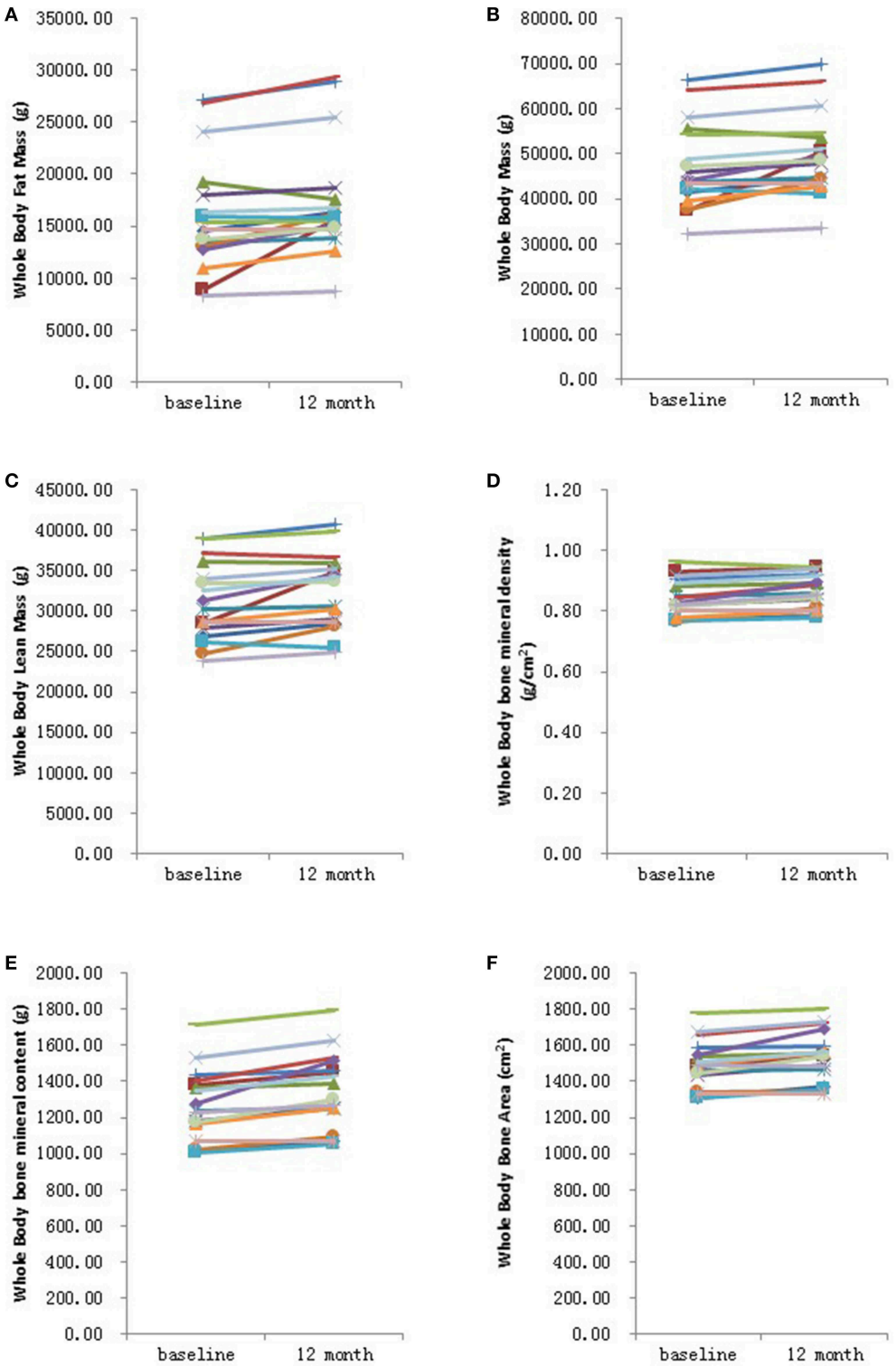

FIGURE 1 | Graphical representation in changes in whole body measurements before and after 12 months HRT. **(A)** Whole body fat mass; **(B)** Whole body mass; **(C)** Whole body lean mass; **(D)** Whole body bone mineral density; **(E)** Whole body bone mineral content; **(F)** Whole bone area. $N = 17$.

strength and can prevent bone fractures (4). In addition, keeping a fit body shape and low fat mass can also help prevent other serious chronic metabolic health problems such as obesity, type 2 diabetes, and cardiovascular disease for TS patients. Therefore, the positive effect of HRT on body composition in TS women may also lead to lifelong health benefits.

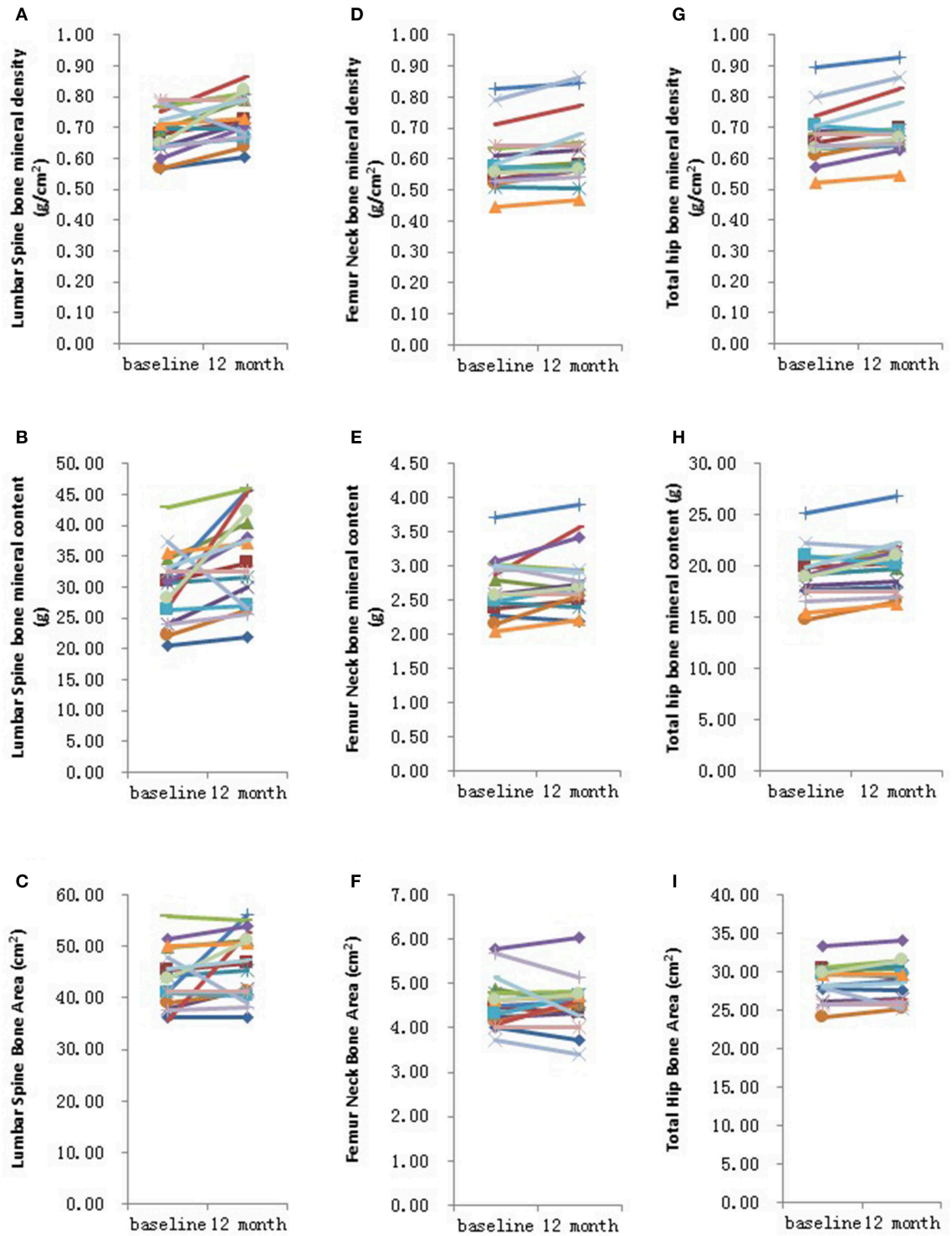

FIGURE 2 | Graphical representation in changes in individual site measurements before and after 12 months HRT. **(A)** Lumbar spine bone mineral density; **(B)** Lumbar spine bone mineral content; **(C)** Lumbar spine bone area; **(D)** Femur neck bone mineral density; **(E)** Femur neck bone mineral content; **(F)** Femur neck bone area; **(G)** Total hip bone mineral density; **(H)** Total hip bone mineral content; **(I)** Total hip bone area. $N = 17$.

The current study demonstrates that HRT was an effective way to increase whole body BMD, femur neck BMD, total hip BMD, but not lumbar spine BMD in Chinese TS girls, which indicated that for whole body BMD, femur neck BMD and total hip BMD, chronic estrogen deficiency may be the major reason for bone loss in these sites, but for lumbar spine BMD,

X-chromosome abnormalities or other genetic factors may be the major reason for bone loss in Chinese adolescent and young adult TS patients. In addition, HRT was an effective way to enhance whole body lean mass and whole body mass in TS girls, and it has no effect on whole body fat mass and android:gynoid ratio. However, it must be noted that due to the rarity of this condition the sample size in the current study was small and larger studies are required. Our present study is a 1-year longitudinal study, which only shows the effect of HRT on BMD and body composition at this time point. Further long-term follow-up studies are needed to evaluate the long-term efficacy of HRT and determine the peak BMD after HRT for TS patients during their life time. In conclusion, our findings suggest that HRT has a significant positive impact on increasing BMD, improving muscle strength, preventing bone fracture and keeping a fitness body shape, which will give TS women lifelong health benefits.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

The study was performed in accordance with institutional guidelines, and written informed consent was obtained from the participants enrolled or the parents of those with a chronological age below 18 years. The study was approved by the ethics committee for human investigation at Guangzhou Women and Children's Medical Center.

AUTHOR CONTRIBUTIONS

LLi and XQ designed and prepared the draft of manuscript. LLi and LLiu conceived the idea, supervised all research. GL revised the manuscript. LY analyzed the data. ZL participated in the experiments. All authors reviewed the manuscript.

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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.2019.00377/full#supplementary-material

Supplementary Table 1 | Basic clinical data of normal 18-year-old girls.

Supplementary Table 2 | Change of bone mineral status and body composition after 6 months HRT.

Supplementary Table 3 | Summary of genotype and change of tanner stage after 1year HRT.

Supplementary Figure 1 | Graphical representation in changes in whole body measurements before and after 6 months HRT. **(A)** Whole body fat mass; **(B)** Whole body mass; **(C)** Whole body lean mass; **(D)** Whole body bone mineral density; **(E)** Whole body bone mineral content; **(F)** Whole bone area. $N = 10$.

Supplementary Figure 2 | Graphical representation in changes in individual site measurements before and after 6 months HRT. **(A)** Lumbar spine bone mineral density; **(B)** Lumbar spine bone mineral content; **(C)** Lumbar spine bone area; **(D)** Femur neck bone mineral density; **(E)** Femur neck bone mineral content; **(F)** Femur neck bone area; **(G)** Total hip bone mineral density; **(H)** Total hip bone mineral content; **(I)** Total hip bone area. $N = 10$.

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The Usefulness of Magnetic Resonance Imaging of the Cardiovascular System in the Diagnostic Work-Up of Patients With Turner Syndrome

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Cardiovascular defects occur in 50% of patients with Turner syndrome (TS). The aim of the study was to estimate the usefulness of cardiac magnetic resonance imaging (CMR) and magnetic resonance angiography (angio-MR) as diagnostics in children and adolescents with TS. Forty-one females with TS, aged 13.9 ± 2.2 years, were studied. CMR was performed in 39 patients and angio-MR in 36. Echocardiography was performed in all patients. The most frequent anomalies diagnosed on CMR and angio-MR were as follows: elongation of the ascending aorta (AA) and aortic arch, present in 16 patients (45.7%), a bicuspid aortic valve (BAV), present in 16 patients (41.0%), and partial anomalous pulmonary venous return (PAPVR), present in six patients (17.1%). Aortic dilatation (Z-score > 2) was mostly seen at the sinotubular junction (STJ) (15 patients; 42.8%), the AA (15 patients; 42.8%), the thoracoabdominal aorta at the level of a diaphragm (15 patients; 42.8%), and the transverse segment (14 patients; 40.0%). An aortic size index (ASI) above 2.0 cm/m^2 was present in six patients (17.1%) and above 2.5 cm/m^2 in three patients (8.6%). The left ventricular end-diastolic volume (EDV), end-systolic volume (ESV), and stroke volume (SV) were diminished (Z-score< -2) in 10 (25.6%), 9 (23.1%), and 8 patients (20.5%), respectively. A webbed neck was correlated with the presence of vascular anomalies ($p = 0.006$). The age and body mass index (BMI) were correlated with the diameter of the aorta. Patients with BAV had a greater aortic diameter at the ascending aorta (AA) segment ($p = 0.026$) than other patients. ASI was correlated with aortic diameter and descending aortic diameter (AD/DD) ratio ($p = 0.002$; $r = 0.49$). There was a significant correlation between the right ventricular ($p = 0.002$, $r = 0.46$) and aortic diameters at the STJ segment ($p = 0.0047$, $r = 0.48$), as measured by echocardiography and CMR. Magnetic resonance can identify cardiovascular anomalies,

dilatation of the aorta, pericardial fluid, and functional impairment of the ventricles not detected by echocardiography. BMI, age, BAV, and elongation of the AA influence aortic dilatation. The ASI and AD/DD ratio are important markers of aortic dilatation. The performed diagnostics did not indicate a negative influence of GH treatment on the cardiovascular system.

Keywords: Turner syndrome, girls, MRI, cardiovascular anomalies, magnetic resonance angiography, cardiac magnetic resonance imaging

INTRODUCTION

Congenital and acquired cardiovascular disease occurs in almost 50% of patients with Turner syndrome (TS) (1) and are the major cause of death in TS, mostly due to dissection of the aorta (2). The incidence of aortic dissection is increased 100 times in TS and is responsible for 2–8% of premature deaths (2–5). Therefore, the evaluation of the cardiovascular system is an important element of the diagnosis in TS. It is essential in terms of the safety aspects of recombinant growth hormone (rGH) treatment and pregnancy planning.

The proven risk factors for aortic dissection are as follows: aortic dilation, bicuspid aortic valve (BAV), aortic coarctation, karyotype 45X, arterial hypertension, and pregnancy (5–9).

Currently, echocardiography is the most popular screening examination for cardiac anomalies in patients with TS. However, it can miss some cardiovascular anomalies (10, 11). Assessment of the aortic arch and descending aorta on echocardiography can be limited by abnormalities of the chest wall and a poor acoustic window (5, 6, 12). Assessment of the aorta is essential due to arteriopathies of aortic arch and descending aorta (7, 13) because ∼20% of aortic dissections occur in the descending aorta (5, 6). The aortic diameter is a risk factor for aortic dissection and can be monitored by cardiac magnetic resonance imaging (CMR).

Cardiovascular magnetic resonance imaging allows a noninvasive assessment of whole aorta without ionizing radiation, enabling recognition of clinically, and sonographically silent anomalies (7, 11). Magnetic resonance, in particular, offers information that is difficult to obtain from other imaging modalities such as complex congenital cardiovascular anomalies and quantitate aspects of regional ventricular function. The guidelines for the care of girls and women with TS recommend that CMR should be used as a screening tool in all children with TS at an age when it can be performed without sedation, even if echocardiography did not reveal any abnormalities (14, 15).

The aortic diameter is determined by age and body size (16), so aortic dimensions must be adjusted for body surface area (BSA). The aortic size index (ASI) calculated as the ratio between the ascending aortic diameter and the BSA is currently commonly used in clinical practice (2, 17). TS patients with an ASI >2 cm/m^2 are at high risk for aortic dissection, and those with an ASI >2.5 cm/m^2 are at a very high risk (2, 17).

There is no consensus on whether dilatation of the aorta may occur during early childhood and which CMR parameters can predict dissection of aorta. There is still discussion about the role of echocardiography in the CMR era for the diagnosis of cardiovascular anomalies in TS.

The aim of the present study is to evaluate the usefulness of CMR and 3D dynamic magnetic resonance angiography (angio-MR) in the diagnosis of anomalies of the aorta and other vessels and to establish risk factors for aortic dilatation in TS patients. The other aims are to compare the usefulness of CMR and echocardiography in TS and to estimate the risk factors of aortic dissection, the correlations of aortic diameter with several clinical factors (age, BMI, karyotype) and CMR parameters.

PATIENTS AND METHODS

Forty-one patients with recognized TS, aged 13.9 ± 2.2 years, were studied. The exclusion criteria were as follows: lack of informed consent, contraindications for magnetic resonance studies, or a lack of cooperation during the CMR study. An ethical review process was not required for this study because it utilizes the standard diagnostic tests for TS. Before CMR and angio-MR, each patient signed the informed consent. Each patient had echocardiography performed before CMR and angio-MR performed ∼6 months after CMR. CMR was performed in 39 patients (95.1%), and angio-MR was performed in 36 (87.8%) patients. One patient did not have angio-MR with contrast, and aortic measurements were not possible. In 34 (82.9%) TS patients, both CMR and angio-MR were performed.

Height and BMI centiles were calculated with the OLAF calculator using normal Polish ranges (18). The height standard deviation score (HtSDS) was calculated using the same ranges. Overweight and obesity were diagnosed according to the International Obesity Task Force Criteria (19). Arterial hypertension was diagnosed when systolic and diastolic blood pressure exceeded or were equal to the 95th centile for age and/or height for the Polish population (18).

Karyotype was established due to conventional cytogenetics analysis by peripheral lymphocytes (20–100 metaphase plates) evaluation.

Twenty patients (48.8%) had a webbed neck, which is defined as redundant cervical skin folds arcing out from mastoid at the level of ear lobe to the acromion. Hypothyroidism was diagnosed in 20 patients (48.8%) and treated with L-thyroxine. In 18 patients, autoimmunologic inflammation of the thyroid gland was detected. Growth hormone therapy was induced in 36 patients (87.8%). The average dose was 0.025–0.055 mg per kg body weight per day. Estrogen replacement therapy was administered to 25 patients (61.0%). Clinical data of the studied population are presented in **Table 1**.

Transthoracic 2D and Doppler echocardiography were performed using a Vivid E9 (GE, Little Chalfont). The standard views and measurements were obtained according to the ESC guidelines. Aortic diameters were measured using an edge-to-edge technique at the aortic ring, sinotubular junction (STJ) and sinus of Valsalva.

CMR and 3D dynamic MR angiography were performed with a 1.5-T scanner (Siemens, Avanto) with the use of a matrix coil for body and cardiac applications combined with a spinal coil. All sequences were performed with ECG triggering during breath-hold. Cardiac MRI included the following sequences: anatomical imaging, ventricular volume and functional assessment and phase-contrast flow quantification. Anatomical imaging was obtained with an echo-planar fast-spin echo sequence (HASTE—Half-Fourier Acquisition Single-shot Turbo spin Echo) in three orthogonal planes (axial, coronal, and transverse). The imaging parameters were as follows: TR/TE 2 R-R intervals/27 ms, field of view 380 × 260 mm, slice thickness 8 mm, gap 2 mm, and matrix size 104 × 256. Cine MRI was performed with a steady-state free precession (SSFP) sequence in two-, three-, and four-chamber views. A short-axis stack was obtained from the cardiac base to apex. The typical parameters were TR/TE 55.88/1.07 ms, field of view 380 × 310 mm, slice thickness, 8 mm, gap 2 mm, matrix size 109 × 192, and in-plane resolution 2.8 × 2.0 mm. The volumetric method was used to evaluate the left ventricular end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), ejection fraction (EF), and mass. The diameters of the right ventricle and the left ventricle (LV) in diastole, the diameter of the left atrium in systole, and the thickness of the interventricular septum and inferior wall were also measured.

Flow imaging was performed with a free breathing ECG-gated flow-sensitive sequence. A through-plane phase-contrast gradient-echo sequence was performed at the level of the AA above the aortic and pulmonary valves, and the imaging parameters were as follows: velocity encoding 150 m/s for the aorta and 120 m/s for the pulmonary artery, TR/TE 29.90/2.18 ms, field of view 380 × 285 mm, slice thickness 5 mm, gap 1 mm, matrix size 192 × 256, and in-plane resolution 1.5 × 1.5 mm. Phase-contrast flow quantification was used to assess the ratio of pulmonary flow (Qp) to systemic flow (Qs) ratio and aortic and pulmonary regurgitation.

TABLE 1 | Clinical characteristics of the study group.

	***n* = 41**
Age at CMR (years)	13.9 ± 2.2
Age at angio-MR (years)	14.6 ± 2.2
Height SDS	−2.16 ± 1.1
BMI (centile)	69.7 ± 25.2
Previous cardiac surgery (*n* %)	6, 14.6%
Karyotype 45,X (*n* %)	18, 44%
Webbed neck (*n* %)	20, 48.8%
Arterial hypertension (*n* %)	3, 7.32%
Hypothyroidism (*n* %)	20, 48.8%
Growth hormone therapy (*n* %)	36, 87.8%
Estrogen replacement therapy (*n* %)	25, 61%

Angio-MR was performed with the use of the dynamic *Time-resolved Angiography With Interleaved Stochastic Trajectories* (TWIST) after the administration of a contrast agent (0.1 mmol/kg) followed immediately by a 20 ml saline flush. The temporal resolution varied between 3 and 5 s, with an overall sequence time of ∼100 s. The time of contrast injection was calculated following the administration of 1 ml of contrast bolus. The typical sequence parameters were: TR/TE 2.3/0.87 ms, field of view, 500 × 310 mm, slice thickness 1.5 mm, gap 0 mm, matrix size 384 × 224, and in-plane resolution 1.40 × 1.30 mm. The TWIST sequence was used for the evaluation of vascular anomalies and the following measurements. The aortic diameter was measured at nine levels including the aortic sinus (AS), STJ, AA, at the origin of the brachiocephalic artery (BCA), first transverse segment (T1), second transverse segment (T2), isthmic region (IR), descending aorta (DA), and thoracoabdominal aorta at the level of diaphragm (D) (**Figures 1**, **2A–C**). Distances between the first transverse segment (T1) and the sternoclavicular joint, the length of the aortic arch between the BCA and the left subclavian artery, and elongation of the AA measured between the aortic ring and the BCA were also estimated (**Figures 3A–E**). All measurements were obtained with dedicated the software Medis Suite MR 3.0.

Measurements of aortic diameters and ventricular volumes were standardized by body surface area. The results for the aorta were compared to ranges developed by Kaiser et al. (20). A

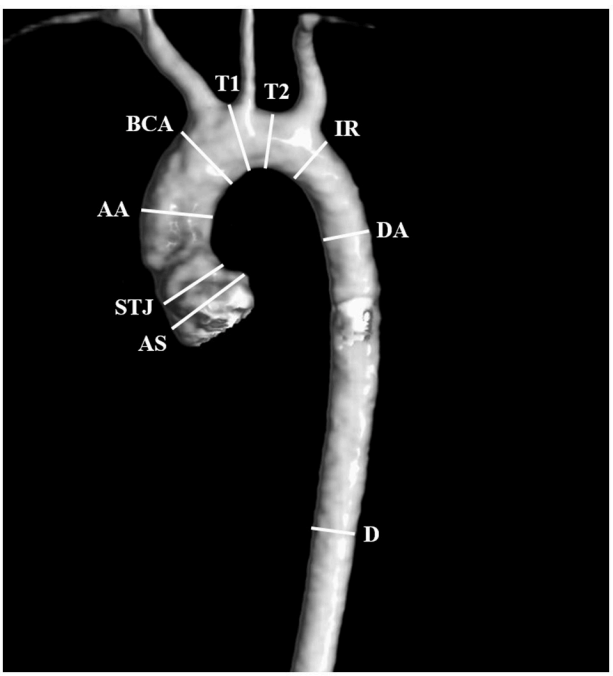

FIGURE 1 | Locations of aortic diameter measurements: aortic sinus (AS), sinotubular junction (STJ), ascending aorta (AA), at the origin of the brachiocephalic artery (BCA), first transverse segment (T1), second transverse segment (T2), isthmic region (IR), descending aorta (DA), and the thoracoabdominal aorta at the level of the diaphragm (D).

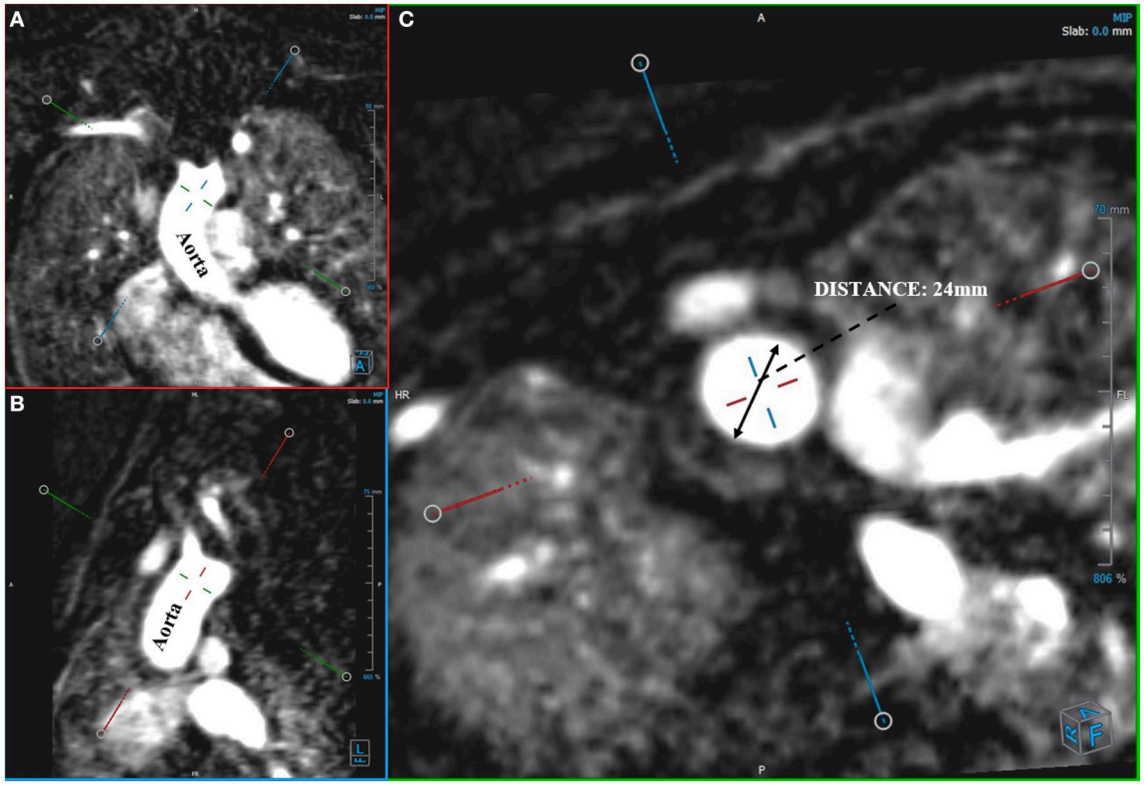

FIGURE 2 | Measurement of aortic diameter. Angio-MR **(A,B)**, -perpendicular planes **(C)**, -diameter of the ascending aorta in a transverse plane.

standardized Z-score for aortic diameter at each segment was calculated with an electronic calculator developed by Kaiser et al. (20), in which the diameter of each segment of the aorta and BSA were used. Ventricular volumes and myocardial mass were compared to ranges estimated by Buechel et al. (21). The ASI, which is defined as the ratio between ascending aortic diameter and the BSA, was calculated. The ratio between the ascending aortic diameter and the descending aortic diameter (AD/DD ratio) was also evaluated.

The calculations were performed using the Statistica 12 program from StatSoft and StaXact from Cytel. An $\alpha = 0.05$ was assumed as the significance level. The results were considered statistically significant when $p < \alpha$. Continuous variables are shown as the mean $\pm$ standard deviation, the minimum and maximum values and the median. Numbers and percentages are given for categorical variables. The normality of the distribution of variables was evaluated using the Shapiro-Wilk test. To compare the variables, Student's *t*-test for unrelated samples was used, if the distribution of the variable was consistent with the normal distribution and the variances were equal; the Mann-Whitney test was used when the variable were not normally distributed. To investigate the relationship between continuous variables, in cases in which both of variables were normally distribution, the Pearson *r* correlation coefficient was calculated, while the Spearman *r* rank correlation coefficient was calculated when the variables were not normally distributed. To test the relationship between categorical variables, the chi-square test, Fisher's exact test or the Fisher-Freeman-Halt test were used. In the cases of dependency, the odds ratio was calculated along with 95% confidence intervals. To estimate whether there was a statistically significant difference between the measurements obtained using two techniques, Student's *t*-test for related samples or the Wilcoxon test were used.

RESULTS

In 18/41 (44.0%) patients the 45,X karyotype was detected, and the rest had different mosaic karyotypes. Thirteen patients (13/41; 31.7%) were overweight, and 1 patient (1/41; 2.4%) was obese. Six patients underwent cardiac surgery; five of these patients (5/41; 12.2%) had coarctation of the aorta, and one (1/41; 2.4%) had a patent ductus arteriosus (PDA). Arterial hypertension was diagnosed in three patients (3/41; 7.3%). Details of the clinical characteristics of the patients and results of ECHO, CMR, and angio-MR are presented in **Table S1**.

The most frequent anomalies diagnosed on CMR and angio-MR were as follows: elongation of the AA and aortic arch in 16/35 (45.7%) patients, BAV in 16/39 patients (41.0%), partial anomalous pulmonary venous return (PAPVR) in 6/35 patients (17.1%), persistent left superior vena cava (PLSVC) in 4/35 patients (11.4%; **Figure 4**) and bovine arch in 3/35 patients (8.6%; **Figure 5**; **Table 2**). Most of

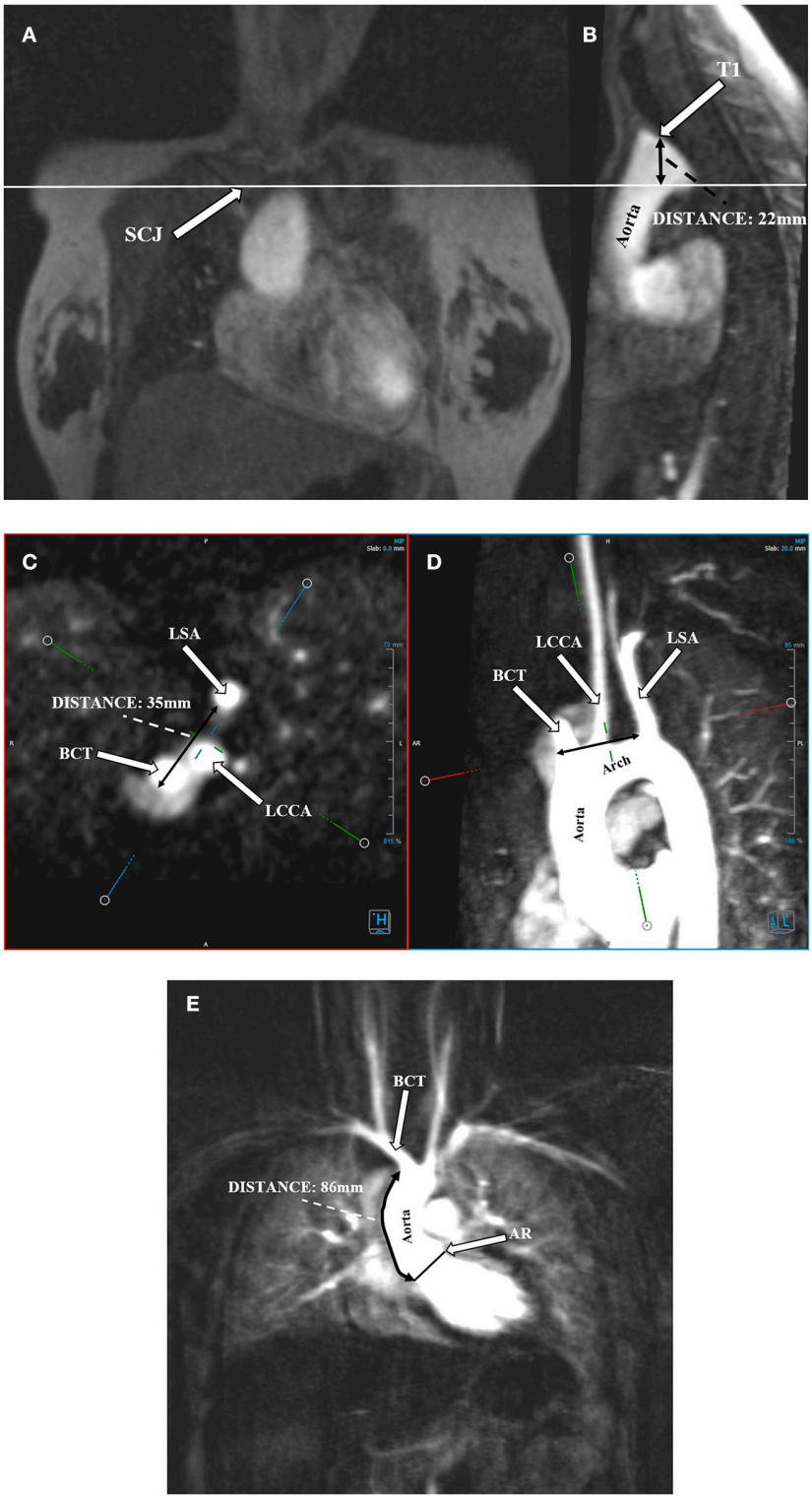

FIGURE 3 | Protrusion of the aortic arch above the sternoclavicular joint. Distance between the highest point of the first transverse segment (T1) and the sternoclavicular joint (SCJ). **(A)** Angio-MR, coronal plane. SCJ (arrow); the perpendicular line shows the level of the SCJ. **(B)** Angio-MR, sagittal plane, showing the distance between the highest point of T1 and SCJ. Measurement the length of the aortic arch between the brachiocephalic trunk (BCT) and the left subclavian artery (LSA). **(C)** Measurements of the BCT, LCCA (left common carotid artery), and LSA were made on Angio-MR in a transverse plane. **(D)** Angio-MR of the aorta showing the spatial orientation of the arch and its branches (BCT, LCCA, LSA) and the measurement locations along the length of the aortic arch (black arrow). **(E)** Elongation of the ascending aorta. Distance between the aortic ring (AR) and the brachiocephalic trunk (BCT). Angio-MR, coronal plane.

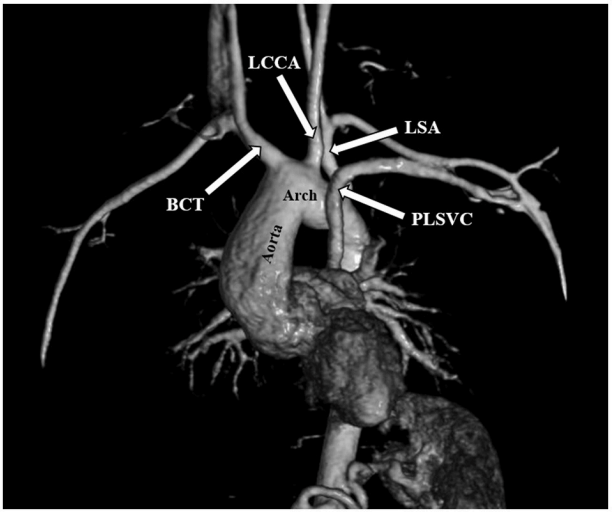

FIGURE 4 | Persistent left superior vena cava (PLSVC). 3D MRA. BCT, brachiocephalic trunk; LCCA, left common carotid artery; LSA, left subclavian artery.

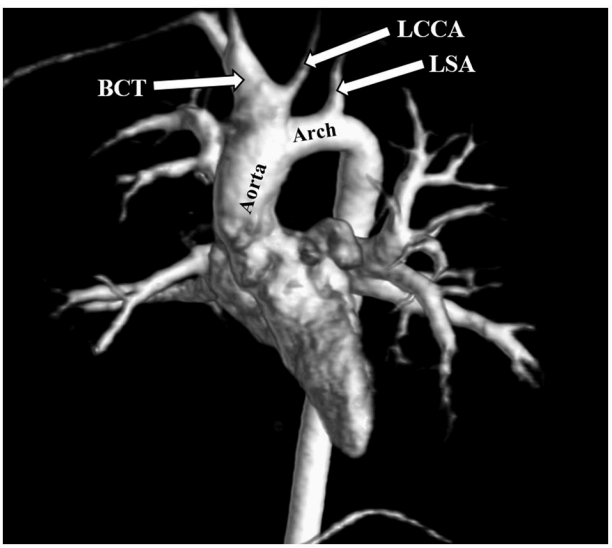

FIGURE 5 | Bovine arch. Left common carotid artery (LCCA) arising from the brachiocephalic trunk (BCT). 3D MRA. LSA, left subclavian artery.

TABLE 2 | Cardiovascular anomalies in patients with Turner syndrome on angio-MR vs. ECHO.

Anomaly	Angio-MR *n*, %	ECHO *n*, %
Elongation of the ascending aorta and aortic arch	16 (45.7%)	Not detected
Bicuspid aortic valve (BAV)	16 (41.0%)	14 (36.1%)
Partial anomalous pulmonary venous return (PAPVR)	6 (17.1%)	Not detected
Persistent left superior vena cava (PLSVC)	4 (11.4%)	Not detected
Common origin of the left common carotid artery (LCCA) and brachiocephalic trunk (BCT) - bovine arch	3 (8.6%)	Not detected
Aberrant right subclavian artery	2 (5.7%)	Not detected
Right sided aortic arch	1 (2.8%)	Not detected
Anomalous left vertebral artery origin	1 (2.8%)	Not detected
Atrial septal defect (ASD)	1 (2.6%)	Not detected

TABLE 3 | Aortic diameters and the number of patients with dilated aortic segment.

Segment of measurement	Diameter (mean, range) (mm)	Aortic diameter index (mean) (mm/m^2)	Number of patients with *Z*-score > 2 (*n*,%)
Aortic sinus (AS)	27.2 (19.0–44.0)	20.0	12 (34.3)
Sinotubular junction (STJ)	23.5 (15.0–37.0)	17.37	15 (42.9)
Ascending aorta (AA)	24.3 (17–41.0)	17.98	15 (42.9)
Brachiocephalic artery (BCA)	22.3 (16.0–34.0)	16.42	8 (22.9)
First transverse segment (T1)	21.0 (14.0–35.0)	15.40	14 (40.0)
Second transverse segment (T2)	18.8 (13.0–31.0)	13.81	11 (31.0)
Isthmic region (IR)	17.4 (12.0–22.0)	12.81	9 (25.7)
Descending aorta (DA)	17.5 (13.0–29.0)	12.95	7 (20.0)
Thoracoabdominal aorta at the level of the diaphragm (DD)	15.7 (12.0–23.0)	11.63	15 (42.9)

these congenital vascular anomalies, except BAV, were missed by echocardiography. Only 3/39 BAV cases (7.7%) were underdiagnosed by echocardiography. In one patient (1/39; 2.6%), BAV was diagnosed on echocardiography but was not confirmed on CMR. In 7/39 patients (17.9%) pericardial fluid seen on CMR was not detected by echocardiography.

The mean aortic diameters and number of patients with enlarged aortic segments (*Z*-score >2) are presented in **Table 3**. Aortic dilatation was most frequently seen at the STJ (15/35 patients, 42.9%), AA (15/35 patients, 42.9%), thoracoabdominal aorta at the level of the diaphragm (15/35 patients, 42.9%), and in the first transverse segment (14/35 patients, 40.0%). The mean ratio between the ascending aortic diameter and the descending aortic diameter (AD/DD ratio) was 1.55 ± 0.21 (range 1.09–2.00). Fifteen patients (15/35; 42.9%) had an AD/DD ratio > 1.5. ASI > 2 cm/m^2 was present in six patients (6/35; 17.1%), and an ASI > 2.5 cm/m^2 was present in three patients (3/35; 8.6%; with a maximum of 2.79 cm/m^2).

The mean EDV of the left ventricle was 94.29 ml, and the mean EDV index was 68.94 ml/m^2. Ten patients (10/39; 25.6%) had an EDV *Z*-score < −2, and two patients (2/39; 5.1%) had an EDV *Z*-score > 2. The mean ESV of the left ventricle was 37.13 ml, and the mean ESV index was 27.14 ml/m^2. Nine patients (9/39;

23.1%) had an ESV Z-score < -2, and two patients (2/39; 5.1%) had an ESV Z-score > 2. The mean SV was 57.15 ml, and the mean SV index was 41.8 ml/m^2. Eight patients (8/39; 20.5%) had an SV Z-score < -2.

Karyotype 45,X was not associated with an increased prevalence of cardiovascular abnormalities. There was no significant difference in the aortic diameters or Z-scores of aortic diameters between patients with or without karyotype 45,X. However, patients with karyotype 45,X more often had an aortic diameter at the T1 segment with a Z-score > 2 compared with patients without karyotype 45,X ($p = 0.023$).

The prevalence of cardiovascular anomalies was higher in patients with a webbed neck, especially BAV ($p = 0.04$), PAPVR ($p = 0.008$), and PLSVC ($p = 0.047$). Age was correlated with the diameter of the aorta at the following segments: BCA ($r = 0.43$, $p = 0.009$), T1 ($r = 0.57$, $p < 0.001$), T2 ($r = 0.61$, $p < 0.001$), IR ($r = 0.69$, $p < 0.001$), and DA ($r = 0.35$, $p = 0.039$). BMI was correlated with the diameter of the aorta at BCA ($r = 0.39$, $p = 0.018$), T2 ($r = 0.36$, $p = 0.032$), and DD ($r = 0.36$, $p = 0.03$).

The length of the AA was correlated with the diameter of the aorta at the following segments: AS ($r = 0.64$, $p < 0.001$), STJ ($r = 0.51$, $p = 0.001$), AA ($r = 0.44$, $p = 0.008$), T1 ($r = 0.41$, $p = 0.014$); it was also correlated with the length of the aortic arch ($r = 0.59$, $p < 0.001$). A total of five patients with corrected coarctation of the aorta had a statistically greater aortic diameter at the STJ ($p = 0.01$) and smaller diameter at the IR ($p = 0.006$). Patients with BAV had significantly bigger aortic diameter at AA ($p = 0.026$). We also found a correlation between the ASI and AD/DD ratio ($r = 0.49$, $p = 0.002$). Patients with an ASI >2 cm/m^2 more often had an AD/DD ratio >1.5 compared with those with an ASI <2 cm/m^2 ($p = 0.015$) (**Figure 6**). Patients with BCA and a DD Z-score >2 had a significantly longer aortic arch than that of other patients ($p = 0.023$ and $p = 0.047$, respectively).

There was agreement of most of the measurements made on echocardiography and CMR, especially the right ventricular diameter ($r = 0.46$, $p = 0.002$) and the aortic diameter at the STJ segment ($r = 0.48$, $p = 0.005$).

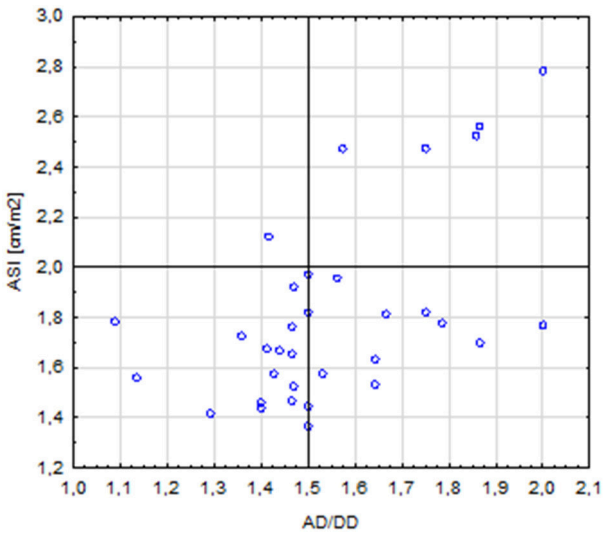

FIGURE 6 | Correlation between the ASI (aortic size index) >2 and AD/DD (aortic ascending/descending diameter) >1.5 ($p = 0.015$).

DISCUSSION

The relatively young age of the studied group is exceptional. Most of the recently published studies have evaluated young adults with TS (7, 12, 22, 23).

We report a high prevalence of vascular anomalies diagnosed on angio-MR and CMR. The pathophysiology of cardiovascular abnormalities in TS is still under debate. There is a hypothesis that jugular lymphatic sac obstruction in 45,X fetuses can lead to distended thoracic ducts, compression of the AA, and reduced intracardiac blood flow (24). Reduced blood flow leads to impaired development of aortic valve and arch. Thus, patients with webbed necks are suspected to have cardiovascular defects. The X-chromosome genes that are responsible for congenital heart defects in TS have not been identified yet. Prakash et al. analyzed 454 TS subjects and found that left-sided congenital heart lesions were associated with a reduced dosage of Xp genes and increased dosage of Xq genes (25). They also showed that genome-wide copy number variation is increased in TS, and they identified a common copy number variant (CNV) in chromosome 12p13.31 that is associated with left-sided congenital heart lesions. The CNV contained three protein-coding genes (*SLC2A3*, *SLC2A14*, and *NANOGP1*) and was previously implicated in congenital heart defects in 22q11 deletion syndrome. Additionally, they identified a subset of rare and recurrent CNVs that are also enriched in asymptomatic BAV cases. There is also a hypothesis that haploinsufficiency of the *FOXC2* gene, which codes a forkhead winged-helix transcription factor, is responsible for cardiovascular defects (2). Several syndromes including congenital heart defects are attributed to X-linked genes. Mutations of the following genes can lead to cardiovascular disease: *Filamin A, EMD* (emerin), *LAMP2* (lysosomal-associated membrane protein 2), *DMD* (dystrophin), *TAZ* (tafazzin), and *VEGF-D* (vascular endothelial growth factor D) (26). Although TS patients have no signs of connective tissue defects, there is a theory that arterial TGF-beta signaling in TS can be disrupted and lead to aneurysm formation and a risk of vascular dissection (27).

In the studied group, karyotype 45,X was not associated with an increased prevalence of cardiovascular abnormalities, and there was no significant difference in the aortic diameters between patients with or without karyotype 45,X. Patients with karyotype 45,X had greater aortic diameter at the T1 segment than patients without karyotype 45,X. It is well known that patients with TS and pure karyotype 45,X are more affected by developmental anomalies including fetal lymphedema, BAV, and aortic coarctation (2). The relationship between karyotype 45,X, BAV, and aortic dilatation were confirmed in a French cohort study (28). However, Cleeman et al. did not find a direct association between karyotype 45,X and the diameter of the aorta (29). The potential reason for cardiovascular anomalies in

karyotype 45,X patients is haploinsufficiency for Xp genes (30). It seems that the correlation between cardiac and aortic anomalies and karyotype should be more profound in patients lacking one Xp arm and not those with karyotype 45,X.

A webbed neck, which is a sign of fetal lymphedema, is strongly associated with cardiovascular defects (1, 31) and it is also confirmed by our study. The age of TS patients is also positively correlated with the diameter of the aorta as documented by others (22, 29, 32). Dissection and rupture of the aorta were not seen in our population due to their young age, while in the Danish and Swedish studies, the median age of onset of aortic dissection or rupture in TS patients was found to be 35 years (5). Our study also confirmed that BMI is associated with the dilatation of the aorta. Obesity is a known risk factor for increased aorta diameter, which is associated with arterial stiffness and a greater carotid intima-media thickness (33). Since patients and women with TS have an increased risk of metabolic disturbances and overweight, they should be carefully monitored.

In our study, the frequencies of cardiovascular anomalies such as aortic coarctation (12.2%), elongation of the AA (45.7%), PAPVR (17.1%), PLSVC (11.4%), bovine arch (8.6%), aberrant right subclavian artery (5.7%), and anomalous left vertebral artery origin (2.8%) were similar to those reported in other studies (1, 23). The prevalence of BAV in our group (41%) was relatively higher than that in previous published studies (25–30%) (7, 10, 13). Only Kim et al. (23) reported a similar BAV prevalence (39%). We observed a high percentage (42.9%) of patients with an increased aortic diameter. Aortic dilatation is a known serious risk factor for aortic dissection (8); thus, the diagnosis of aortic dilatation is essential in clinical practice. Aortic dilatation has been reported in 32–42% of patients and women with TS (8, 11, 34, 35). Kim et al. reported aortic dilation at the AS segment in 30% of patients and at the STJ in 26% (23). However, in the Danish study, the diameters of aorta in TS patients were significantly smaller at the aortic arch and descending aorta than those of control subjects (29). The standardization of aortic diameter measurements in TS is crucial because TS patients are usually shorter than the healthy population. Therefore, in our study, the diameters and ventricular volumes were indexed with BSA. Approximately 17.1% of our patients had an ASI >2 cm/m^2, which is also assumed to be a high-risk factor for aortic dissection (2, 17). Three patients had an ASI >2.5 cm/m^2, which is an extremely high-risk factor and prompts the need for surgical intervention (8, 26). If the descending aortic diameter is within the normal range, another ratio can be used to estimate the AA dilatation: an AD/DD ratio >1.5 (11). Some studies showed that the ASI is a more reliable parameter in TS than the AD/DD ratio (8). In a study by Matura et al. 33% of women with an ASI >2.5 cm/m^2 experienced aortic dissection within 3 years, and 3% of those with an AD/DD ratio >1.5 had aortic dissection (8). In our study, the presence of BAV was also associated with a greater diameter of the AA. Isolated BAV is usually associated with larger proximal aortic diameters, apart from normal valve function (36). Identifying BAV in asymptomatic individuals is also important because they are at increased risk for valvular dysfunction, infective endocarditis, and aortic aneurysm (10). In our study, BAV was diagnosed on CMR in three patients (7.7%) but was missed on echocardiography.

The association between elongation of the aorta and aortic dissection is still debated. In our study, elongation of the AA was associated with a greater aorta diameter. Thus, aortic elongation may be a contributing factor for aortic dilatation. A recent study from Germany showed that aortic elongation may play a role in the pathogenesis of aortic dissection (37).

Left ventricular volumes (EDV, ESV, and SV) as evaluated by CMR were decreased in 20–25% of our population. Considering that 87.8% of the studied patients were treated with rGH, the safety of which might be questioned. Neither myocardial hypertrophy nor ventricular dysfunction was found in our population, similar to the findings of previous studies on the safety of rGH treatment (38–40). The results concerning left ventricle volumes are similar to those found by Van den Berg et al. (38) but contrast those of other papers that report comparable LV volumes between healthy controls and TS patients (39, 40). The decreased left ventricle volumes may be explained by the different effects on the growth of various organs as shown in the experimental models of GH and IGF-I deficiency (41, 42). However, a recent echocardiographic study in TS showed no evidence for disproportionate cardiac growth between patients treated with GH and those not treated with GH (43). Van den Berg et al. (38) found neither myocardial nor ventricular hypertrophy in a TS population treated with rGH. Smaller LV volumes may reflect cardiac hypoplasia in TS rather than a GH effect (44).

Another interesting finding was that in seven patients (17.9%), pericardial fluid was observed on CMR. Pericardial effusion is rarely described in TS (45, 46). However, past study results were based on echocardiography. In our study, pericardial effusion was recognized on CMR. It can be associated with hypothyroidism and thyroiditis, but in our group, only three out of seven patients had euthyroid Hashimoto's disease. Pericardial effusion and pericarditis may be a sign of systemic inflammatory disease or autoimmune vasculitis. Although none of our patients were diagnosed with systemic disease during the time of the study, a more specific diagnosis should be performed. The increased risk of autoimmune diseases in TS patients has been confirmed (47), and screening for celiac and Hashimoto's diseases or diabetes has become standard practice. The estimation of specific vasculitis antibodies should also be considered in TS.

Improvements in imaging techniques facilitate the diagnosis of cardiovascular anomalies in TS. Most of the cardiovascular anomalies diagnosed on angio-MR were not detected by echocardiography. Additionally, the dilation of the aorta was estimated more precisely with angio-MR. Because of the poor acoustic window and chest wall abnormalities in TS patients, only the AA and aortic arch can be visualized by echocardiography.

CONCLUSIONS

Our study shows that CMR and angio-MR provide a detailed diagnosis of arterial and venous anomalies that have important clinical implications. Magnetic resonance

can identify cardiovascular anomalies, dilatation of the aorta, pericardial fluid and functional impairment of ventricles that are missed by echocardiography. BMI, age, BAV, and elongation of the AA influence aortic dilatation. The ASI and AD/DD ratio are important markers of aortic dilatation. The performed diagnostics did not indicate a negative influence of GH treatment on the cardiovascular system. The cardiac measurements made on MRI and echocardiography were comparable in most cases.

AUTHOR CONTRIBUTIONS

MO-M contributed to the study design, acquisition of data, analysis and interpretation of data, and writing of the manuscript. JR-T contributed to the study design, acquisition of data, analysis and interpretation of data, and critical revision of the manuscript. SR contributed to the acquisition of data, graphics design, and analysis and interpretation of data. AK contributed to the acquisition of data and drafting of the manuscript. KK-K, BR-P, MJ, AG-S, BM, and AS contributed to the acquisition of data. MN and MP contributed to the study design, drafting of the manuscript, and critical revision of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00609/full#supplementary-material

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MINI REVIEW
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Can Brain Natriuretic Peptides and Osteoprotegerin Serve As Biochemical Markers for the Detection of Aortic Pathology in Children and Adolescents with Turner Syndrome?

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Turner syndrome (TS) is a chromosomal disorder that affects 1:2,000 females. It results from either the complete or partial loss of the X chromosome as well as other aberrations. Clinical features of TS include short stature, delayed puberty, and congenital cardiac malformations. TS children also have an increased prevalence of cardiometabolic risk factors, which predisposes them to complications like coronary artery disease, cerebrovascular-related deaths, and aortic dissection. Early cardiac imaging, such as echocardiography and cardiac magnetic resonance imaging, are recommended to detect underlying aortic pathology. However, these modalities are limited by cost, accessibility, and are operator dependent. In view of these shortcomings, alternative methods, like vascular biomarkers, are currently being explored. There are only a few studies that have examined the relationship between B-type natriuretic peptide (BNP), N-terminal pro BNP (NT pro-BNP), and osteoprotegerin (OPG) and aortic disease in TS, and thus the data are only in proof-of-concept stages. Further meticulous longitudinal studies are required before BNP, NT pro-BNP, and OPG are used as vascular biomarkers for the detection of aortic disease in childhood and adolescent TS.

Keywords: Turner syndrome, vasculopathy, B-type natriuretic peptide, N-terminal pro BNP, osteoprotegrin

INTRODUCTION

Turner syndrome (TS) is the commonest chromosomal abnormality in females affecting approximately 50 in 100,000 live female births (1). TS commonly results from either the complete or partial loss of a single X chromosome and though the phenotype varies with the type of chromosomal abnormality, it is hallmarked by short stature and ovarian failure (2, 3). Left-sided cardiac malformations such as co-arctation of the aorta and bicuspid aortic valves (BAV) are seen in 50% of TS patients (4, 5). The presence of BAV in TS is associated with an increased incidence of aortic dissection

(Ao D) such that 95% of TS cases who develop Ao D have underlying BAV. Hypertension, overweight, glucose intolerance, and vascular endothelial dysfunction also contribute to the pathogenesis of Ao D (6–21). Children with TS have abnormal aortic distensibility and carotid intima media thickness which are present even in the absence of cardiometabolic risk factors. Furthermore, the functional changes in the vascular endothelium are evident prior to the structural changes in children with TS (22, 23).

Guidelines recommend regular cardiac surveillance with echocardiography and cardiac MRI as part of the structured care of young children with TS. It is aimed in detecting early aortic valve disease and any underlying adverse cardiometabolic risk factors in an attempt to reduce the risk of fatal Ao D (3). However, it is likely that these modes may not be sensitive enough to detect the early vasculopathic changes that are inherent to TS, given that functional endothelial changes precede structural changes.

Soluble vascular biomarkers (SVBs) may offer viable alternatives to assessing vascular health in TS. SVBs are measureable substances, which serve as indicators of clinical end points. They can be used to monitor and predict response to therapy. Some SVBs have clinical utility in the diagnosis of certain aortic and cardiac conditions in adults. The American Heart Association lists nine criteria that SVBs should fulfill before they can be considered as clinical tools (**Table 1)** (24). Some biomarkers, such as low-density lipid cholesterol, B-type natriuretic peptide (BNP), and atrial natriuretic peptide, fulfill these criteria and they are currently used in clinical practice. Similarly, there are SVBs that are associated with vasculopathy. These include BNP, N-terminal pro BNP (NT pro-BNP), and osteoprotegerin (OPG). On the premise that TS is an inherently vasculopathic condition, an intriguing question that rises is whether these biomarkers have a potential role to detect subclinical aortic disease, monitor treatment response, and predict outcome childhood TS associated with early aortic disease (25, 26). This review will discuss data from studies on B type natriuretic peptide (BNP), NT pro-BNP, and OPG in childhood TS. **Table 2** summarizes studies on biochemical markers in aortic pathology in TS.

TABLE 1 | AHA criteria for soluble vascular biomarkers.

AHA biomarker criteria
1. Proof of concept
Is the biomarker different in subjects with and without the clinical outcome?
2. Prospective validation
Does the biomarker predict the development of future outcomes?
3. Incremental value
Does the biomarker add predictive information over current risk markers?
4. Clinical utility
Does the biomarker predict risk sufficiently to guide management?
5. Clinical outcomes
Does the use of the biomarker improve clinical outcomes?
6. Cost-effectiveness
Does the use of the biomarker justify its additional costs?
7. Ease of use
Is the biomarker widely applicable?
8. Methodological consensus
Can the biomarker be used to make comparisons between studies?
9. Reference values
Are there established reference ranges?

B TYPE NATRIURETIC PEPTIDES

B type natriuretic peptides and NT pro-BNP are neurohormones that are encoded by the BNP gene, NPBB. These neurohormones are released in response to cardiac muscle stretch, ventricular ischemia, or volume overload and mediate their effects through the renin–angiotensin–aldosterone system act to lower systemic blood pressure. BNP is noted to be elevated in clinical conditions such as congestive cardiac failure, hypertension, and coronary artery disease in adults (27). BNP levels and NT pro-BNP levels also correlate with the clinical severity of aortic stenosis (AS), predict the need for aortic valve replacement in AS, and predict mortality associated with AS (28–33). BNP can be sampled easily in the clinical setting with the use of commercially available kits ensuring timely measurements for diagnosis and therapeutic monitoring (34). However, interpretation of BNP levels should be done with caution using sex and age appropriate reference ranges and also take into account BMI and glomerular filtration rate as these factors influence circulating BNP and NT pro-BNP levels. BNP and NT pro-BNP appear as promising SVBs in non-TS-related cardiac disease and aortic disease in non-TS patients. Thus, presenting a logical starting point from which to assess if there are sufficient data supporting BNP and NT pro-BNP as potential biomarkers in childhood TS.

B NATRIURETIC PEPTIDES AND TS

The relationship between NT pro-BNP and TS was first investigated by Gravholt et al. (11). The primary aim of this study in nine TS women, all karyotype confirmed and aged between 29.7 ± 5.6 years, was to determine the impact of hormone replacement therapy (HRT) on sympathovagal tone in TS patients. B natriuretic peptide levels, which were quantified in both the TS patients and healthy peers, were noted to be elevated in all TS subjects but particularly in the subset of TS subjects who had nocturnal hypertension. None of the participants had an ECHO during the study, so it is difficult to ascertain whether the TS cohort with nocturnal hypertension had underlying aortic disease, even though they all reportedly had normal systolic function on an ECHO done prior to the study (11). Interestingly, over the 2 years while on HRT, an increase in the NT pro-BNP levels was not observed in the TS group, suggesting that NT pro-BNP levels are not influenced by HRT and so may be of value in TS children undergoing pubertal induction. Despite the lack of a discernible connection between NT pro-BNP and aortic disease in this TS cohort, the study did report the novel finding that NT pro-BNP levels are distinctly abnormal in TS individuals, thus posing the question for future studies regarding the utility of NT pro-BNP as a potential biomarker in TS.

Another study by Gutin et al. (35) examined the relationship between NT pro-BNP and aortic disease in 114 karyotype

TABLE 2 | Summary of studies examining B-type natriuretic peptide (BNP), N-terminal pro BNP (NT pro-BNP), and osteoprotegerin (OPG) in Turner syndrome (TS) patients.

Reference	Biomarker	Population	Study design	Main findings
Gravholt et al. (11)	NT pro-BNP Renin Aldosterone	TS (n = 9) Mean age 29.7 ± 5.6 years Controls (n = 8)	Randomized placebo controlled cross over study	– NT pro-BNP levels were higher in the TS cohort. – Renin and aldosterone levels were comparable between the 2 groups. – Treatment with hormone replacement therapy (HRT) did not influence the levels of NT pro-BNP.
Gutin et al. (35)	NT pro-BNP	TS (n = 114) Controls (n = 27) Age 18–67 years (mean age 37.4 ± 12 years)	Cross-sectional	– NT pro-BNP levels are significantly different between the 2 groups. – NT pro-BNP levels are significantly higher in the TS group after excluding TS patients with dilated aorta. – Highly significant correlation between NT pro-BNP levels and descending aortic diameter. – TS participants with dilated ascending aorta had significantly high mean NT pro-BNP levels as compared to those who did not have dilated ascending aorta.
Uçar et al. (36)	BNP ANP hsCRP PRA IGF1 IGFBP3	TS (n = 61) Age 6.6–21.3 years (mean age 12.6 years) Controls (n = 61)	Cross-sectional	– TS cohort and healthy controls were matched for age, sex, 24-h ambulatory BP, Nocturnal BP dipping. – TS cohort had significantly high cIMT, β-index, Einc SDS values. – TS cohorts had significantly higher levels of BNP, atrial natriuretic peptide (ANP), hsCRP even after correcting for BMI and puberty. – BNP maintained a significant positive correlation with all measures of arterial stiffness (cIMT, Einc, β-index, and Distensibility coefficient). – ANP only retained a positive correlation with cIMT and hsCRP had a positive correlation with β-index, Einc, and distensibility coefficient.
Buzi et al. (46)	OPG RANKL	Controls (n = 46) Age 1–14 years (Mean 7.8 years SD 3.76) TS (n = 10) Mean age 10.8 years No estrogen replacement On human GH therapy	Cross-sectional	– OPG levels in normal children (aged 1–14 years) were highest in infancy and decreased with increasing age. – No correlation between OPG levels and BMI, height, weight, puberty. – OPG levels in TS cohort were lower than normal in age and sex-matched controls. – No difference in RANKL levels between TS and controls.
Trolle et al. (47)	OPG	Controls (n = 68) TS (n = 99) Age matched	Prospective	– TS patients were significantly different from healthy controls in terms of BMI, BSA, blood pressure measurements. – OPG levels were significantly lower in the TS cohort at baseline and at follow-up and at the end of the study. – Lower levels of OPG were more pronounced in the Monosomy X compared to mosaics, but not significant. – OPG levels correlated with BSA-indexed distal descending aortic diameter at all 3 time points.

confirmed *normotensive* adult TS patients. This study demonstrated that NT pro-BNP levels were elevated in TS subjects as compared to healthy controls and that the NT pro-BNP levels were high irrespective of the presence of underlying aortic disease, confirming the findings from Gravholt et al. (11), but further elucidating that high NT pro-BNP levels are intrinsic to TS in the absence of underlying aortic disease and clinically detectable hypertension. A sub-analysis of the TS group with aortic dilatation demonstrated a strong positive correlation between NT pro-BNP levels and the diameter of the ascending aorta as well as BSA-indexed ascending aortic dilatation confirming that there is indeed a relationship between NT pro-BNP levels and radiologically detectable aortic disease in TS women.

Uçar et al. (36), more recently, examined the association between BNP and arterial stiffness in *young* normotensive TS patients. Sixty-one TS subjects, with a mean age of 12.6 years, normal cardiac anatomy, and matched for systolic and diastolic BP SD scores, were compared with healthy controls on measures of arterial stiffness and BNP levels. The TS cohort, the youngest participant being 6.6 years old, had significantly higher measures of arterial stiffness than healthy peers, such as cIMT, β-index, distensibility coefficient, and incremental elastic modulus (Einc). Furthermore, sub-analyses revealed a robust correlation between BNP levels and *all* measures of arterial stiffness: cIMT, β-index, Einc, and distensibility coefficient. This study reports several important findings: first, that BNP levels can be assessed in very young TS children; second, that BNP levels are increased in young TS individuals who do not have clinical hypertension, suggesting the idea that elevated BNP levels in young TS patients may signify an inherent vasculopathy; third, that BNP levels were not influenced by BMI nor puberty, making it a potentially useful tool in childhood, a period during which BMI is fluctuating and pubertal induction is ongoing.

OPG IN TS

Osteoprotegerin is a glycoprotein from the tumor necrosis factor family of cytokines, which is linked to several cardiac conditions. OPG functions as a decoy receptor for receptor activator of

nuclear factor kb ligand (RANKL) and thus blocks the interaction between RANKL and NFkB. NFkB activation is a key regulator of inflammatory, vascular, and skeletal gene transcription pathways. OPG levels are increased in advanced atherosclerosis and abdominal aortic aneurysm, silent myocardial infarction, unstable angina, and heart failure (37–42). OPG levels can also predict outcome and mortality in individuals with coronary artery disease (42–44). These data suggest that circulating OPG levels can be quantified in disease states and that they are distinctly abnormal in *adult* cardiovascular disease.

In children, OPG levels have been quantified in specific cohorts, such as type 1 diabetes mellitus (T1DM) and chronic renal failure. Fekih et al. (45) quantified circulating OPG levels in a childhood T1DM cohort ($n = 143$; mean age 12 years) with cardiometabolic risk factors such as prolonged duration (≥4 years) of diabetes, HbA1c ≥ 7%, dyslipidemia, and microalbuminuria (≥30 mg/24 h). Their study revealed that OPG levels were significantly higher ($p < 0.0001$) in the T1DM cohort. OPG levels also correlated significantly with several cardiovascular risk factors, in particular, OPG levels were significantly increased in children who had more than three cardiovascular risk factors. Though this study is not specific to a TS cohort, it nonetheless demonstrates several important findings that can be applied to future TS cohorts. First, it shows that OPG is quantifiable in children and that normative ranges exist. Second, it highlights that OPG levels are influenced by cardiometabolic risk factors, which are prevalent in the TS childhood population also (7). Finally, given that OPG levels are significantly raised in T1DM children with more cardiometabolic risk factors, raising the question of whether elevated OPG levels can identify those TIDM children who are likely to have a poor prognosis. This question is equally applicable to a TS childhood cohort, given their underlying cardiometabolic risk factor profile.

The relationship between OPG and TS is only being examined more recently. The first, a cross-sectional study by Buzi et al. (46) examined the difference between OPG levels in healthy controls, *children* with precocious puberty, rheumatoid arthritis, and TS aged between 1 and 14 years. Interestingly, they showed that the TS cohort had significantly *lower* OPG levels than age-matched controls but superimposable to the cohort with precocious puberty. It is difficult to draw definitive conclusions from this study about the value of OPG as a biomarker in TS as neither the cardiac nor the metabolic bone health profile of the TS cohort was reported. Nonetheless, it is the first study to demonstrate that OPG levels in the least are deranged in childhood TS.

Trolle et al. reported on a cohort of 99 *adult* TS subjects over a 5-year period (47) with the aim of establishing any differences between OPG and aortic pathology in TS. They corroborated the finding by Buzi et al. that TS individuals have significantly *lower* levels of OPG levels as compared to healthy volunteers, but they also added to the previous study by conducting aortic measurements. They showed that there was a positive correlation between OPG levels and aortic diameter, in particular those individuals with aortic dilatation. Together, these findings confirm that OPG levels are abnormal in adult TS cohorts, in particular those who have underlying aortic dilatation. However, contrary to the study by Fekih et al. (45), OPG levels did not correlate with traditional cardiometabolic factors like hypertension, hsCRP, cholesterol levels, or nocturnal hypertension, all of which are associated with TS. Nonetheless, it suggests that OPG levels are a biomarker worth examining in *childhood* TS in relation to cardiometabolic risk factors and aortic disease.

BNP, NT pro-BNP, AND OPG AS SVBs IN CHILDHOOD TS

Several studies have reported on the association between TS and the levels of certain biomarkers, namely BNP, NT pro-BNP, and OPG. However, only two of these studies specifically examine childhood TS cohorts, thus highlighting the paucity of data on this topic. Given the dearth of information in childhood TS and vascular biomarkers, conclusions about their usefulness as clinical tools are difficult to assert. Despite the lack of studies in this field, several conclusions can still be drawn on which future studies should be based. First, these studies demonstrate that certain vascular biomarkers are indeed abnormal in TS patients and that they do fulfill some of the criteria of SVBs as set out by the AHA (**Table 2**). The studies, though few in number, also demonstrate that the biomarker levels are deranged in childhood TS before the onset of aortic complications. This highlights the importance of investigating further with respect to their usefulness as predictive and prognostic tools. In order to determine the predictive ability of BNP, NT pro-BNP, and OPG for aortic disease, prospective longitudinal studies are needed. Most of the studies discussed in this review are cross-sectional studies and so are unable to draw conclusions on whether these SVBs are useful in predicting aortic disease in TS. Quantifying SVB levels in childhood TS in conjunction with cardiac imaging and measures of endothelial function could give valuable information on the diagnostic ability of these biomarkers and whether they can serve as complementary tools to traditional cardiac imaging techniques. Furthermore, though normative values exist for BNP, NT pro-BNP, and OPG, and it is known that certain variables, such as BMI or puberty, can influence these values, which are relevant to the TS cohort as are variables like growth hormone therapy and pubertal induction (48). Hence, the impact of these common therapies used in TS on SVB levels need to be elucidated. Future studies should also take the opportunity to examine whether these SVBs can be used to monitor aortic disease progression and response to conventional medical and surgical therapy for aortic disease. None of the studies have examined the cost-effectiveness of SVBs in diagnosing TS-related aortic disease. This needs to be determined before they are widely adopted as tools in clinical practice, in addition to determining whether their widespread use can positively impact on aortic disease management and outcomes in childhood TS. Finally, none of the studies have attempted to determine the physiological mechanisms underpinning the aberrations in SVB levels in childhood TS. Elucidating these mechanisms may assist in developing new targeted therapies for the management of aortic disease in childhood TS.

CONCLUSION

The utility of SVBs, in particular BNP, NT pro-BNP, and OPG, in childhood TS to predict aortic pathology is limited. Data from studies examining these SVBs in adult TS cohorts support the fact that their levels are distinct in the TS cohort, in particular those with underlying aortic pathology. At the present time, the data are insufficient to make the claim that these SVBs can be used as clinical tool. Nonetheless, this dearth of information presents numerous opportunities for future research which should focus on consolidating them as biomarkers as per the AHA criteria and also by determining their usefulness as clinical tools for diagnosis, predicting outcomes, and prognosis.

AUTHOR CONTRIBUTIONS

MM contributed to the idea of the article and conducted the literature search and drafting the manuscript. CO contributed to idea of the article, the drafting of the manuscript, and assisted in editing the manuscript prior to final submission.

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The Natural History of Metabolic Comorbidities in Turner Syndrome from Childhood to Early Adulthood: Comparison between 45,X Monosomy and Other Karyotypes

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Objective: Patients with Turner syndrome (TS) are at increased risk for metabolic disorders. We aimed to delineate the occurrence and evolution of metabolic comorbidities in TS patients and to determine whether these differ in 45,X monosomy and other karyotypes.

Methods: A longitudinal and cross-sectional retrospective cohort study was conducted in a tertiary pediatric endocrine unit during 1980–2016. Ninety-eight TS patients, 30 with 45,X monosomy were followed from childhood to early adulthood. Outcome measures included weight status, blood pressure (BP), glucose metabolism, and lipid profile.

Results: Longitudinal analysis showed a significant change in body mass index (BMI) percentiles over time [$F(3,115) = 4.8$, $P = 0.003$]. Age was associated with evolution of elevated BP [systolic BP: odds ratio (OR) = 0.91, $P = 0.003$; diastolic BP: OR = 0.93, $P = 0.023$], impaired glucose metabolism (HbA1c: OR = 1.08, $P = 0.029$; impaired glucose tolerance: OR = 1.12, $P = 0.029$), and abnormal lipid profile (cholesterol: OR = 1.06, $P = 0.01$; low-density lipoprotein cholesterol: OR = 1.07, $P = 0.041$; high-density lipoprotein cholesterol: OR = 1.07, $P = 0.033$). The occurrence of metabolic comorbidities was similar in 45,X monosomy and other karyotypes. Coexistence of multiple metabolic comorbidities was significantly higher in 45,X monosomy [$F(1,72) = 4.81$, $P = 0.032$]. BMI percentiles were positively correlated with metabolic comorbidities (occurrence and number) in each patient ($r = 0.35$, $P = 0.002$ and $r = 0.383$, $P = 0.001$, respectively).

Conclusion: Our longitudinal study provides unique insights into the evolution of weight gain and metabolic disorders from childhood to early adulthood in TS patients. Since overweight and increasing age aggravate the risk for metabolic comorbidities, careful surveillance is warranted to prevent and control obesity already from childhood. The more prominent clustering of metabolic comorbidities in 45,X monosomy underscores the importance of a more vigorous intervention in this group.

Keywords: Turner syndrome, karyotype, metabolic disturbances, obesity, impaired glucose metabolism

INTRODUCTION

Turner syndrome (TS) is the most common chromosomal abnormality in girls, affecting approximately 1:2,500 of female live births (1). TS results from complete X chromosome monosomy, structural abnormality of the second X chromosome, or mosaicism, and has a characteristic phenotype and various comorbidities (2–4). Young adult women with TS are susceptible to a wide range of medical problems, including autoimmune disorders (5, 6), overweight and obesity (7), an increased risk for metabolic disorders such as glucose intolerance or dyslipidemia (8, 9), and osteopenia/osteoporosis (10). The coexistence of increased weight, impaired glucose metabolism, lipid abnormalities and hypertension ultimately increase their risk for acquired cardiovascular disease (11, 12). While the association between distinctive metabolic derangements and various TS karyotypes has been demonstrated in previous studies (4, 8, 9) the evolution of these comorbidities from childhood to young adulthood in TS patients with various karyotypes has not been thoroughly assessed.

Our institutional policy is to offer continuing surveillance to TS patients till the mid-twenties by a multidisciplinary team aware of the complex and interrelated issues impacting on the health of these individuals. This practice has enabled us to carry out a comprehensive metabolic assessment of TS patients from childhood through puberty to young adulthood and to characterize the natural history of the metabolic comorbidities occurring in young adult women with TS.

The objectives of this longitudinal retrospective study were to assess the occurrence and evolution of overweight/obesity, hypertension, impaired glucose metabolism, and dyslipidemia in TS patients from childhood to early adulthood and to determine whether the prevalence and the natural history of these comorbidities differ in TS patients with 45,X monosomy and with other karyotypes.

PATIENTS AND METHODS

Patients

The medical files of 103 patients with TS confirmed by karyotyping who were followed at the Institution for Pediatric Endocrinology at the Schneider Children's Medical Center of Israel between the years 1980 and 2016 were reviewed. Ninety-eight cases fulfilled the inclusion criteria: karyotype documentation in the medical files; referral to our clinic prior to pubertal induction; and regular auxologic and blood pressure (BP) surveillance. Five patients [45,X ($n = 3$) and mosaicism 45,X/46,XX ($n = 2$)] were excluded from the study due to severe congenital malformations predisposing to hypertension (severe congenital cardiovascular disease and congenital anomalies of kidneys and urinary tract). The study cohort was categorized by karyotype into two groups: (1) 45,X (monosomy); (2) other karyotypes.

This study was approved by our institutional ethics committee. Because there was no identification of the patients for whom data was retrieved, informed consent by the patients was waived.

Methods

Our institutional policy consists of continuing clinical and laboratory surveillance of TS patients from referral to the mid-twenties. Girls with TS are routinely scheduled for clinic visits every 4–6 months until attainment of full puberty and adult height, after which they are seen every 6 months until transition to adult endocrine clinics. Clinical assessment includes anthropometric measurements (height, weight), vital signs (heart rate and BP), complete physical examination, and dose adjustment of chronic medications, e.g., growth hormone (GH), estrogen and progesterone, or L-thyroxine. Screening for autoimmune thyroiditis (thyroid function test, antithyroid peroxidase, thyroglobulin antibodies) and celiac disease (anti-tissue transglutaminase, and immunoglobulin A levels) is performed from the age of 4 years onward. Screening for impaired glucose metabolism [fasting plasma glucose, hemoglobin A1c (HbA1c)], dyslipidemia, and liver disease is performed from the age of 8 years onward. All TS patients in whom cardiovascular defects were not identified at diagnosis undergo a reassessment of the cardiovascular system at 5-year intervals.

Data Collection through Childhood and Early Adulthood

The data obtained from the medical files included age at diagnosis, age at initiation and cessation of GH therapy, age at onset of spontaneous puberty and/or initiation of estrogen treatment, age at first menstrual bleeding, age at diagnosis of associated disorders (autoimmune diseases, hypertension, impaired glucose metabolism, dyslipidemia), and the use of chronic medications. The clinical and laboratory data (height, weight, pubertal stage, BP, glucose, both fasting and in 2-h postoral glucose tolerance, fasting insulin levels and HbA1c, lipid profile, and liver function tests) were extracted from the medical files at four timepoints: childhood (prior to pubertal induction), adolescence (1–2 years following onset of spontaneous puberty or initiation of estrogen replacement therapy), young adulthood (fully pubertal; age < 21 years) and early adulthood (age > 21 years; prior to transfer to adult endocrine clinics).

Body Mass Index (BMI) Assessment

Body mass index (weight in kilograms/square of height in meters) was calculated using the anthropometric measurements documented in the medical files. The evolution of BMI of the TS cohort from childhood through adolescence and young adulthood to early adulthood was assessed by using BMI percentiles. In childhood and adolescence, BMI values were converted to age- and sex-specific percentiles according to the CDC2000 (13). In adulthood, BMI values were converted according to the anthropometric reference data for all ages of the US population in 2003–2006 found in the National Health and Nutrition Examination Survey and National Center for Health Statistics (14). BMI percentiles were used as the index of body weight: underweight, <5th percentile; normal weight, ≥5th to <85th percentiles; overweight, ≥85th to <95th percentiles; and obese, ≥95th percentile (15).

BP Assessment

Blood pressure was measured according to the recommendations of the National High Blood Pressure Education Program

(NHBPEP) (16). In childhood, percentiles for systolic BP and diastolic BP were calculated according to height, sex, and age (16). Normal BP, prehypertension, and hypertension were defined according to the NHBPEP: BP was defined as normal when BP values were <90th percentile, prehypertension—when either systolic and/or diastolic BP levels were ≥90th to the 95th percentile, hypertension—when either systolic and/or diastolic BP level ≥95th percentile. Hypertension was classified as either stage 1 (≥95th to the <99th percentile plus 5 mm Hg) or stage 2 (≥99th percentile plus 5 mm Hg). In adulthood, BP values were classified as: normal, <120/80; prehypertension, 120–139/80–89; stage 1 hypertension, 140–159/90–99; stage 2 hypertension, ≥160/100 (17).

Glucose Metabolism Assessment

Fasting glucose levels were defined as follows: normal, <100 mg/dl; impaired fasting glucose, 100–125 mg/dl; diabetes, ≥126 mg/dl. In those cases which underwent an oral glucose tolerance test (OGTT), 2-h postprandial glucose levels following OGTT were defined as: normal, <140 mg/dl; impaired glucose tolerance, 140–199 mg/dl; diabetes, ≥200 mg/dl. HbA1c levels were categorized as: normal, <5.7%; prediabetes, 5.7–6.4%; diabetes, ≥6.5%. Homeostasis model assessment-insulin resistance (HOMA-IR) was utilized as an insulin resistance index and was calculated by the following equation: [fasting glucose (mg/dl) × fasting insulin (μU/ml)]/405; resistance was defined as HOMA-IR ≥ 3 (18).

Lipid Profile Assessment

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TGs) were converted to age- and sex-specific percentiles according to the criteria of AAP Lipids in children aged 5–19 years (19). The lipid profile was classified as: desirable <75th percentile/borderline high 75th–90th percentile/high >90th (TC, LDL-c, and TG) and low level <10th percentile/average level 10th–25th/high level >50th percentile (HDL-c). In adults, hypercholesterolemia was defined when TC levels were above 240 mg/dl; elevated LDL-c was defined when LDL-c levels were above 130 mg/dl; hypertriglyceridemia was defined when TG were above 150 mg/dl and low HDL-c was defined as levels <50 mg/dl.

Statistical Analysis

Data were analyzed using the IBM SPSS software (IBM SPSS Statistics for Windows, Version 24; IBM Corp., Armonk, NY, USA) and the HLM software version 7 (HLM Software). Data are presented as mean and SD or number and percentile, as appropriate. One-way analysis of variance (ANOVA) was used to test for group differences in quantitative, continuous variables; the χ^2 test (or Fisher's exact test for small count tables) was used to compare groups in categorical variables. ANOVA with repeated measures was used to test for change in BMI percentile over time and the Friedman test was used to test for change over time in non-quantitative ordinal variables. Growth Curve Models with Logit link functions were used to test odds ratio (OR) of age on the risk of developing various metabolic comorbidities at early adulthood. The Pearson's correlation coefficient was used to test for association between quantitative variables. Logistic regression was used to test for risk factors for the occurrences of metabolic diseases. A *P*-value of ≤0.05 was considered significant.

RESULTS

Clinical Characteristics

The study group consisted of 98 patients with TS, 30.6% ($n = 30$) with 45,X monosomy and 69.4% ($n = 68$) with other karyotypes: mosaicism 45,X/46, XX ($n = 21$), isochromosomes (Xq), or deletions ($n = 29$); a marker or ring chromosome ($n = 10$); karyotypes with Y chromosome material ($n = 8$).

The characteristics of the study population are shown in **Table 1**. The mean duration of follow-up in this cohort was 11.7 years, with no significant difference between 45,X monosomy and other karyotypes. At diagnosis patients with monosomy were significantly younger than those displaying other karyotypes ($P = 0.05$). Spontaneous puberty occurred less frequently in TS girls with monosomy ($P = 0.01$). Age at onset of spontaneous puberty or age at initiation of pubertal induction were similar in both TS groups, while age at first menstrual bleeding was older in those with monosomy ($P = 0.006$). GH therapy was administered to 56% of the cohort; the rate of GH treatment, the age at initiation of therapy and the duration of therapy were similar in the two groups. Autoimmune thyroiditis was diagnosed in 33% and celiac disease in 8.2% of the cohort, with no significant differences between groups. Liver diseases were found in 22.5% ($n = 22$) of the patients: primary biliary cirrhosis in 2, sclerosing cholangitis in 1, autoimmune hepatitis in 6, fatty liver in 8, and undetermined etiology in 4 patients. In 10 patients, elevated liver enzymes were present already from adolescence.

TABLE 1 | Characteristics of Turner syndrome cohort.

	All karyotypes	45,X monosomy	Other karyotypes	*P*
Number of subjects, *n* (%)	98	30 (30.6)	68 (69.4)	
Duration of follow-up (years)	11.7 ± 5.9	11.7 ± 5.8	11.7 ± 6.1	0.996
Age at diagnosis (years)	6.7 ± 4.5	5.4 ± 4.6	7.3 ± 4.4	**0.05**
Spontaneous puberty, *n* (%)	27 (27.6)	3 (10)	24 (35.3)	**0.01**
Age at spontaneous puberty (years)	11.8 ± 1.3	11.5 ± 1.6	11.9 ± 1.3	0.611
Age at pubertal induction[a] (years)	13.7 ± 1.3	13.8 ± 1.3	13.6 ± 1.4	0.465
Age at first menstrual bleeding[b] (years)	16.1 ± 1.8	17.0 ± 1.6	15.8 ± 1.8	**0.006**
Growth hormone therapy, *n* (%)	55 (56.1)	20 (66.7)	35 (51.5)	0.162
Age at growth hormone initiation (years)	8.4 ± 2.8	9.1 ± 2.9	8.0 ± 2.7	0.517
Age at last clinic visit (years)	21.3 ± 6.0	21.4 ± 5.8	21.2 ± 6.0	0.900
Autoimmune thyroiditis, *n* (%)	32 (32.7)	13 (43.3)	19 (27.9)	0.193
Celiac disease, *n* (%)	8/94 (8.5)	4/30 (13.3)	4/64 (6.3)	0.262
Liver disease, *n* (%)	22 (22.5)	5 (16.7)	17 (25.0)	0.362

Data expressed as mean and SD unless otherwise specified.
Bold font represents P values which are statistically significant.
[a]*3 of the TS cohort were <10 years of age at data collection.*
[b]*19 of the TS cohort have not attained menarche; 3 prepubertal and 16 pubertal girls (≤Tanner 4).*

Evolution of Weight Status and Metabolic Comorbidities

In childhood, metabolic impairments were detected in 20–25% of the studied girls: overweight/obesity in 15%, elevated systolic and/or diastolic BP in 22%, impaired glucose metabolism in 10%, and increased lipid levels (TG and TC) in 27%.

Longitudinal analysis of weight status (expressed as BMI percentile) at three times points (from childhood to young adulthood) showed a significant change over time in both those with 45,X monosomy and those with other chromosomal abnormalities [$F(2,132) = 3.1$, $P = 0.05$], with a marginally significant difference between groups [$F(1,66) = 3.7$, $P = 0.06$] and with no significant between-group interactions. Longitudinal analysis from childhood to early adulthood showed a significant change in BMI percentiles over time [$F(3,115) = 4.8$, $P = 0.003$]. Sidak *post hoc* analysis showed that BMI percentile at early adulthood was significantly lower than at adolescence or young adulthood.

Longitudinal analysis (using Growth Curve Models) of metabolic comorbidities from childhood to early adulthood showed that age was associated with evolution of elevated BP percentiles (SBP: OR = 0.91, $P = 0.003$; DBP: OR = 0.93, $P = 0.023$), impaired glucose metabolism (HbA1c: OR = 1.08, $P = 0.029$; IGT: OR = 1.12, $P = 0.029$), and abnormal lipid profile (TC: OR = 1.06, $P = 0.01$; LDL-c: OR = 1.07, $P = 0.041$; HDL-c: OR = 1.07, $P = 0.033$).

Cross-sectional Analysis of Weight Status and Metabolic Comorbidities

Weight Status

At childhood and adolescence, the mean BMI percentile of the 45,X monosomy was significantly higher than that of the other chromosomal abnormalities ($P = 0.037$ and $P = 0.035$, respectively), while at young adulthood and early adulthood it was similar in both groups (**Tables 2** and **3**). The distribution of BMI weight categories (underweight, normal weight, overweight, and obese) at the follow-up timepoints was similar for both groups.

Blood Pressure

The distribution of BP categories (normal BP, pre-hypertension, stage 1 and stage 2 hypertension) at the follow-up timepoints was similar for all groups. The prevalence of elevated BP at childhood, adolescence, young adulthood, and early adulthood was 21.8%, 35.2%, 25.7%, and 21.8% for SBP and 23%, 33%, 32.4%, and 14.5% for DBP, respectively.

TABLE 2 | Cross-sectional analysis of weight status and blood pressure in 45,X monosomy and other karyotypes at childhood, adolescence, young, and early adulthood.

	Childhood			Adolescence			Young adulthood			Early adulthood		
	45,X	**Other karyotypes**	***P***	**45,X**	**Other karyotypes**	***P***	**45,X**	**Other karyotypes**	***P***	**45,X**	**Other karyotypes**	***P***
Number	27	60		29	62		24	50		18	37	
Age (years)	9.2 (1.0)	9.1 (1.4)	0.777	13.5 (1.3)	13.7 (1.2)	0.455	19.1 (1.2)	19.2 (1.4)	0.852	25.6 (2.3)	25.9 (1.7)	0.514
Weight status												
BMI (kg/m^2)	18 (2.7)	17.0 (2.4)	0.065	21.8 (4.2)	20.3 (3.1)	**0.044**	25.7 (6.1)	23.8 (4.5)	0.132	25.6 (5.1)	25.2 (5.7)	0.784
BMI percentile	65.0 (20.6)	52.5 (27.2)	0.037	68.3 (23.8)	56.1 (26.0)	**0.035**	68.6 (29.1)	61.4 (25.4)	0.279	50.1 (25.3)	48.1 (22.8)	0.763
Weight categories, %												
Underweight	0	0		3.4	1.6		8.3	2.0		0	2.7	
Healthy weight	81.5	86.7	0.173	65.5	85.5	0.117	54.2	78.0	0.151	83.3	89.2	0.539
Overweight	7.4	11.6		20.7	9.7		20.8	12.0		16.7	5.4	
Obesity	11.1	1.7		10.3	3.2		16.6	8.0		0	2.7	
Blood pressure												
SBP (mmHg)	96.5 (8.2)	95.8 (9.6)	0.761	108.1 (13.5)	105.2 (12.6)	0.319	113.5 (13.3)	110.4 (11.9)	0.318	115.9 (10.9)	112.7 (14.5)	0.420
SBP percentile	57.4 (15.8)	59.9 (17.9)	0.540	62.9 (20.4)	65.0 (21.0)	0.667	63.1 (21.0)	60.6 (19.1)	0.602	NA	NA	
SBP categories, %												
Normal SBP	81.5	75		65.5	64.5		70.8	76.0		72.2	81.1	
Pre-HTN	18.5	18.3	1	10.3	16.1	0.864	12.5	10.0	0.736	27.8	13.5	0.528
HTN 1	0	1.7		13.8	9.7		4.2	8.0		0	2.7	
HTN 2	0	3.3		10.3	9.7		12.5	6.0		0	2.7	
DBP (mmHg)	60.4 (6.8)	59.1 (7.5)	0.443	67.3 (10.0)	65.6 (8.2)	0.386	71.5 (8.3)	69.2 (7.9)	0.247	72.0 (10.1)	71.0 (9.2)	0.714
DBP percentile	60.4 (17.9)	59.0 (17.1)	0.731	62.8 (20.4)	64.3 (20.3)	0.717	64.8 (20.8)	63.6 (19.7)	0.809	NA	NA	
DBP categories (%)												
Normal DBP	74.1	76.7		71.4	66.1		66.6	68.0		83.3	86.5	
Pre-HTN	25.9	18.3	1	7.1	21	0.282	16.7	24.0	0.432	11.1	8.1	0.837
HTN 1	0	3.3		19.7	9.7		16.7	6.0		5.6	5.4	
HTN 2	0	0		3.6	3.2		0	2.0		0	0	

Data are presented as mean (SD) or percentage of patients.

Bold font represents P values which are statistically significant.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, hypertension; NA, not applicable.

TABLE 3 | Cross-sectional analysis of glucose metabolism and lipid profile in 45,X monosomy and other karyotypes at childhood, adolescence, young, and early adulthood.

	Childhood			Adolescence			Young adulthood			Early adulthood		
	45,X monosomy	**Other karyotypes**	***P***	**45,X monosomy**	**Other karyotypes**	***P***	**45,X monosomy**	**Other karyotypes**	***P***	**45,X monosomy**	**Other karyotypes**	***P***
Age (years)	9.2 (1.0)	9.1 (1.4)	0.777	13.5 (1.3)	13.7 (1.2)	0.455	19.1 (1.2)	19.2 (1.4)	0.852	25.6 (2.3)	25.9 (1.7)	0.514
Glucose metabolism												
Number	27	60		29	62		24	50		18	37	
FPG (mg/dl)	87.9 (7.1)	82.1 (8.2)	**0.002**	88.2 (9.5)	85.5 (9.5)	0.205	85.4 (9.3)	85.6 (13.3)	0.954	83.1 (9.4)	83.2 (8.4)	0.960
Impaired FPG (%)	0	1.7	1	6.9	6.5	1	8.3	8.0	1	5.6	2.7	0.535
HbA1c (%)	5.3 (0.4)	5.1 (0.3)	0.278	5.2 (0.4)	5.3 (0.4)	0.251	5.3 (1.3)	5.2 (0.3)	0.212	5.3 (0.4)	5.4 (0.4)	0.428
Oral glucose tolerance test												
Number	7	13		15	33		15	27		8	9	
2 h postload glucose (mg/dl)	117.5 (36.9)	88.4 (14.2)	**0.029**	111.4 (35.5)	119.6 (23.4)	0.352	115.8 (37.8)	125.3 (47.0)	0.25	128.9 (47.7)	132.5 (24.4)	0.838
IGT (%)	14.3	7.7	1	20.0	15.1	0.676	26.7	18.5	0.537	50.0	33.0	0.637
HOMA-IR > 3 (%)	14.3	23.1	1	20.0	21.2	1	33.3	33.3	1	12.5	22.2	1
Lipid profile												
TC	26	55		27	61		24	44		16	37	
TC (mg/dl)	170 (28.9)	173.6 (26.1)	0.574	180.8 (37.4)	175.8 (32)	0.522	194.4 (36.5)	172.5 (36.1)	**0.020**	197.6 (42.7)	183.1 (33.7)	0.192
≤50th centile (%)	50	43.6		29.2	45.5		29.2	45.5		37.5	35.1	
75th centile (%)	23.1	32.7	0.555	16.7	18.2	0.436	16.6	18.2	0.436	6.3	24.3	0.496
90th centile (%)	15.4	7.3		16.7	15.9		16.6	15.9		12.5	8.1	
≥95th centile (%)	11.5	16.4		37.5	20.5		37.5	20.5		43.8	32.4	
LDL-c, *n*	21	38		22	46		19	37		13	35	
LDL-c (mg/dl)	96.4 (21.8)	101.8 (20.8)	0.360	106.3 (30.6)	102.8 (24.7)	0.616	115.0 (32.2)	100.3 (24.6)	0.063	113.3 (29.7)	108.2 (29.8)	0.599
≤50th centile (%)	61.9	55.3		36.8	51.4		36.8	51.4		38.5	40	
75th centile (%)	28.5	26.3	0.935	36.8	35.1	0.271	36.8	35.1	0.271	15.4	28.6	0.716
90th centile (%)	4.8	7.9		0	5.4		0	5.4		15.4	8.6	
≥95th centile (%)	4.8	10.5		26.3	8.1		26.3	8.1		30.8	17.1	
HDL-c, *n*	21	39		23	47		19	38		13	35	
HDL-c (mg/dl)	56.8 (13.7)	55.4 (11.6)	0.677	56.5 (16.3)	57.4 (11.9)	0.790	56.2 (13.8)	54.2 (15.3)	0.646	63.6 (17.7)	56.7 (12.5)	0.134
≥50th centile (%)	76.2	76.9		73.7	60.5		73.7	60.5		84.6	71.5	
25th centile (%)	14.2	10.3	0.945	15.8	39.5	0.718	15.8	21.1	0.718	7.7	20.0	0.720
10th centile (%)	4.8	7.7		0	18.4		0	7.9		7.7	5.7	
≤5th centile (%)	4.8	5.1		10.5	10.5		10.5	10.5		0	2.9	
TG, *n*	24	43		23	49		20	39		15	35	
TG (mg/dl)	80 (34.8)	84.8 (34.7)	0.587	97.7 (52.3)	85 (33.5)	0.219	104.9 (55.5)	81.9 (25.4)	**0.032**	115.7 (81.3)	91.4 (46)	0.198
≤50th centile (%)	54.2	41.8		20.0	56.4		40.0	54.4		46.1	40.0	
75th centile (%)	20.8	30.2	0.718	15.0	30.8	**0.039**	15.0	30.8	**0.039**	7.7	22.9	0.434
90th centile (%)	8.3	14.0		10.0	5.1		10.0	5.1		7.7	17.1	
≥95th centile (%)	16.7	14.0		35.0	7.7		35.0	7.7		38.5	20.0	

Data expressed as mean (SD) unless otherwise specified.

Bold font represents P values which are statistically significant.

n, number; FPG, fasting plasma glucose; IGT, impaired glucose tolerance; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TG, triglycerides.

Glucose Metabolism

Fasting glucose, 2-h post-OGTT glucose, and HbA1c levels were within the normal range in most of the studied girls at all follow-up timepoints. Impaired fasting glucose was documented in 6.6% of the girls during adolescence and in 8.1% at young adulthood. Impaired glucose tolerance was documented already from childhood, with increasing prevalence during adolescence, young adulthood and early adulthood (10%, 16.7%, 21.4%, and 41.2%, respectively). Insulin resistance (HOMA-IR) was detected in 20% of the patients tested during childhood and adolescence, and in 33.3% of the patients tested at young adulthood. Glucose metabolism parameters showed no statistical difference between the patients with the 45,X monosomy and those with all other karyotypes.

Lipid Profile

At the four follow-up timepoints the mean TC, LDL-c, HDL-c and TG levels of the entire cohort were relatively normal, with no significant difference between the two groups with the exception of higher mean TG levels at young adulthood in the 45,X monosomy group ($P = 0.032$). The distribution of TC and LDL-c percentiles (≤50th%, 75th%, 90th%, and ≥95th%) and of HDL-c percentiles (≥50th%, 25th%, 10th%, and ≤5th%) at the four follow-up timepoints was similar in the two groups; the prevalence of elevated TG categories at adolescence and young adulthood was higher in the 45,X monosomy group ($P = 0.039$ for both timepoints).

Cooccurrence of Metabolic Comorbidities

Cooccurrence of metabolic comorbidities was found in 55.4% of the cohort. There was a significant difference between those with 45,X monosomy and those with other chromosome abnormalities in both the frequency [79.2% and 44%, $\chi^2(1) = 8.1$, $P = 0.004$] and number of comorbidities [2.4 ± 1.5 and 1.6 ± 1.3, $F(1,72) = 4.81$, $P = 0.032$].

Correlations

Body mass index percentiles of the cohort at childhood were positively correlated with BMI percentiles during adolescence ($r = 0.69$; $P < 0.001$), young adulthood ($r = 0.43$; $P < 0.001$), and early adulthood ($r = 0.33$; $P = 0.021$); BMI at adolescence was positively correlated with BMI percentiles during young adulthood ($r = 0.76$; $P < 0.001$) and early adulthood ($r = 0.65$; $P < 0.001$); BMI at young adulthood was positively correlated with BMI percentiles during early adulthood ($r = 0.76$; $P < 0.001$) (**Figure 1**).

Body mass index percentiles were positively correlated with the metabolic comorbidities: SBP percentiles (childhood $r = 0.21$, $P = 0.05$; young adulthood $r = 0.32$, $P = 0.005$, early adulthood $r = 0.35$, $P = 0.009$), DBP percentiles (young adulthood $r = 0.24$, $P = 0.043$), FPG (adolescence $r = 0.24$, $P = 0.020$; young adulthood $r = 0.31$, $P = 0.007$; early adulthood $r = 0.33$, $P = 0.016$), HbA1c (early adulthood $r = 0.38$, $P = 0.010$), and TG levels (childhood $r = 0.27$, $P = 0.028$; young adulthood $r = 0.25$, $P = 0.053$). The occurrence of coexistent metabolic comorbidities and the number of comorbidities in each patient were positively correlated with BMI percentile ($r = 0.35$, $P = 0.002$ and $r = 0.383$, $P = 0.001$, respectively).

Logistic regression analysis showed that karyotype, GH therapy, HRT, autoimmune thyroid disease, or celiac disease were not associated with the occurrence of metabolic comorbidities.

DISCUSSION

Although the cardinal manifestations of TS during childhood and adolescence are short stature and sexual infantilism, a wide spectrum of cardiometabolic risk factors (overweight, hypertension, impaired glucose metabolism and dyslipidemia) also has its start in childhood, increasing the risk for atherosclerosis and cardiovascular disease across the patient's lifespan (11, 12, 20–23). The evolution of these metabolic disorders from childhood to adulthood has not yet been completely defined. In this longitudinal retrospective study of a relatively large cohort of TS patients followed in our institution from childhood to early adulthood, we found that the prevalence of overweight/obesity and cardiometabolic disorders identified in childhood increased with age. Since adolescence the increased rate and cooccurrence of the metabolic disorders in each patient were associated with overweight/obesity. A more prominent clustering of metabolic comorbidities in 45,X monosomy were found. Our findings confirm previous observations that an abnormal metabolic profile appears at a relatively young age in girls with TS (8, 11, 12, 24–27).

Although the development of the comorbidities from childhood through adolescence to young/early adulthood was similar in all these TS patients, both those with 45,X monosomy and those with other karyotypes, clustering of metabolic risk factors was more prominent in those with 45,X monosomy. It has been suggested that X chromosome gene dosage (complete absence of the second X chromosome or haploinsufficiency of genes on the X chromosome) has an impact on the occurrence of metabolic disorders (9, 28, 29). Our data, however, showed a similar prevalence of overweight, elevated BP, impaired glucose metabolism, and abnormal lipid profile in TS patients with 45,X monosomy and those with other karyotypes, not only during childhood but also through adolescence and young adulthood. These findings are consistent with those of Irzyniec and Jeż, who demonstrated a similar rate of metabolic risk factors in all the karyotype subgroups (30), but in contrast to previous studies reporting a higher rate of metabolic disorders among patients with 45,X monosomy (4, 9, 28, 29). The parental origin of the single X-chromosome has also been linked to the incidence of metabolic disorders. Women with a single maternal X-chromosome were found to be more prone to excessive visceral adiposity and dyslipidemia (9, 31). Regrettably, such an analysis was not performed in our studied cohort and therefore we cannot confirm or negate possible X-chromosome gene imprinting.

A plausible explanation for the early development of metabolic impairments could have been overweight and obesity. However, in a substantial number of the girls in our study already

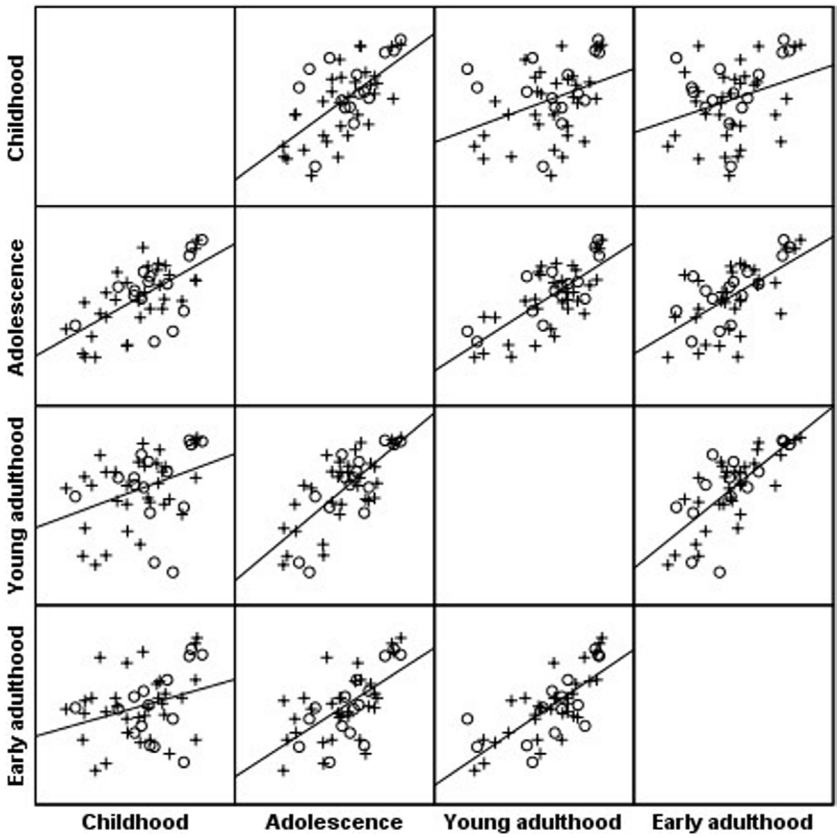

FIGURE 1 | Scatterplot matrix of body mass index (BMI) percentile along four timepoints: BMI percentiles of the Turner syndrome cohort at childhood were positively correlated with BMI percentiles during adolescence ($r = 0.69$; $P < 0.001$), young adulthood ($r = 0.43$; $P < 0.001$), and early adulthood ($r = 0.33$; $P = 0.021$); BMI at adolescence was positively correlated with BMI percentiles during young adulthood ($r = 0.76$; $P < 0.001$) and early adulthood ($r = 0.65$; $P < 0.001$); BMI at young adulthood was positively correlated with BMI percentiles during early adulthood ($r = 0.76$; $P < 0.001$). o, 45,X monosomy, x, all other karyotypes.

displaying cardiometabolic risk factors during childhood the BMI percentile was normal. As in other studies, our findings showed that hypertension, impaired glucose metabolism and dyslipidemia were not associated with body weight in young girls with TS (7, 24–27). It would therefore appear that these early metabolic derangements may be attributed to risk factors inherent to TS: impaired glucose metabolism—to pancreatic beta cell dysfunction and decreased insulin secretory response to glucose (8, 12); abnormal circadian BP rhythm and elevated BP—to autonomic dysfunction with altered vascular tone (7, 32). It was, however, clear that increased weight aggravated the metabolic profile of these patients in adolescence and young adulthood.

The evolution of weight gain observed in our TS patients was characterized by an increasing rate of overweight and obesity from childhood to young adulthood in the entire cohort, with BMI percentile consistently higher in girls with 45,X monosomy. It is noteworthy that a high BMI percentile in childhood predicted overweight and obesity in adolescence and young/early adulthood. These findings agree with those of earlier and more recent studies (33–36). The natural history of the metabolic risk factors in our study cohort revealed a sustained increase in the prevalence of the metabolic comorbidities from childhood to young/early adulthood. In agreement with previous studies, we found that the occurrence of hypertension, impaired glucose metabolism and hypertriglyceridemia were positively correlated with the increased BMI (7, 12). Interestingly, clustering of several metabolic risk factors was more prevalent in those of the TS patients with 45,X monosomy who displayed increased BMI since adolescence.

In this observational longitudinal study, the issue of causality of the metabolic comorbidities could not be addressed. There is, however, an indication that increased weight during adolescence and young adulthood aggravated insulin resistance, glucose intolerance and hypertriglyceridemia, and contributed to the development of hypertension. Importantly, and similarly to previous reports, we found no evidence that GH treatment, age at onset of spontaneous puberty or at induction of puberty, or estrogen treatment (oral or transdermal) had any impact either on weight gain or on the development of metabolic comorbidities in our patients with TS (37–39).

Obesity, hypertension, impaired glucose metabolism and hyperlipidemia when identified in childhood are all modifiable risk factors. Thus, our findings come to emphasize the importance

of regular assessment of weight status and metabolic risk factors in girls with TS from early childhood. Early detection of increased weight gain and of metabolic risk factors allows timely intervention to prevent the overweight and the development of metabolic impairments and their progression toward overt metabolic comorbidities.

The main strength of our study was the longitudinal data obtained from a relatively large cohort of TS patients followed in our tertiary center from childhood to early adulthood. The main limitation of our study is the lack of clinical measures of body adiposity such as skinfold thickness, waist circumference, bioelectrical impedance, or dual-energy X-ray absorptiometry as well as the lack of quantitatively determined atherosclerosis (intimal–medial thickness) by carotid arterial ultrasound. Despite several limitations, BMI is considered a reliable and clinically valid screening tool for obesity. Furthermore, our data include markers of impaired glucose metabolism associated with adiposity such as measures of insulin resistance (HOMA-IR) and IGT. Another limitation is lack of information regarding the origin of the missed X-chromosome.

In conclusion, TS patients followed in our tertiary center from childhood to early adulthood showed a sustained increase in the prevalence of their metabolic comorbidities. While the abnormal metabolic profile during childhood most likely stemmed from risk factors inherent to TS, the aggravation of the metabolic derangements was associated with the increased weight observed during adolescence and young adulthood. Therefore, regular screening of weight and metabolic risk factors and efforts to prevent and control obesity in young TS patients should be accorded a high priority already from childhood. The more prominent clustering of metabolic comorbidities in TS girls with 45,X monosomy and those with overweight/obesity underscores the importance of a still more vigorous intervention in these groups. Future research should address whether modification of these variables at a young age can alter the metabolic outcomes in adulthood.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Rabin Medical Center Institutional Review Board. The protocol was approved by the institutional review board, because there was no identification of the patients for whom data was retrieved, informed consent by the patients was waived.

AUTHOR CONTRIBUTIONS

YL and LL contributed to the conception and design of the study, acquisition of the data and interpretation of data, drafted the article, revised it, and gave their final approval of the version to be published. SL contributed to the data analysis, data interpretation, and gave final approval of the version to be published. ES-D contributed to the conception of the study, acquisition of data, and gave her final approval of the version to be published. NN contributed to acquisition of data and gave final approval of the version to be published. NW, SS, LV, AT, and MP contributed to acquisition of data, revised the manuscript critically for important intellectual content, and gave their final approval of the version to be published.

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REVIEW
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Glucose Metabolism in Turner Syndrome

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Turner syndrome (TS) is one of the most common female chromosomal disorders. The condition is caused by complete or partial loss of a single X chromosome. Adult patients with TS have a high prevalence of diabetes mellitus (DM). Deranged glucose metabolism in this population seems to be genetically triggered. The traditional risk factors for DM in the general population may not play a major role in the pathogenesis of DM in patients with TS. This review focuses on the latest research studies pertaining to abnormalities of glucose metabolism in TS. We extensively review the available evidence pertaining to the influence of insulin secretion and sensitivity, obesity, autoimmunity, lifestyle, growth hormone, and sex hormone replacement therapy on the occurrence of DM in these patients.

Keywords: turner syndrome, glucose metabolism, insulin resistance, diabetes mellitus, growth hormone, estrogen

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INTRODUCTION

Turner syndrome (TS) is one of the most common chromosomal disorders of female development. The estimated prevalence of TS is 25–50 per 1,00,000 females (1). The condition is caused by complete or partial deletion of an X chromosome in all or some of the somatic cells (2, 3). The diagnosis is based on the karyotype analysis of peripheral blood lymphocytes (4). About 50% of patients have haplotype 45, X, while about 20–30% have chimerism 45, X/46, XX, 45, X/47, XXX, and some chromosomal structural abnormalities. The typical symptoms of TS include short height, webbed neck, low hairline at the back of the neck, low-set ears, markedly elevated levels of follicle stimulating hormone (FSH), chronic otitis media (OM), lymphedema of extremities, small mandible, and multiple pigmented nevi (4). Patients with TS are often affected by many other comorbidities, including autoimmune diseases (AD), hypothyroidism, kidney dysfunction, loss of ovarian function or other reproductive disorders, neurological or ophthalmological abnormalities, osteoporosis, diabetes mellitus (DM), dyslipidemia, hypertension, and heart disease (2, 5–10).

The increased incidence of DM in TS patients was first reported almost 50 years ago by Ann Forbes and Eric Engel (11). Abnormal glucose metabolism is found in >70% of adults affected by TS (12, 13); the abnormalities include impaired glucose tolerance (IGT), hyperinsulinaemia, and reduced insulin sensitivity. Increased prevalence of DM coupled with higher incidence of cardiovascular disease in patients with TS may contribute to increased mortality in this population. The specific DM phenotype associated with TS remains unclear. An epidemiologic study in Denmark showed that the incidence of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) in TS patients is 11 times and 3–4 times greater than that in healthy people, respectively (14). However, clinical endocrinal studies in adult populations suggest that the phenotype of gradually progressive, adult-onset glucose intolerance is more likely to be T2DM (13, 15). Indeed, the link between TS and T1DM is not well characterized. In the National Cooperative Growth study

in the United States, the incidence of T1DM among girls with TS (n = 5,220) was greater than that in the general population; however, the standardized incidence ratio (SIR) was not statistically significant (SIR, 0.92–4.18) (16). According to the latest clinical practice guidelines for TS, the prevalence of glucose intolerance and T2DM in patients with TS is 15–50 and 10%, respectively; however, the prevalence of T1DM is yet to be determined (2). It is hypothesized that occurrence of DM in TS is linked to insulin resistance (15) or impaired β-cell function (17). However, the precise mechanism of development of DM in this patient population is not clear.

This review focuses on the latest updates about the pathogenesis of deranged glucose metabolism in TS and the effect of routine TS therapy on the glucose metabolism in these patients. Beginning with current controversies related to the mechanisms, we review the latest evidence pertaining to the intrinsic risk factors, potential confounding variables, and the effect of growth hormone (GH) and EP (Estrogen/Progestin) therapies on DM incidence. The objective of this review is to provide insights into the treatable and non-treatable risk factors for development of DM in TS patients.

OVERVIEW OF GLUCOSE METABOLISM IN TS PATIENTS

Recent work has highlighted that abnormal glucose metabolism is common in patients with TS. Currently, most researchers tend to use oral glucose tolerance test (OGTT) to study glucose metabolism in TS patients. Studies have shown that OGTT is superior to other tests (fasting blood glucose, postprandial blood glucose, or glycosylated glycoprotein levels) for diagnosis of early abnormalities of glucose metabolism in these patients (18).

The incidence of IGT in TS patients is about 10–34%, which is higher than that in the healthy population. Moreover, even though some patients with TS have normal fasting blood glucose and glycated hemoglobin levels, the incidence of IGT is generally higher than that in the healthy subjects, irrespective of the age of the patient (TS girls or TS adult women) (19). In order to rule out the negative effects of obesity and gonadal dysplasia on glucose metabolism, Bakalov et al. compared TS patients with age- and body mass index (BMI)-matched control group with normal karyotype but premature ovarian failure (17). The incidence of IGT in TS group was still significantly higher than that in the control group.

Some studies have shown a stronger correlation of age with the occurrence of abnormal glucose metabolism in TS patients. In a study by Cicognani et al. (20), the incidence of IGT in TS children (age: 5–12 years) was 40%, while the incidence in TS adolescents (age: 12–16 years) was 23.5%, however, the corresponding incidence in adult women was 25–78% (7, 12, 17). In a recent cohort study of 103 patients with TS by Lebenthal et al. (1), the proportion of patients with elevated fasting blood glucose level was 6.6%, while the corresponding proportion among children and adolescents was 8.1%. In contrast, the proportion of patients with IGT was found to increase with increase in age (children, 10%; adolescents, 16.7%; young adults, 21.4%; and adults, 41.2%). Similar findings were reported by Ibarra-Gasparini et al. (13); they also found no association of the traditional risk factors for T2DM (BMI, body composition, family history) or history of growth hormone or sex hormone replacement therapy with impaired glucose tolerance. In addition, age was the only independent predictor of DM in patients with TS.

Genetic Mechanisms of Impaired Glucose Metabolism in TS Patients

It seems that the disordered glucose metabolism in TS patients is caused by the characteristic changes associated with the disease itself. It has been hypothesized that this may result from deletion of some genes related to insulin signal transduction and β cell function located on the X chromosome.

Bakalove et al. (12) explored the possible mechanisms at the gene level; the incidence of T2DM in patients with TS was 25% (56/224), while only 1 patient had T1DM. After karyotyping, patients with 45, X and X short arm deletion (delXp) had a relatively high incidence of DM (17 and 23%, respectively), while those with X chromosome long arm loss (delXq) had a lower incidence of DM (9%). The results showed that the Xp chromosome haploid gene deficiency increases the risk of DM in the TS population.

The major X-chromosome pseudoautosomal region (PAR1) is located at the Xp end, and the lack of expression of the haploid gene is thought to be related to certain phenotypes of TS. For example, the *SHOX* gene plays a critical role in bone growth and development and its deficiency is liable to cause short height of patients with TS. *PAR1* genes (21) encode several types of receptors, phospholipases, protein phosphatases, GTP binding proteins, ATP transporter, and transcription factors. Therefore, PAR1 haploid gene deficiency may affect the insulin response by affecting the expression of the above molecules. Studies have also shown that the long arm of the X chromosome (iXq) is associated with a higher incidence of DM (43%) as compared to the 45 and X groups. Therefore, it is speculated that additional Xq copies may trigger escape-inactivated gene overexpression, including diabetes-related genes such as islet cell antigen (*ICA*), C-reactive protein (*CRP*), insulin-like growth factor-II (*IGFI-II*), and other genes related to the normal physiological functions of islet cells (*GLIS3, KLF11*) (12). This hypothesis was confirmed by comparing the relative gene expressions of 45, X ($n = 10$) with 46, X, i (X) (q) ($n = 5$) TS patients. Some researchers have found that genes encoding GAD and ICA overexpression are closely related to immunologic injury of β-cells. Therefore, it was speculated that high incidence of DM in the 46, X, i (X) (q) group was linked to the production of β-cell autoantigen. Supernumerary copies of Xq may increase the risk of diabetes even in those who do not exhibit monosomy X, e.g., among men with Klinefelter syndrome (47, XXY) and 48, XXYY (22).

In summary, it is currently believed that the high incidence of DM in the TS population may be due to Xp haplotype gene deficiency, which leads to impaired β-cell function; in addition, overexpression of some genes of Xq may aggravate the problem.

Insulin Sensitivity in TS Patients

Studies pertaining to insulin sensitivity in patients with TS have yielded inconsistent results. Most studies suggest that TS patients have impaired insulin sensitivity. Choi et al. evaluated

insulin sensitivity using the insulin sensitivity quantification index (QUICKI) in two groups of patients with impaired glucose tolerance and normal glucose tolerance; they found reduced insulin sensitivity in the IGT group. In addition, Mazzanti et al. (19) investigated the use of growth hormone therapy and insulin sensitivity in TS patients; the results showed that patients with TS have mild insulin resistance even prior to initiation of growth hormone (GH) replacement therapy. Decreased insulin sensitivity in TS patients was also uncovered in a study that compared TS patients with age- and BMI-matched controls with premature ovarian failure (17). Salgin et al. (15) also provided evidence of insulin resistance in TS patients; they suggested that insulin resistance in TS patients is not a consequence of changes in body fat or body mass. However, other studies that employed the same methods for assessment of insulin sensitivity assessment showed normal insulin sensitivity in TS patients. For example, both Hjerrild et al. (23) and Bakalov et al. (17) (QUICK study) used insulin clamp technique and found that the insulin sensitivity of TS patients is similar to that in the age- and BMI-matched normal control group.

Women with TS who have X-monosomy and X mosaicism were found to develop higher levels of insulin resistance at a younger age relative to that in age-matched controls (20). Insulin receptor substrate 4 is encoded in the Xp22.3-23 region, and it may be linked to insulin resistance in other contexts (15, 24). More work is required to identify the specific genes on the X chromosome that contribute to impaired insulin sensitivity.

Insulin Secretion in TS Patients

Several studies have demonstrated that abnormal insulin secretion is the main mechanism of development of DM in TS patients. In a cross sectional study of TS patients by Hjerrild et al. (23), insufficient compensatory insulin production during GTT was shown to result in an overall reduction in the insulin-to-glucose ratio; in addition, there was evidence of declining β-cell function. Ibarra-Gasparini et al. (13) conducted a clinical study of 113 patients with TS. During OGTT in TS patients with DM, there was insufficient insulin secretion in the first phase (60 min) after the glucose load; however, in the subsequent 60 min, there was a certain increase in insulin levels. A recent study further indicated that in TS women, traditional factors underlying insulin resistance may not be the reason of their phenotype; instead, deficiency of pancreatic beta cells may lead to impaired glucose tolerance, thereby leading to development of DM over a period of time (9). It appears that decreased insulin secretory response may be the underlying mechanism of the observed increase in the risk of DM in TSpatients.

OBESITY AND BODY COMPOSITION IN TS PATIENTS

Obesity, increased waist circumference, and high adipose content may further contribute to the increased prevalence of DM in TS patients. Patients with TS have a high prevalence of obesity owing to the impaired fitness and typically sedentary life as a result of the syndrome (25). The average waist circumference of these individuals is larger, consistent with an increase in abdominal fat, which is a risk factor for T2DM as well (9). Higher BMI values during childhood strongly predict the occurrence of obesity at later ages (26). In a large retrospective cohort study of TS patients, patients who developed obesity and cardiovascular diseases in early childhood and who showed an increasing tendency with age, were more likely to suffer from metabolic disorders such as T2DM during adolescence than non-obese healthy people (1).

Nonetheless, whether BMI, adiposity, and waist circumference are truly correlated with T2DM in patients with TS as they do in the general population remains controversial. Hamilton et al. (9) suggested that while BMI can be a good measure of fat tissue levels in a large population, in a smaller subgroup, the BMI standard deviation score (BMI-SDS) offers superior insights as it is better controlled for age in such settings. Interestingly, BMI-SDS scores of patients with TS and matched controls were found to be comparable, and this finding was further supported by an MRI assessment of adipose tissue levels. In the same study, some of the TS patients that presented cardiovascular and metabolic problems in childhood exhibited normal BMI, which further raises doubts on the link between BMI and T2DM in this population (1). Indeed, age and specific karyotype seem to be more predictive of abnormal glucose metabolism in patients with TS (20). TS may itself increase metabolic abnormalities during childhood, which consequently increases the prevalence of obesity among TS patients (1). Obesity may in turn further disrupt normal glucose metabolism and induce insulin resistance in a feedback loop, which ultimately accelerates the development of T2DM in patients with TS.

The average height of TS patients is 20 cm lower than that of their age-matched peers; the altered growth is restricted to height and does not affect the horizontal growth (27). As a consequence, the arm span and sitting height are roughly 3 standard deviations lower than that of the general population, while head circumference and hand/foot size are typically comparable. Therefore, the total and visceral fat mass in TS patients is generally elevated, and lean body mass (LBM) as well as skeletal muscle mass are decreased. General physical activity and VO2 max (maximal oxygen consumption) are also substantially decreased in those with TS (7). Similarly, fat mass in the arms, legs, and the torso of TS patients is higher than that in controls (28); this may potentially promote insulin resistance and T2DM in these patients. Gravholt et al. (7) also identified enlargement of type IIa muscle fibers in TS patients, which was related with lower requirement of oxygen and glucose content for normal metabolic activity.

Adipose tissue is currently considered as an endocrine tissue, which actively produces a wide range of bioactive adipokines including adiponectin, chemerin, and vaspin. These adipokines can regulate fat mass and the activity of adipocytes. These chemokines can broadly influence the cardiovascular and neuroendocrine systems, leading to altered glucose and lipid metabolism (29). Due to the broad regulatory roles, adipokine dysregulation in patients with TS may further contribute to the abnormal metabolic phenotypes. One study detected higher levels of adiponectin and chemerin in girls with TS (30). Another

study detected elevated interleukin (IL)-6 levels in patients with TS (31), while elevated CRP levels were found in another TS population (32); these findings suggest an ongoing chronic state of inflammation in these individuals. Further work is required to assess the mechanism by which adipokine or inflammatory cytokines alter metabolic function in TS patients.

Autoimmunity in TS Patients

The risk of AD in patients with TS is approximately 2-fold higher than that in the female general population and 4-fold higher than that in male general population. Goldacre et al. analyzed 2,459 women with TS and discovered that Hashimoto's thyroiditis, diabetes mellitus, and coeliac disease were significantly more common than that in the general population (33). Antithyroid antibodies are common, whereas celiac, diabetic, and adrenal antibodies are rare. Excessive autoimmune antibodies may be caused by defects in the X chromosome. Genes on the X chromosome, including the major histocompatibility complex located in the long arm, have been shown to regulate immune responses and alter immune tolerance (34). Recent studies have shown that the pathogenesis of TS is associated with HLA haplotypes, genetic factors, single nucleotide polymorphisms, and cytotoxic T lymphocyte-associated protein-4. The increased prevalence of AD in patients with TS is also attributable to X-chromosome haploinsufficiency, maternal origin of X chromosome, overproduction of pro-inflammatory cytokines (IL-6), reduction in anti-inflammatory cytokines (IL-10, TGF-β), or hypogonadism (35).

The proportion of TS patients with anti-GAD65 was shown to be 4%, which is slightly higher than the 1.1% prevalence among adults in the general population. A cross-sectional study of 107 Danish TS patients by Mortensen et al. suggested that non-diabetic anti-GAD-65-positive TS patients may eventually develop diabetes (36). Thus, the increased risk of DM in TS patients may be attributable to autoimmune damage of β-cells. Hence, GAD-65 antibody testing is recommended for all TS patients with newly developed diabetes. Because the occurrence of diabetes in TS patients is related to the immune system, studies based on Treg have investigated the prevention of type 1 diabetes in patients with TS (35). It is expected to reduce immune-related islet β-cell damage in patients with TS via immunological regulation, thereby reducing the incidence of diabetes in patients with TS.

Lifestyle of TS Patients

TS is likely to lead to various complications including lifestyle-related diseases such as diabetes mellitus, hypertension, and dyslipidemia. In a survey of 492 patients with TS (age ≥17 years), the prevalence of diabetes in patients aged ≥20 years was 6.3%. This survey also demonstrated a close association of lifestyle-related diseases with the severity of obesity rather than the karyotypes (11). As TS is associated with an increased risk of obesity; obesity may in turn further disrupt normal glucose metabolism, lead to development of insulin resistance, and eventually accelerate the development of T2DM. Over the past 40 years, the incidence of preobesity and obesity has increased dramatically across all age-groups worldwide (12). Therefore, we speculate that the increased prevalence of diabetes among TS patients may be associated with the incidence of preobesity and obesity. Sienkiewicz-Dianzenza et al. (37) indicated that the level of physical activity of girls with TS was not affected; however, further research suggests that the typical features of TS such as growth deficit and abnormal body proportion may contribute to physical weakness in these girls. We believe that patients with TS have unique lifestyle, such as reduced physical activity and sedentary lifestyle. Other common risk factors for T2DM, such as social environment and unhealthy dietary habits, are also risk factors for development of obesity and hyperglycemia. For TS women, especially those who are obese, it is important to follow the nutritional instructions and exercise therapy to control their body weight to prevent or alleviate diabetes.

EFFECT OF GROWTH HORMONE (GH) OR EP (ESTROGEN/PROGESTIN) THERAPY

Short stature is the dominant physical characteristic of TS, which has been linked to the *SHOX* gene (38). Current data suggests that the short stature is not due to a lack of GH (39); however, GH replacement therapy was shown to increase the height of these patients (19). Clinically, the use of GH therapy is now a standard means to achieve normal height of TS patients, typically starting at the age of 4–6 years.

GH can disrupt normal insulin signaling, and may potentially alter glucose metabolism. High level of GH can reduce skeletal muscle glucose uptake and increase hepatic glucose production, which aggravates insulin resistance (40).

Whether GH replacement therapy increases the risk of T2DM in TS patients is not clear. A recent retrospective study of children with TS who used GH replacement therapy for 7 years found

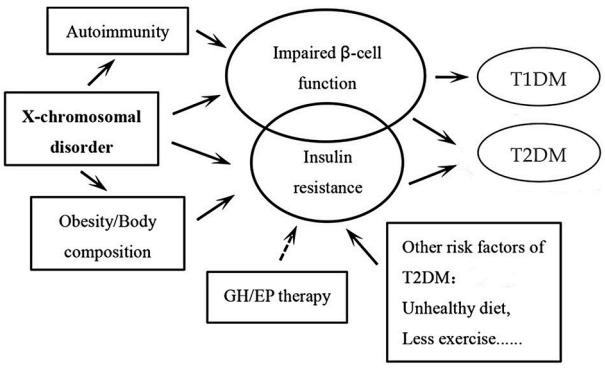

FIGURE 1 | Schematic illustration of the pathogenetic mechanisms of the development *of diabetes mellitus* (DM) in patients with Turner syndrome. The circle of impaired β-cell function is bigger than the one of insulin resistance, as we believe that insulin secretory response to glucose is a facet of TS that likely underlies the elevated risk of DM in these patients. It is the same for the circles of type 1 DM and type 2 DM. Among all the pathogenetic factors, X-chromosomal disorder may play the most important role. The arrows "→ " indicate that more evidence may still be needed in this respect. GH: growth hormone; EP: Estradiol and progesterone; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

no significant change in insulin sensitivity or β-cell secretory capacity (41). In a study of rhGH replacement therapy in 28 Chinese children, follow-up of children with TS and IGT did not reveal aggravation of deranged glucose metabolism (42). It is hypothesized that GH may have an insulin-like function, with the exception of increasing blood sugar. GH-induced IGT may indirectly promote insulin secretion and enhance arginine and glucose-stimulated insulin secretion, which may explain the lack of worsening of glucose metabolism during treatment. In another study (43), TS children treated with GH were found to have lower subcutaneous and visceral fat content and improved glucose tolerance than those who did not receive GH treatment; this suggests a protective effect of GH against metabolic dyshomeostasis in TS patients.

In another study, insulin sensitivity was slightly reduced in patients with TS at 4 years of initial GH therapy, especially during the first 6–12 months; the insulin sensitivity remained relatively stable in the subsequent 7–8 years of GH treatment, which may be related to an increase in lean body mass and a decrease in fat content (19). However, with the completion of GH treatment, insulin sensitivity is likely to recover slightly, although it cannot be restored to the pre-treatment level. Studies have shown that this increase in insulin sensitivity is not affected by BMI or triglyceride levels, but is related to the age at which GH therapy is discontinued. Although hyperinsulinemia and insulin resistance caused by GH therapy are reversible, the long-term effects are still unknown. Bannik et al. followed up TS patients after the completion of GH therapy (mean: 4.8 ± 1.9 years). Compared with pre-treatment, fasting insulin level was still high and insulin sensitivity was still low (44).

Another common characteristic of TS is hypergonadotropism, resulting in amenorrhea. As such, in order to induce puberty and development of female sex characteristics, EP (Estrogen /Progestin) therapy is typically required for TS patients. Estrogen replacement therapy in these individuals is initiated around the age of 12 years, with progesterone added to the therapeutic program within the first 2 years (2).

Seventeen-estradiol signaling is beneficial for energy homeostasis, skeletal muscle, adipose tissue, liver, pancreas, as well as the cardiovascular system (45). Animal research suggests that estrogen may suppress β-cell apoptosis (46) and may further regulate gene expression pertaining to insulin secretion and glucose uptake (47, 48). Human studies have further suggested that HRT may protect postmenopausal women against DM by counteracting the deficiency of insulin (49, 50).

Whether HRT may have some additional effect on glucose intolerance or development of DM in TS patients is not well-understood. Some studies have shown that estrogen replacement therapy can aggravate insulin resistance (7, 51). Gravholt et al. compared 24 women with TS treated with EP therapy and age-matched healthy women; they found that sex hormone replacement therapy increased IGT and the incidence of insulin resistance in the TS group. Giordano et al. conducted a study of adult women with TS (mean age: 32.4 ± 1.3 years) who received EP therapy and age-matched healthy women; they found that therapy increased coronary heart disease, IGT, insulin resistance, and hypertriglyceridemia in patients with TS (14). However, other similar studies found no impact of EP therapy on metabolic parameters (such as weight gain or glucose tolerance) in TS patients (52, 53). A study showed that 24-month estrogen replacement therapy has no significant effect on blood glucose, insulin sensitivity, or insulin levels in patients with TS; in addition, blood glucose levels may even be slightly reduced with increasing estrogen dose. The authors claimed that EP therapy may have a slight beneficial effect on glucose metabolism in patients with TS. In addition, the macrophage marker SCD 163, which is associated with insulin resistance and is elevated in TS patients, may reduce to some extent in patients receiving estrogen therapy (54). Thus, estrogen may alleviate the inflammatory state in TS patients and play a beneficial role in the maintenance of glucose homeostasis. Another study (55) did detect a trend of reduced arm fat mass ($p = 0.054$) and increase in total lean mass ($p = 0.054$) and trunk lean mass ($p = 0.074$) in patients utilizing estradiol gel. This may have been the consequence of the mode of percutaneous administration that bypasses the first liver metabolism, thereby allowing for smaller doses that are less likely to disrupt normal hepatic metabolic activity. However, guidelines established in 2017 (2) indicated that the superiority of transdermal (TD)estrogen to oral estrogen administration still remains controversial.

Given this uncertainty and the definitive efficacy of GH and EP treatment, annual monitoring for glucose tolerance based on glycosylated hemoglobin levels is recommended for all TS patients.

CONCLUSION

Women affected by TS are at a significantly higher risk of DM. The underlying pathogenetic mechanism of DM development in TS is illustrated in **Figure 1**. Glucose intolerance appears to be an intrinsic defect associated with TS, although the genetic mechanisms are yet to be completely understood. Autoimmune factors may be involved in this process, although there is no definitive evidence in this respect. The traditional risk factors for type 2 DM in the general population (obesity, high visceral adipose content) do not seem to be a major factor in TS, especially in children. Growth hormone or estradiol/prosgesterone therapy in TS patients during childhood or adolescence may not alter the metabolic derangement or lead to development of DM in a destructive feedback cycle (1). Additional long-term prospective studies are required to verify these results.

In addition to disease-intrinsic DM risk factors, unhealthy lifestyle can also influence the development of DM. It is therefore important for TS patients to undergo regular weight monitoring, assessment of metabolic risk factors, and counseling pertaining to nutrition and physical activity. Further high quality RCT studies will provide additional evidence for the effective management of metabolic diseases in patients with TS.

AUTHOR CONTRIBUTIONS

GW devised the main concept of the manuscript. LS and XG collected data and wrote the first draft of the manuscript. YaW and TZ contributed to editing this work. XZ and YiW contributed to the revision of draft. All the authors have read and approved the submitted version.

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Immunological Profile and Predisposition to Autoimmunity in Girls With Turner Syndrome

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Objective: The risk of autoimmune diseases (AD) in patients with Turner Syndrome (TS) is twice higher than in the general female population and four times higher than in the male population. The causes of the increased incidence of AD in TS are still under discussion. We hypothesized the presence of a specific humoral, cellular, and regulatory T cell (Treg) immunity profile which predisposes to AD, disorders of immunity, and disorders of immune regulation.

Methods: The study encompassed 37 girls with TS and with no signs of infection. The control group included 11 healthy girls with no hormonal disorders. A medical history focused on AD and immunity disorders was taken from all participants. The levels of: immunoglobulins IgG, IgA, IgM, total lymphocytes, lymphocytes subpopulations CD3+, CD4+, CD8+, CD19+, natural killer cells, Treg cells (CD4+ CD25+ CD127− FOXP3+), anti-inflammatory cytokines (interleukin-10, transforming growth factor-β), anti-nuclear antibodies, glutamic acid decarboxylase (GAD65 Abs), anti-thyroid peroxidase (anti-TPO Ab), and anti-thyroglobulin (anti-TG Ab) autoantibodies were determined in each participant.

Results: The mean age and BMI in the TS group and in controls were comparable (11.9 ± 4.1 vs. 12.5 ± 4.0 years; 19.2 ± 3.4 vs. 19.7 ± 4.6, $p > 0.05$). Mean hSDS was significantly higher in controls (−2.2 ± 0.9 vs. −0.4 ± 1.5, $p < 0.0001$). AD and recurrent otitis media with complications were previously confirmed in 9 (24.3%) and 10 (27.0%) girls with TS. The TS group had significantly lower levels of IgG ($p = 0.02$), lower%CD4 ($p < 0.001$) and a significantly lower CD4:CD8 ratio than the controls ($p < 0.001$). There were no differences in mean Treg% between girls with TS and healthy controls. However, comparing Treg% between the TS group with coexisting autoimmunity and the remaining participants, a statistically significant difference was observed (2.09 ± 0.5 vs. 2.77 ± 1.6, $p = 0.048$). Patients with iXq had lower CD4% and more frequently had positive anti-TPO Ab and anti-TG Ab compared to the remaining girls with TS and controls ($p = 0.001$, $p < 0.001$, $p = 0.01$).

Conclusion: TS predisposes to AD, especially if associated with coexisting iXq. Our preliminary findings show that patients with TS may present a specific profile of humoral and cellular immunity markers, different from healthy girls.

Keywords: Turner syndrome, autoimmunity, lymphocytes subpopulation, T regulatory cells

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INTRODUCTION

With an incidence of 1 in 2,000–2,500 liveborn female infants, Turner Syndrome (TS) is one of the most frequent chromosomal aberrations. It is characterized by short stature, incorrect gonadal development with puberty disorders, kidney, and circulatory system diseases, recurrent otitis, impaired hearing and an increased incidence of autoimmune diseases (AD).

The risk of ADs in patients with TS is approximately twice as high as in the general female population and four times higher than in males. The most common autoimmune disorder associated with TS is Hashimoto's thyroiditis; other disorders include: celiac disease, type 1 diabetes mellitus, vitiligo, alopecia areata, ulcerative colitis, Crohn's disease, psoriasis, idiopathic thrombocytopenic purpura, and juvenile rheumatoid arthritis (1–3). The pathogenesis of AD is complex and depends on numerous mechanisms, such as family factors connected with the HLA haplotype, genetic factors related to single nucleotide polymorphism or cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), present for example on regulatory T cells (Tregs). One of the mechanisms leading to disorders of immune regulation is lack of Tregs-dependent immunosuppression (4).

Regulatory T cells are specialized T cells with the phenotype CD4+ CD25+ FoxP3+, whose function is to maintain self-tolerance and immune homeostasis by suppressing the activation, proliferation, and effector functions of various immune cells. Tregs mediate suppressive function through a variety of mechanisms and functional specialization depending on the type and location of the immune response. Tregs express CD4 and CD25 surface antigens, and intracellular forkhead family transcription factor (FoxP3)—crucial for their suppressor function. FoxP3 is coded by *AIRE*, which is located on the X-chromosome. Mutation in this gene results in disorders of tolerance to organ-specific antigens. One function of Tregs involves the secretion of interleukin-10 (IL-10), which inhibits effector T cells responses. Tregs also secrete transforming growth factor-β (TGF-β) to induce conventional T cell by FoxP3 to differentiate into Treg. As Tregs control the peripheral immune response, their potential role in the development of AD has been hypothesized.

The increased risk of autoimmunity in patients with TS has also been attributed to X-chromosome haploinsufficiency, maternal origin of the X-chromosome, excessive production of proinflammatory cytokines (IL-6), decrease in anti-inflammatory cytokines (IL-10, TGF-β), or hypogonadism (5, 6).

The impact of three copies of genetic material on the long arm of the X-chromosome and an increased incidence of AD in girls with the iXq karyotype have also been suggested (7, 8). Despite the importance of early detection and treatment of AD, literature reports are ambiguous, and studies related to girls with TS are very few. Accordingly, the aim of our study was to analyze the importance of markers of humoral, cellular, and Treg lymphocyte immunity profiles in girls with TS in determining their predisposition to AD or disorders of immunity/immunoregulation. Additionally, the impact of karyotype on autoimmunization in TS was assessed.

PATIENTS AND METHODS

The study included 37 unselected, consecutive girls with TS treated at the Department of Pediatric Endocrinology, and 11 healthy controls. The immunological profile and the presence of clinical or preclinical autoimmune disorder markers were assessed.

All the girls with TS were under treatment with recombinant growth hormone (GH, 47–66 μg/kg/day); the control group was composed of healthy girls with no hormone-related disorders. A detailed history of chronic diseases was taken from all study participants. The exclusion criteria were: an ongoing inflammatory process or lack of informed consent to participate in the study.

Laboratory Parameters

Venous blood samples were drawn from the antecubital vein in the morning, after overnight fasting. Immunoglobulins IgG, IgA, IgM, total lymphocytes, lymphocytes subpopulations CD3+, CD4+, CD8+, CD19+, natural killer (NK) cells, Treg cells (CD4+ CD25+ CD127− FOXP3+), anti-inflammatory cytokines (IL-10, TGF-β), anti-nuclear antibodies (ANA), glutamic acid decarboxylase (GAD65 Abs), anti-thyroid peroxidase (anti-TPO Ab), and anti-thyroglobulin (anti-TG Ab) autoantibodies were analyzed. Additionally, insulin-like growth factor 1 (IGF1) was assessed in TS patients.

The numbers and percentages of lymphocyte subpopulations were determined using a standardized 4- color FACS-analysis on Becton-Dickinson cytometer (BD FAX-Caliber) and commercial reagents. CD19+ was marker for B-cells and CD3+ for T-cells, CD3+ CD4+ for helper T-cells, CD3+ CD8+ for cytotoxic T-cells, CD56+ CD3− for NK-cells. The ratio of CD4+/CD8+ was also calculated. The Human Treg cocktail (BD Pharmingen™), a three-color reagent, was used to identify the natural Treg cell population. The expression pattern of CD4+ CD25*int*/brightCD27*dim* correlated with the expression of the transcription factor Forkhead box P3 (FoxP3), a specific marker of Tregs.

Transforming Growth Factor-β was analyzed using a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle (DRG TGF-β ELISA Kit). Human interleukin -10 (IL-10) was analyzed by immunoenzymetric assay, a solid-phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate (DIAsource IL-10-EASIA). Enzyme immunoassay (Medizym anti-GAD) was used to determine GAD65 Abs in human serum. Anti-TPO Ab and anti-TG Ab concentrations were determined with radioimmunoassay (Izotop, Hungary). Values above the manufacturer-defined assay cutoff points were considered positive. IGF1 was measured by solid-phase enzyme-labeled chemiluminescent immunometric assays (IMMULITE, DPC).

The results were compared to published age-related in-house reference ranges, except for Treg, IL-10, and TGF-β, which were compared between TS and control group.

Analysis of Karyotype Impact

In order to determine the impact of the presence of isochromosomes for the long arm of the X-chromosome (iXq) in the karyotype of blood lymphocytes on the presence of autoimmunity disorders, a subgroup of girls with iXq was identified and

compared with the remaining girls with TS (TS non-iXq) and controls.

Statistical Analysis

Statistical analyses were performed with STATISTICA version 13. Comparisons between two groups were performed with two-sided Student's *t*-test or Student's *t*-test with separate variance estimates, as appropriate. Comparisons between three groups were performed using ANOVA or Kruskal–Wallis rank ANOVA, as appropriate. Linear correlation analyses were used to asses IGF1 influence. Data are presented as means and SDs, and percentages. *P*-Values of <0.05 were considered significant.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Medical University of Silesia. Written informed consent was obtained from the participants' parents or legal custodians and from all participants aged over 16.

RESULTS

The study encompassed 48 girls aged from 3.4 to 18.2 years old. The median (range) age was 12.8 years (min. 3.4, max. 18.2) in the TS group and 12.8 years (min. 6.3, max. 17.9) in the control group. The detailed characteristics of the study group and controls are presented in **Table 1**. Out of the 37 girls with TS (study group), 9 (24.3%) had a previously confirmed AD: Hashimoto's disease (7), psoriasis (1), celiac disease (1), and vitiligo (1); two girls had coexisting thyroiditis and celiac disease or vitiligo. Except for one girl with coexisting Hashimoto's diseases and celiac disease (both diagnosed 6 years earlier), all the remaining girls with a confirmed AD had high levels of anti-thyroid antibodies.

Positive anti-TPO Ab and/or anti-TG, together with normal thyroid function (clinical and laboratory assessment) were observed in five girls in the study group. The controls showed no signs of an AD and their antibodies test was negative. No ANA and anti-GAD were observed in any of the girls. Recurrent otitis media with complications was observed in 10 (27.0%) girls in the study group and in none of the controls.

All the study participants had a normal leukocyte count ($\geq 4.0 \times 10^3/\mu l$), and none had neutropenia. Six (16.2%) girls from the study group and one (9.1%) control had a slightly lowered lymphocyte count, ranging from $1.5 \times 10^3/\mu l$ to $4.6 \times 10^3/\mu l$, but not below $1 \times 10^3/\mu l$.

The mean leukocyte and lymphocyte counts did not differ significantly ($p > 0.05$) between the study group and controls: 6.68×10^3 and $2.23 \times 10^3/\mu l$ vs. 7.25×10^3 and $2.03 \times 10^3/\mu l$.

TABLE 1 | Clinical parameters—girls with TS vs. healthy controls (mean ± SD).

	TS (n = 37) mean ± SD	CG (n = 11) mean ± SD	p-Value
Age (years)	11.9 ± 4.1	12.5 ± 4.0	NS
Weight (kg)	36.7 ± 14.8	45.2 ± 19.3	NS
Height (cm)	134.8 ± 20.0	146.3 ± 21.3	NS
hSDS	−2.16 ± 0.95	−0.41 ± 1.5	<0.0001
BMI	19.2 ± 3.3	19.7 ± 4.6	NS

TS, Turner syndrome; CG, control group; n, number of patients; BMI, body mass index; hSDS, height standard deviation score; NS, not significant.

Severe immune deficiency was excluded in all the study participants based on the analysis of concentrations of all immunoglobulin classes, and the percentages and absolute counts of lymphocyte subpopulations. The comparison of mean immunoglobulin levels showed significantly lower levels of IgG in the study group than in controls; no difference was observed for the remaining immunoglobulin classes (**Table 2**). None of the immunoglobulin levels were lower than normal.

With regard to lymphocyte subpopulation, the level of CD4+ lymphocytes was significantly lower in girls with TS than in controls (**Table 2**). There were no significant deficiencies in lymphocyte subpopulations. The levels of absolute counts of lymphocytes CD3+, CD4+, CD8+, and CD19+ were slightly lower than normal for age in 4 (10.8%) girls with TS and in 1 (2.7%) control.

The study group and the controls differed significantly with regard to the CD4:CD8 ratio (1.20 vs. 1.77, $p < 0.001$). In 10 girls, the CD8+ cell count was higher than the CD4+, or equal: 9 (24.3%) girls with TS and 1 (9.1%) control. In five (13.5%) girls with TS, an abnormal CD4:CD8 ratio was observed; four of the girls presented clinical and/or laboratory signs of AD.

The percentage of Treg cells (Treg%) was between 1.2 and 7.42% in the study group and between 1.2 and 4.9% in the control group. The mean values of IL-10 and TGF-β concentrations did not differ between the study group and the controls ($p > 0.05$) (**Table 2**). The mean values of Treg% in girls with TS and coexisting autoimmunity were significantly lower than Treg% in the remaining participants (2.09 ± 0.5 vs. 2.77 ± 1.6, $p = 0.048$). No correlation was observed between Treg% and the levels of cytokines IL-10 and TGF-β.

Weak correlations between IGF1 concentrations (in reference ranges) and some markers of the immune profile in TS were found: negative with CD3+, CD8+, CD19+, NK cell counts ($r^2 = 0.22$, $p = 0.016$; $r^2 = 0.17$, $p = 0.04$; $r^2 = 0.23$, $p = 0.014$;

TABLE 2 | Laboratory markers of humoral and cellular immunity, and anti-inflammatory cytokines in girls with TS vs. healthy controls (mean ± SD).

	TS (n = 37) mean ± SD	CG (n = 11) mean ± SD	p Value
IgA (g/l)	1.08 ± 0.39	1.15 ± 0.42	NS
IgM (g/l)	0.84 ± 0.30	1.01 ± 0.42	NS
IgG (g/l)	9.14 ± 2.10	11.15 ± 3.29	0.019
TGF-β (pg/ml)	260.2 ± 121.9	195.2 ± 113.7	NS
IL-10 (pg/ml)	7.38 ± 2.30	19.23 ± 38.39	NS
CD3 (%)	66.9 ± 7.3	68.2 ± 6.0	NS
CD3 (cells/μl)	1369.5 ± 504.8	1387.8 ± 531.4	NS
CD4 (%)	32.6 ± 5.5	40.4 ± 5.8	NS
CD4 (cells/μl)	656.3 ± 227.3	817.3 ± 350.1	<0.001
CD8 (%)	28.6 ± 6.4	24.3 ± 5.4	NS
CD8 (cells/μl)	597.6 ± 272.6	494.8 ± 188.3	NS
CD19 (%)	16.4 ± 4.4	15.7 ± 4.9	NS
CD19 (cells/μl)	326.0 ± 108.9	329.4 ± 189.7	NS
NK (%)	14.3 ± 6.6	13.9 ± 6.5	NS
NK (cells/μl)	290.9 ± 171.2	263.8 ± 132.7	NS
Treg (%)	2.76 ± 1.56	2.37 ± 1.18	NS

TS, Turner syndrome; CG, control group; n, number of patients; NS, not significant; IgA, IgM, IgG, immunoglobulins IgA, IgM, IgG; CD3, CD4, CD8, CD19, lymphocytes subpopulations CD3+, CD4+, CD8+, CD19+; NK, natural killer cells, Treg cells, regulatory T cells (CD4+ CD25+ CD127− FOXP3+); IL-10, interleukin-10; TGF-β, transforming growth factor-β.

$r^2 = 0.25$, $p = 0.009$, respectively) and positive with anti-TG Ab ($r^2 = 0.17$, $p = 0.015$).

Seven girls with iXq were identified in the study group. They presented significantly lower CD4% and more frequently had elevated anti-TPO Ab and anti-TG Ab antibody titers compared to the remaining girls with TS and controls ($p = 0.001$, $p < 0.001$, $p = 0.01$). Girls with three copies of genes from Xq also presented the lowest Treg and the highest CD8, though the levels were not statistically significant (**Table 3**).

DISCUSSION

Every fourth girl with TS in our study group was diagnosed with an AD. This is in line with our previous findings and literature data, according to which approx. 20–50% of patients with TS, depending on their age, are diagnosed with an AD (1, 8–13). Chronic thyroiditis (Hashimoto's disease) was confirmed in almost a fifth of the patients; few also suffered from psoriasis, celiac disease, or vitiligo (the last two were associated with thyroiditis).

In compliance with the protocol, test exclusion criteria were: an acute infection, a recent acute disease, or a recent vaccination. Recurrent otitis, frequently with complications, was noted in the history of every fourth girl with TS. However, in only two of them, a slight deviation in immunological tests was noticed, though it was not significant from a clinical point of view. We did not observe significant immunodeficiency regarding the levels of immunoglobulins or the main lymphocyte subpopulations, which is in line with the Karolinska University Hospital reports from 2004 (14). As observed in the early 80s by Cacciari et al., also in our study, girls with TS had relatively lower IgG levels than the controls (15).

TABLE 3 | Laboratory markers of humoral and cellular immunity, and anti-inflammatory cytokines in girls with TS and the iXq karyotype vs. girls with TS non-iXq and vs. healthy controls (mean ± SD).

	TS iXq ($n = 7$) mean ± SD	TS non-iXq ($n = 30$) mean ± SD	CG ($n = 11$) mean ± SD	*p* Value
IgA (g/l)	0.92 ± 0.43	1.11 ± 0.37	1.15 ± 0.42	NS
IgM (g/l)	0.87 ± 0.32	0.83 ± 0.31	1.01 ± 0.42	NS
IgG (g/l)	9.27 ± 3.07	9.11 ± 1.88	11.15 ± 3.29	NS
TGF-β (pg/ml)	209.8 ± 93.6	271.9 ± 126.0	195.2 ± 113.7	NS
IL-10 (pg/ml)	7.70 ± 2.09	7.30 ± 2.38	19.23 ± 38.39	NS
CD3 (%)	67.2 ± 8.1	66.8 ± 7.2	68.2 ± 6.0	NS
CD3 (cells/μl)	1374.3 ± 613.6	1368.2 ± 488.6	1387.8 ± 531.4	NS
CD4 (%)	30.8 ± 5.8	33.1 ± 5.5	40.4 ± 5.8	NS
CD4 (cells/μl)	625.7 ± 287.6	664.3 ± 216.0	817.3 ± 350.1	0.001
CD8 (%)	30.2 ± 6.5	28.2 ± 6.4	24.3 ± 5.4	NS
CD8 (cells/μl)	620.0 ± 294.1	591.8 ± 273.4	494.8 ± 188.3	NS
CD19 (%)	14.2 ± 2.0	16.9 ± 4.7	15.7 ± 4.9	NS
CD19 (cells/μl)	279.3 ± 102.0	338.1 ± 109.4	329.4 ± 189.7	NS
NK (%)	16.2 ± 7.3	13.8 ± 6.5	13.9 ± 6.5	NS
NK (cells/μl)	295.2 ± 112.8	289.7 ± 185.5	263.8 ± 132.7	NS
Treg (%)	1.84 ± 0.40	2.99 ± 1.66	2.37 ± 1.18	NS

TS, Turner syndrome; iXq, isochromosome Xq; CG, control group; n, number of patients; NS, not significant; IgA, IgM. IgG, immunoglobulins IgA, IgM, IgG; CD3, CD4, CD8, CD19, lymphocytes subpopulations CD3+, CD4+, CD8+, CD19+; NK, natural killer cells, Treg cells, regulatory T cells (CD4+ CD25+ CD127– FOXP3+); IL-10, interleukin-10; TGF-β, transforming growth factor-β.

Stenberg's analyses of patients with an increased risk of disorders of immune tolerance revealed a low percentage of CD4 and a lower CD4:CD8 ratio, just as in our study group (14). A low CD4:CD8 ratio in the population of patients with TS has also been confirmed by Maureen et al. (16).

One of the mechanisms of immune regulation disorders may be the loss of control by Tregs as a result of their absence or dysfunction. Therapeutic administration of Tregs (experimental stage) with a view to stop the development of AD, e.g., type 1 diabetes, is being tested (17). We determined the percentages of CD4+ CD25+ CD127− FoxP3+ T cells phenotype and the results obtained in our study were lower than those reported in the literature (18). Like Maureen et al. (16), we observed no differences in Treg% between girls with TS and the controls. However, the percentage of Tregs in girls with TS and autoimmunity was significantly lower than in the remaining study participants.

Similar results were obtained in two studies on juvenile idiopathic arthritis (JIA) (19, 20). In Wei's study, a lower T phenotype CD4+ $CD25^{high}$ lymphocyte count, with simultaneous lower expression of CTLA-4 was confirmed in a population with JIA. The opposite was observed by Sznurkowska et al. (21), whereby the percentage of Tregs was higher in children with JIA than in controls; however, the tests were performed in newly diagnosed children before treatment.

There are contradictory results concerning Tregs depending on the selected study group (19–21). It is highly probable that the results may be influenced by the length of the autoimmunity process, degree of compensation, and organ damage.

Similar to our analysis, also other studies with a control group (21) did not confirm the presence of a relationship between the autoimmunity process and anti-inflammatory cytokine secretion (IL-10 and TGF-β). Additionally, in our study we did not observe any differences in the levels of these cytokines between girls with TS and coexisting AD, and girls with TS without coexisting AD.

In the subgroup of girls with isochromosome Xq, we observed a significantly higher frequency of autoimmunity, particularly regarding the thyroid. Similar results were obtained by other authors (7, 8). The Oxford centre (7) confirmed that in a group of 145 women with TS, over 80% of those with iXq had positive anti-thyroid antibodies. In an Italian study (8), nearly 40% of a group of 66 patients with TS had thyroiditis, significantly more frequently in the subgroup with the iXq karyotype.

We are aware of the limitations of our study. Our results may be biased by the relatively small number of participants, especially in the control group. The power analysis showed that 25 controls were needed for our TS group of 37 patients in order to have a strong effect size with Cohen's *d* of 0.8 in the *t*-test. Unfortunately, we were only able to recruit 11 controls, which gives the power of 0.63. The study was conducted in a population of children and only age-matched healthy girls were involved. The small number of girls with TS could be explained by the incidence of TS in the general population and the prospective nature of the study. Future work should also consider comparing laboratory autoimmune markers between TS patients and non-TS population with autoimmune disorders. Patients with confirmed hypogonadism or hypothyroidism were under hormone therapy. Most of our

girls with TS were treated with GH or had already completed the treatment. The GH receptor expression on immune cells (on more than 90% of B lymphocytes and monocytes, but only variably on T lymphocytes and NK cells) could suggest the presence of an impact of GH on the immune system, though it has not been fully explored yet (22–24). The low GH receptor number expressed on peripheral blood lymphocytes was confirmed by Bresson et al. (25). Studies on the effect of GH therapy both in GH deficient and non-GH deficient children on immune functions have given discrepant results; however, in most of them without significant changes (26–30). Similarly, little attention has been given to the interaction between GH and cytokines, and the published results seem to be ambiguous, or even contradictory (31). At this point, it is difficult to give a definite answer as to whether the therapy used in our patients had any impact on the obtained results. Our analysis showed only weak correlation between IGF1 and some of the immunological parameters: higher normal IGF1 concentrations corresponded with lower counts of CD3+, CD8+, CD19+, NK cells and higher anti-TG Ab.

Our results confirm a higher incidence of AD in the population with TS, especially with predisposition to autoimmunity in patients with iXq. Among the laboratory markers confirming abnormalities of humoral and cellular immunity, our attention was drawn to the low levels of immunoglobulin G, low percentage of Tregs and the low CD4:CD8 ratio. However, in view of the study limitations, our results should be considered preliminary.

The latest guidelines emphasize that the risk of AD increases with age; therefore, regular follow-up and screening are recommended, both in children and adults (e.g., thyroid function at diagnosis and thereafter annually; celiac screen starting at the age of 2, and thereafter every 2 years) (32). Identifying a specific immunological profile in patients with TS and autoimmune disease(s) could potentially be relevant in everyday clinical practice.

At present, AD are mainly treated with supplementation, in the case of organ-specific diseases, or by inflammation suppression, in the case of systemic diseases. Broadening the knowledge about disorders of immune regulation and loss of immune tolerance, especially with regard to Tregs activation, may provide new methods of therapy, both for the prevention and suppression of autoimmune disorders.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the Medical University of Silesia. Written informed consent was obtained from the participants' parents or legal custodians and from all participants aged over 16.

AUTHOR CONTRIBUTIONS

AG and EB-S designed the study, analyzed the database, and wrote the manuscript, their contribution was equal. TG, EB, MH, and EM-T prepared and analyzed the patient database and wrote the manuscript. DB and RK collaborated in designing the work and performed laboratory analyses.

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Normal Performance in Non-Visual Social Cognition Tasks in Women with Turner Syndrome

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Turner syndrome (TS) is a chromosomal disorder in women resulting from a partial or complete absence of the X chromosome. In addition to physical and hormonal dysfunctions, along with a unique neurocognitive profile, women with TS are reported to suffer from social functioning difficulties. Yet, it is unclear whether these difficulties stem from impairments in social cognition *per se* or from other deficits that characterize TS but are not specific to social cognition. Previous research that has probed social functioning in TS is equivocal regarding the source of these psychosocial problems since they have mainly used tasks that were dependent on visual-spatial skills, which are known to be compromised in TS. In the present study, we tested 26 women with TS and 26 matched participants on three social cognition tasks that did not require any visual-spatial capacities but rather relied on auditory-verbal skills. The results revealed that in all three tasks the TS participants did not differ from their control counterparts. The same TS cohort was found, in an earlier study, to be impaired, relative to controls, in other social cognition tasks that were dependent on visual-spatial skills. Taken together these findings suggest that the social problems, documented in TS, may be related to non-specific spatial-visual factors that affect their social cognition skills.

Keywords: Turner syndrome, social cognition, visual-spatial skills, emotional expressions, theory of mind, faux-pas

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INTRODUCTION

Turner syndrome (TS) is a genetic disorder, with an occurrence rate of approximately 25–50 per 100,000 females, resulting from a partial or complete absence of an X chromosome in a phenotypic female [a karyotype referred to as X-monosomy or 45, X; (1–5)]. This chromosomal absence leads to haplodeficiency of genes, which are normally expressed from both chromosomes. The physical appearance of women with TS is featured by short stature, webbed neck, and high-arched palate. They suffer from ovarian dysfunction, which leads to estrogen and androgen deficiency [e.g., Ref. (6)], and have significantly higher risks for hypertension, hypothyroidism, cardiac and renal defects, diabetes, and cancer. Treatment of TS includes induction of puberty by estrogen, and estrogen/progesterone replacement therapy in adulthood (1, 5).

Individuals with TS also demonstrate a unique psychosocial functioning profile. In childhood, girls with TS have difficulties in forming and maintaining social relations, and are more socially withdrawn than their typically developing (TD) peers. In adulthood, several studies also reveal that women with TS are less likely of achieving independent living and professional achievements that are on par with their education level [Ref. (7–10) but see Ref. (11)]. These

problems may be the consequence of the social rejection that is experienced by individuals with TS, due to their syndrome-related physique and other abnormalities. However, they may be also related, at least in part, to their impairments in social cognition. Social cognition is an omnibus term which covers several psychological processes [Ref. (12, 13); see Ref. (14) for a detailed map of components of social cognition space]. Two important aspects of it are emotion perception and theory of mind: emotion perception is the ability to detect and perceive emotionally relevant information in one's surroundings (15). Theory of mind is the ability to infer the contents of own and others mental states, including beliefs, intentions, emotions, thoughts, plans, and behavioral reactions (16). Both emotion perception and theory of mind are essential components of social functioning and, if impaired in TS, may account for their poor behavior in society.

Several studies have examined social cognition in TS. Specifically, women with TS were found to have difficulties, compared with normal controls, in recognition of emotions from facial expressions (17–20). Deficits were also detected in TS in recognizing emotional states from a restricted region of the face containing only the eyes (17). In addition, problems in theory of mind were also observed. For example, in one study participants were asked to describe short animations involving geometrical shapes (21–23). Description of these animations usually elicits mental-state descriptions, but TS women produced less mental-state descriptions than TD controls [see also Ref. (20) for similar results].

Although the accrued evidence indicates that women with TS are impaired in different aspects of social cognition it is noteworthy that all of the studies that examined the issue have used visual stimuli (e.g., faces, eyes, and animated shapes). This may be problematic since visual-spatial deficits have been widely recorded in women with TS [e.g., Ref. (24), see Ref. (3) for review]. Thus, it may be claimed that social cognition difficulties, demonstrated in TS, are restricted to visual stimuli and may be lacking, or at least attenuated when assessed through other modalities. The first goal of the present study was to investigate emotion perception and theory of mind in women with TS using tasks that consist of auditory-verbal stimuli. If social cognition impairments in TS are general, they would be seen across domains. However, if these difficulties arise or are exacerbated by the visual impairments in TS, comparable performance to TD controls is expected in non-visual social cognition tasks.

The second goal of the study was to examine the different aspects of theory of mind in TS. Theory of mind has been suggested to consist of two components, affective, and cognitive [e.g., Ref. (25)]. *Affective* theory of mind is the ability to acquire knowledge about the emotional states of others while *cognitive* theory of mind is the capacity of understanding other's beliefs and thoughts. This distinction has been supported by several studies that have shown a dissociation between affective and cognitive theory of mind among different clinical populations [e.g., Ref. (26, 27)]. In women with TS, however, it was not explored, to the best of our knowledge, and it is yet to be determined how the affective and cognitive aspects of social cognition are expressed in TS compared with TD women.

MATERIALS AND METHODS

Participants

Twenty-six women with TS and 26 TD controls participated in the study. The TS participants were recruited from the endocrinology clinic at the Ruth Rappaport Children's Hospital, Rambam Medical Center. Twenty-four of them had chromosome Xp monosomy while two others had mosaic karyotypes. Fourteen women had taken growth hormone in childhood, and 20 received estrogen/progesterone replacement therapy in adulthood. The TS and TD groups were matched on age, education, and marital status (see **Table 1**). Both the TS and TD groups belong to the same sample as in the Anaki et al. (20) study. All participants had normal or corrected-to-normal vision. The study was conducted with approval of the hospital Institutional Review Board and after obtaining informed written consent from the participants, in accordance with the declaration of Helsinki.

Materials and Procedure

Verbal and Performance IQ

Verbal IQ and performance IQ were assessed with the Similarities and the Block Design subtests, respectively, taken from the Wechsler adult intelligence scale III (28). In the similarities subtest, participants are presented with pairs of words and are asked to identify the relationship between each pair. This subtest assesses verbal reasoning, concept formation capacities, and abstract thinking. The Block Design subtest consists of two-dimensional designs which the participants construct using three-dimensional blocks. This subtest reflects visual-motor analytic and synthetic skills.

Social Cognition Tasks

Three tasks were administered to address the two goals of the present study: first, an auditory expression task, in which TS and

TABLE 1 | Demographic characteristics of the TS and typically developing groups.

	TS ($N = 26$)			TD ($N = 26$)			Significance
	Mean	SD	Range	Mean	SD	Range	
Age (years)	30.58	7.36	18–45	29.07	5.76	20–44	$t(50) = 0.67$, $p > 0.51$
Education (years)	13.90	1.90		14.04	1.71		$t(50) = 0.25$, $p > 0.80$
Marital status (% married)	35			38			$\chi^2(1) = 0.08$, $p > 0.77$
Performance IQ (WAIS-III Block Design SS)	8.88	2.41		11.38	2.86		$t(50) = -3.4$, $p < 0.001$
Verbal IQ (WAIS-III Similarities SS)	10.77	1.95		10.32	2.17		$t(49) = 0.78$, $p > 0.37$

TS, Turner syndrome; TD, typically developing; WAIS-III, Wechsler adult intelligence scale III; SS, scaled scores.

TD women were asked to identify different vocal expressions (29). In addition, participants also performed the *false belief task* (30), where short vignettes are presented and the participants have to infer the mental states of one of the characters. Finally, participants were given the *faux-pas* recognition task, where short stories are introduced and participants have to judge whether someone had said something which should not have been said (31). This task taps both affective and cognitive components of theory of mind as in order to understand that a wrong behavior has occurred one has to acknowledge two mental states: that of the addressee that feels insulted by the hurting utterance, and that of the addresser that does not know that he/she should not have said the faux-pas.

Auditory Expression Identification

The auditory stimuli used in this task were taken from the *Montreal affective voices* database, a standardized set of emotional vocal expressions designed for research on auditory affective processing with the avoidance of potential confound from linguistic content (29). From this set, we selected 6 expressions (happy, sad, fear, anger, surprise, and disgust), produced by 5 actors, and 5 actresses (60 vocalizations in total). The acoustic characteristics of the vocalizations were as follows: the median of the fundamental frequency ($f0$) was 333 Hz (SD = 103), the median sound duration was 1,094 ms (SD = 663 ms), and the median power was 72 decibels (SD = 8.56). The voices were presented on an IBM color monitor controlled by E-Prime software (Psychological Software Tools, Inc., 2000), implemented in an IBM PC-compatible computer.

Each trial began with a fixation point for 750 ms, followed by the auditory stimuli. The names of the six expressions appeared at the bottom of the screen, each with a corresponding number, and participants were asked to press the matching key. For each type of expression, the proportion of correct recognition was calculated.

The False Belief Task

The false belief task was comprised of two subtests; the first-order and the second-order false belief tasks. The former task assessed participant's ability to infer that someone can have a mistaken belief that is different from the factual reality and the participant's true belief. For example, Person A puts an object in a certain place in the presence of person B. Then, Person B leaves the room and Person A puts the object in a new location in the room. Person B then returns to the room. The participant is asked four questions: (a) a belief question—where Person B thinks the object is?, (b) a reality question—where the object is really located at the time of the return of Person B to the room?, (c) a memory question—what was the location of the object in the beginning?, and (d) a reference question—referring to some detail in the story which requires physical inference, in contrast to the mentalistic inference in the belief question.

The second-order false belief task evaluates one's ability to understand what someone else thinks about what someone else thinks. For example, when Person B leaves the room he peeks back and sees how Person A moves the object, without the latter knowing that he is being seen. Person B then returns to the room. The participant is asked the same four questions as detailed above. Importantly, the belief question in this task probes the ability to grasp that individuals can represent the mental states of other people. Specifically, the question asked is: when Person B comes back to the room, where Person A will think person B thinks the object is? Participants completed four stories in each task (first- second-order false belief). Each story was read to the participants by the experimenter, followed immediately by the four questions.

Recognition of Faux-Pas

The faux-pas task consists of 10 stories in which a faux-pas situation occurs, and 10 control stories which depict interpersonal conflict but not of a faux-pas nature (30). After each story, the participant is asked two questions to ascertain whether he recognized a faux-pas situation ("Did someone say something he should not have said?" "Who said it?"). The two follow-up questions address whether the participant understands both the affective aspect of the faux-pas (i.e., "Why shouldn't the individual in the story have said what he did?"), and the cognitive aspect of it, relating to the lack of intentionality from the viewpoint of the speaker (i.e., "Why do you think they said it?"). Finally, the participant is asked about an important detail in the story as a control condition to ensure that the story was understood. In the control stories, no faux-pas breach is made and the participants are required to provide a negative answer to the question about whether a faux-pas deed occurred. The control stories were not scored and were used as distracters. The stories were read to the participants but the printed version was placed before them to prevent any influence of memory, attention, or working memory. The answers, given by the participants, were written down by the experimenter.

Research Design

The data were analyzed using independent samples *t*-tests for the verbal and performance IQ, as well as some demographic variables, with the participants' group as a factor. The three social cognition tasks were analyzed using repeated-measures ANOVAs, with the participants' group as a factor, and specific variables, unique for each task, as within-subject and dependent variables. The significance level was set to 0.05.

RESULTS

Verbal and Performance IQ

Analysis of the similarities subtest did not reveal any differences between the TS and TD groups (see **Table 1**). In the Block Design subtest, however, TS women were less accurate than TD women.

Auditory Expression Identification

Mean accuracy for the different conditions is presented in **Figure 1**. A repeated-measures ANOVA as a function of Group (TS and TD) and Expression Type (happy, sad, fear, anger, surprise, and disgust) was performed for the accuracy measures. The analysis revealed a significant main effect of Expression Type [$F(5, 46) = 123.45$, $p < 0.0001$, $\eta^2 = 0.93$]. Pairwise comparisons

(with Bonferroni corrections) revealed that the identification of happiness (M = 0.96, SD = 0.09), followed by sadness (M = 0.90, SD = 0.10), and disgust (M = 0.81, SD = 0.10) were the easiest to identify. The three other emotions, namely, surprise (M = 0.54, SD = 0.20), anger (M = 0.62, SD = 0.21), and fear (M = 0.53, SD = 0.20) were harder to identify and their accuracy level was comparable. Importantly, the group difference was not significant [$F(1, 50) < 1$], as well as the interaction between Group and Expression Type [$F(4, 46) < 1$].

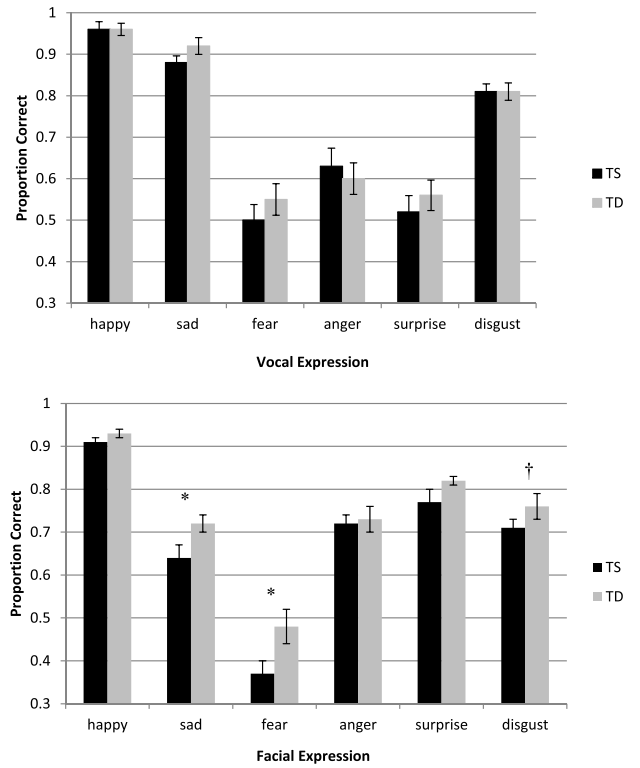

FIGURE 1 | Top—accuracy in the auditory expression identification task as a function of group (TS and TD). Bottom—accuracy in the facial expression identification task as a function of group (TS and TD). The bottom graph is reprinted from Anaki et al. (20). Face perception in women with TS and its underlying factors, *Neuropsychologia*, *90*, 274–285, with permission from Elsevier. Note—*$p \leq 0.05$, †significant only in a three-way interaction which included Group, Expression Type, and Morphing Level. TS, Turner syndrome; TD, typically developing.

The False Belief Task

The mean accuracy for the different conditions is presented in **Figure 2**. A repeated-measures ANOVA on performance accuracy was performed as a function of Group (TS and TD), False Belief Task (first-, second-order), and Question Type (belief, reality, memory, and reference). The analysis yielded a main effect of the False Belief Task variable, resulting from higher accuracy for the first- (M = 0.99, SD = 0.07) than for the second-order questions [M = 0.96, SD = 0.09, $F(1, 49) = 7.34$, $p < 0.01$, $\eta^2 = 0.13$]. Question Type was also found significant [$F(3, 47) = 4.13$, $p < 0.01$, $\eta^2 = 0.21$], with reality questions less accurate (M = 0.95, SD = 0.09) than memory or reference questions (M = 0.99, SD = 0.04 and M = 0.99, SD = 0.03, respectively). Importantly, Group was not significant, neither by itself, as the main effect, nor by interaction with other variables (all Fs < 1).

Recognition of Faux-Pas

A repeated-measures ANOVA on performance accuracy was performed as a function of Group (TS and TD), Story Type (faux-pas occurring/missing), and Question Type (faux-pas and control). The mean accuracy for the different conditions is presented in **Figure 3**.

Participants were more accurate in the control questions (M = 0.99, SD = 0.13) than in the faux-pas questions [M = 0.91, SD = 0.02, $F(1, 50) = 63.81$, $p < 0.0001$, $\eta^2 = 0.56$]. In addition, participants were more accurate answering the questions in the non-faux-pas conditions (M = 0.98, SD = 0.05) than in the faux-pas conditions [M = 0.93, SD = 0.14, $F(1, 50) = 17.52$, $p < 0.0001$, $\eta^2 = 0.26$]. Finally, a Story Type X Question Type

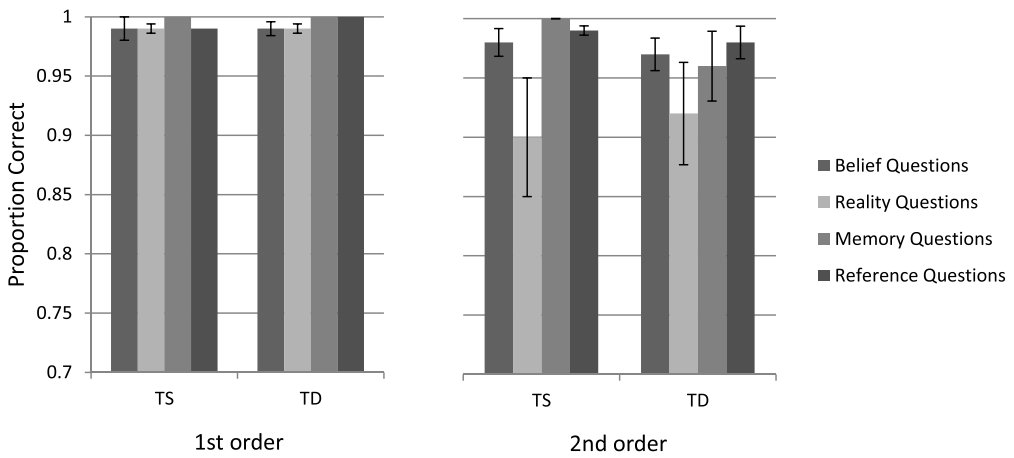

FIGURE 2 | Turner syndrome (TS) and typically developing (TD) groups performance in the false belief task (first-order, second-order questions).

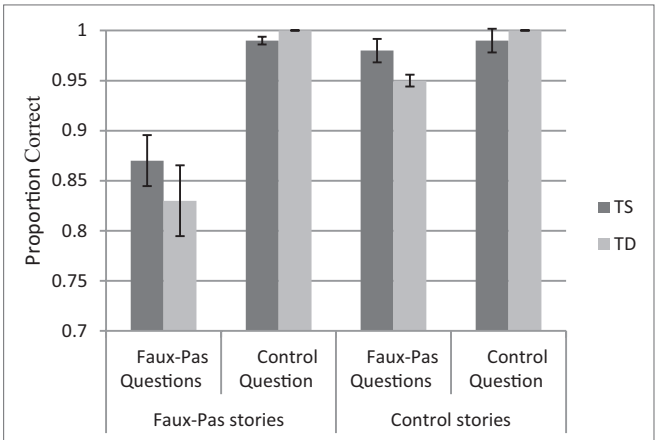

FIGURE 3 | Turner syndrome (TS) and typically developing (TD) groups performance (proportions) in the recognition of faux-pas task.

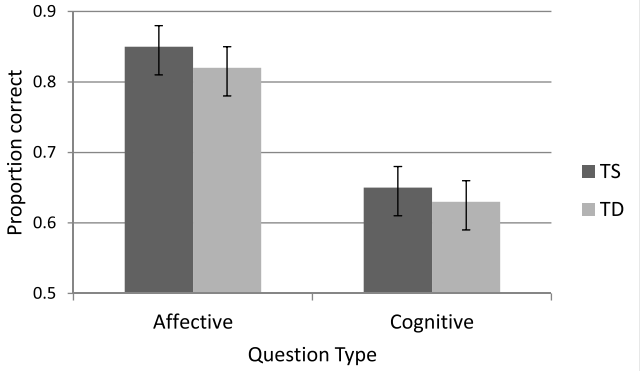

FIGURE 4 | Accuracy in faux-pas affective and cognitive questions for Turner syndrome (TS) and typically developing (TD) women.

interaction was observed [$F(1, 50) = 19.18$, $p < 0.0001$, $\eta^2 = 0.28$], indicating that for control questions accuracy was comparable across story type [M = 0.97, SD = 0.06 and M = 0.99, SD = 0.01, for faux-pas and non faux-pas stories, respectively, $t(51) = 0.57$, $p < 0.0001$]. However, accuracy for the faux-pas questions was lower in scenarios where faux-pas occurred compared with when it did not occur [M = 0.85, SD = 0.16 and M = 0.99, SD = 0.01, respectively, $t(51) = 4.35$, $p < 0.0001$]. Importantly, as in previous tasks, the Group variable was not significant by itself or with other variables.

A second ANOVA analysis was performed on the two faux-pas questions that refer to the affective and cognitive aspects of the faux-pas. As can be seen in **Figure 4** responses to the affective questions were more accurate than the cognitive ones [M = 0.83, SD = 0.17 and M = 0.64, SD = 0.21, respectively, $F(1, 50) = 126.75$, $p < 0.0001$, $\eta^2 = 0.72$]. TS women were as accurate as TD women for both affective and cognitive questions ($F < 1$).

DISCUSSION

The present study aimed to examine social cognition in TS. Although this topic was addressed to some extent in TS research, past studies have used tasks that required reliance on the visual modality (e.g., the perception of facial expressions). However, in the current study, the social cognition tasks entailed auditory-verbal capacities. In addition, the present study sought to explore affective and cognitive aspects of theory of mind and whether a unique pattern may characterize TS. The findings revealed a comparable performance of TS and TD women in all the three tasks examined. Specifically, the performance of women with TS was similar to TD women in recognizing auditory expressions, in identifying situations in which a faux-pas behavior occurred and in mentalizing the thoughts of different individuals described in short vignettes. In addition, both groups showed more cognitive than affective errors in the faux-pas task, yet no difference was found between TS and TD women in understanding cognitive and affective aspects of the theory of mind. We believe that these findings shed new light on the social cognitive capacities of women with TS, and provide an alternative interpretation of the difficulties that they experience in social functioning. In the following, we will elaborate on the theoretical implications of the present results.

Abundant findings suggest that social functioning is compromised in TS, at least to some extent [e.g., Ref. (32)]. Girls with TS are involved in fewer social activities than their peers and exhibit lower than normal competence in social interactions and interpersonal relationships (33–35). They are evaluated by their caregivers as performing poorly in social awareness, cognition, and communication (7). Women with TS are reported to have fewer intimate partners, they stay longer at their parental homes and marry at a lower rate than their control peers [e.g., Ref. (36–38)]. Some studies find that women with TS perceive themselves as having less social competence compared with controls, and report higher levels of shyness and social anxiety (39, 40).

Several accounts were proposed to explain the social difficulties that exist in TS. One hypothesis attributes these problems to social stigma arousing from body deformities related to the syndrome, such as short stature, webbed neck, and other physical deformations (e.g., cubitus valgus). However, studies have failed to find a relationship between physical appearance and social performance (41). Another potential underlying factor of the deficient social functioning may be related to the poor psychological well-being of individuals with TS due to the burden of coping with the medical, cognitive, and physiological consequences of the syndrome (42). Finally, the impaired social functioning in TS can be part and parcel of the disorder itself, reflecting an inherent deficit in social cognition [e.g., Ref. (3, 7, 32, 43)].

Past studies have provided evidence favoring this last account. For example, women with TS are less accurate than normal controls in recognizing facial expressions, perceiving eye gazes, and inferring mental states from animated objects (17–21, 44). However, the social cognition impairment hypothesis is based mainly on tasks that required visual-spatial capacities, known to be impaired in TS. Thus, these latter findings supporting specific social cognition impairment in TS may be confounded with

non-specific variables such as visuospatial factors. This possibility raises an alternative account that social functioning may be hindered by non-specific factors that are prevalent in TS, such as visuospatial difficulties (5). In order to disentangle the two accounts, the present study sought to assess social cognition in TS with non-visual stimuli. We conjectured that if TS women will show difficulties in these tasks as well, it will support the notion that social cognition is impaired in TS, regardless of modality or specific TS-related deficits. On the other hand, equivalent performance in these tasks of TS women and TD controls will support the claim that social cognition deficits in TS stem, at least to some degree, from other impairments that characterize TS.

The present findings are not compatible with the account of social cognition deficits in TS. In addition, the findings are at odds with our previous study, where the same cohort of TS women was found to be impaired, compared with TD women, in social cognition tasks (20). Specifically, in the facial expression recognition task, they were less accurate than control participants in identifying facial expressions, especially fearful, sad and, in some conditions, disgust expressions (see **Figure 1**). In addition, in the animated triangles task, women with TS were less accurate in providing descriptions of the animations. Moreover, their responses contained less mental-states portrayals of the unfolding events and more external physical descriptions of the objects' movements.

A couple of potential interpretations can be offered to explain this discrepancy. First, the tasks may have differed in difficulty and therefore TS impairments emerged only in the more challenging tasks, namely, the facial expression and animated triangles task. However, this interpretation does not seem plausible since some of the tasks were similar in difficulty, and still yielded different results. For example, the facial visual expression and auditory expression identification tasks were of similar difficulty, as indicated by the same level of accuracy obtained by TD women. TS women performed the auditory expression identification task as well as their controls. Yet, they displayed reduced proficiency in the visual expression identification task, compared with their own performance in their auditory task. The performance of the two groups in the faux-pas task was also far from reaching ceiling effects. Thus, the enhanced performance of TS women in the non-visual social cognition tasks compared with the visual tasks does not appear to result from differential levels of tasks' difficulty.

An alternative account to the discrepancy observed between the different tasks attributes the conflicting findings, regarding TS social cognition capacities, to the different modalities that have been used. According to this interpretation, TS impairments in social cognition tasks are exacerbated in tasks that are based on the visual modality. This is because TS individuals are plagued by visual-spatial impairments, reflected empirically in their lower Wechsler Performance IQ score compared with their normal Wechsler Verbal IQ score [see Ref. (3) for review, see Ref. (45)]. In contrast, their social cognition deficits are less discernible (or even non-existent) in tasks based on non-visual stimuli. Indeed, in our TS cohort we have found poorer TS performance in the Block Design subtest of the Wechsler, which is part of the Performance IQ score. The TS women were also less accurate than the TD women in performing subtests from the Birmingham Object Recognition Battery that assesses different levels of visual object perception (46). However, both TS and TD women were comparable in their performance in the Wechsler's Similarities subtest. Thus, our comprehensive findings appear to support the view that social cognition may not be impaired *per se* in TS but rather affected by non-specific visual impairments that characterize this syndrome.

The proposed explanation suggests that social cognition and its various facets (e.g., theory of mind, expression perception) are independent abilities (14). However, social cognition functioning harness other processes as well, processes that are involved in social cognition but are not specific to it. The auxiliary processes that have been the focus of the present study and Anaki et al. (20) study are visual-spatial, auditory-verbal, and facial perception capacities (**Figure 5**). In TS auditory-verbal capacities are relatively intact and therefore they are able to support aural and linguistic aspects of social cognition. In contrast, visual-spatial processes are impaired in TS, and consequently, the direct or indirect support (namely through face perception), that they can provide to social cognition, is more limited. Due to the substantial involvement of visuospatial abilities in social cognition, the social behavior of women with TS will be compromised. Indeed, partial compensation would be possible through other sensory modalities but it would not allow the full practical expression of their social skills.

The claim that social cognition by and of itself is not impaired in TS is purportedly inconsistent with neuroimaging studies conducted in TS, that have shown abnormalities in several brain areas, some of them known to be involved in emotional processing. Specifically, structural imaging studies in TS have found greater brain volumes in the amygdala, cingulate, and insular cortices, but also reduced cortical thickness in other brain areas, such as left frontal lobe and bilateral parahippocampal gyrus [Ref. (1, 7, 24, 43, 47, 48), see Ref. (49) for review]. In a recent study, Lepage et al. (50) found that socio-emotional functioning in TS [measured with the Emotional Quotient Inventory (51)]

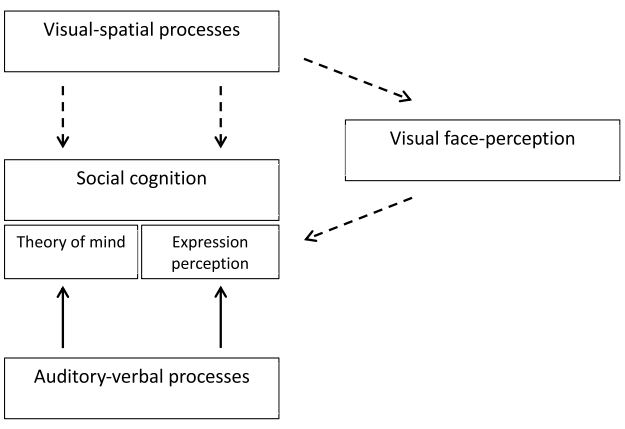

FIGURE 5 | A theoretical model of social cognition in Turner syndrome (TS) and its relationship to other perceptual and cognitive capacities. The broken lines represent TS impairments while the full lines represent intact capacities.

was related to their aberrant brain morphological structures of the social brain. In addition, functional imaging studies have found anomalous patterns of amygdalar activation in response to fearful stimuli (52), as well as reduced neural activity in frontal areas, such as the anterior dorsal anterior cingulate cortex and the dorsolateral prefrontal cortex (44). These findings appear to be more consistent with the claim that the social problems in TS are due to anomalies in brain regions related to social processing.

However, as already pointed out [e.g., Ref. (50)], it is hard to determine whether the brain's unique morphological and activation patterns, observed in TS, are the cause of their social difficulties or the consequence of it. Moreover, the inclusion of other factors claimed to be involved in social cognition, such as visual-spatial skills, establishes a complex network which consists of potential mediating and interactive relationships among different variables. For example, TS visual-spatial deficits, apparent at birth, may influence the structural and functional development of social-related regions, and consequently social behavior. Alternatively, an atypical TS social brain could exist from an early stage, but visual-spatial difficulties could shape its trajectory and accelerate the rate of its abnormal development. For now, the present results emphasize the importance of visual-spatial factors in the development of TS deficits in social cognition. Future longitudinal studies are therefore required to determine the exact structure and functioning of the social brain in girls and women with TS and its impact on their social functioning.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ruth Rappaport Children's Hospital Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ruth Rappaport Children's Hospital Institutional Review Board.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study; VG and ZH selected the TS sample; TZ-M conducted the research and collected the data; TZ-M and DA performed the statistical analysis; DA wrote the first draft of the manuscript. All authors contributed to the manuscript first draft, read, and approved the submitted version.

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