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ORIGINAL ARTICLE *Reproductive endocrinology*

Anti-Müllerian hormone levels in girls and adolescents with Turner syndrome are related to karyotype, pubertal development and growth hormone treatment

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STUDY QUESTION: In girls and adolescents with Turner syndrome (TS), is there a correlation between serum AMH levels and karyotype, spontaneous puberty and other biochemical markers of ovarian function, or growth hormone (GH) therapy?

SUMMARY ANSWER: Serum anti-Müllerian hormone (AMH) correlates with karyotype, pubertal development, LH, FSH and are measurable in a higher percentage of TS patients under GH therapy.

WHAT IS KNOWN ALREADY: Most girls with TS suffer from incomplete sexual development, premature ovarian failure and infertility due to abnormal ovarian folliculogenesis. Serum AMH levels reflect the ovarian reserve in females, even in childhood.

STUDY DESIGN, SIZE, DURATION: Cross-sectional study investigating 270 karyotype proven TS patients aged 0–20 years between 2009 and 2010.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Studies were conducted at three University Children's hospitals in Europe. Main outcome measures were clinical data concerning pubertal development as well as laboratory data including karyotype, serum AMH, LH, FSH, estradiol (E2), inhibin B and IGF.

RESULTS AND THE ROLE OF CHANCE: Serum AMH was detectable in 21.9% of all TS girls and correlated strongly with karyotypes. A measurable serum AMH was found in 77% of TS girls with karyotype 45,X/46,XX, in 25% with 'other' karyotypes and in only 10% of 45,X TS girls. A strong relationship was also observed for measurable serum AMH and signs of spontaneous puberty such as breast development [adjusted odds ratio (OR) 19.3; 95% CI 2.1–175.6; $P = 0.009$] and menarche (crude OR 47.6; 95% CI 4.8–472.9; $P = 0.001$). Serum AMH correlated negatively with FSH and LH, but did not correlate with E2 and inhibin B. GH therapy increased the odds of having measurable AMH in TS (adjusted OR 4.1; 95% CI 1.9–8.8; $P < 0.001$).

LIMITATIONS, REASONS FOR CAUTION: The cross-sectional design of the study does not allow longitudinal interpretation of the data; for that further studies are needed. High percentage of non-measurable AMH levels in the cohort of TS require categorized analysis.

WIDER IMPLICATIONS OF THE FINDINGS: Serum AMH levels are a useful marker of the follicle pool and thus ovarian function in pediatric patients with TS. These findings are in line with the published literature. The finding that GH therapy may affect AMH levels is novel, but must be confirmed by future longitudinal studies.

Key words: Turner syndrome / anti-Müllerian hormone / ovarian reserve / puberty / growth hormone treatment

Introduction

Turner syndrome (TS) is found in ~1:2000 newborn girls. The classic form is associated with the 45,X karyotype (50%), whereas variants either show a mosaic of 45,X/46,XX (25%) or have structural anomalies of the X chromosome such as deletions, iso- or ring chromosomes (25%) (Saenger *et al.*, 2001; Bondy, 2007). The clinical spectrum of TS is broad and highly variable but short stature and gonadal dysgenesis are characteristic features. Gonadal dysgenesis in TS results in pubertal delay or failure and infertility in most patients. However, up to 30% of girls with TS have spontaneous pubertal development and 2–5% have regular menstrual cycles before the onset of premature menopause (Abir *et al.*, 2001). Spontaneous pregnancies occur only in ~2% of women with TS, mostly at a young age (23–24 years) and appear to be associated with the mosaic karyotype (Hovatta, 1999).

Primary ovarian insufficiency (POI) in patients with TS is due to an accelerated loss of follicles from the ovaries which may start as early as 18 weeks into fetal life (Weiss, 1971; Santoro, 2003; Reynaud *et al.*, 2004). More recent studies found that there may be viable follicles even in the ovaries of 12–13-year-old girls with classical TS without spontaneous pubertal development (Gravholt *et al.*, 2002; Hreinsson *et al.*, 2002), although the quality of these follicles is doubtful. Thus, preservation of fertility in patients with TS may be feasible through cryopreservation of ovarian tissue before follicles begin to disappear (Rutherford and Matthews 2000; Huang *et al.*, 2008). However, to determine the possible candidates among girls and young adolescents with TS and the right time point for such interventions, biochemical markers reflecting the ovarian reserve in childhood are needed.

Antral follicle count (AFC) and early follicular phase serum levels of FSH, inhibin B and estradiol (E2) are measured to assess a woman's ovarian reserve (Burger *et al.*, 1995). However, all these methods to assess the ovarian reserve are based on the mature, adult female hypothalamic–pituitary–gonadal axis. In contrast, GnRH is switched off after birth and remains silent until the age of ~10 years when healthy girls start puberty. During this time FSH, inhibin B and E2 levels are low. Although gonadotrophin levels (FSH and LH) tend to be higher in patients with TS, especially in the first 2–5 years of life, a significant overlap exists between TS and normal girls especially during mid-childhood (Conte *et al.*, 1975; Chrysis *et al.*, 2006; Hagen *et al.*, 2010b). Therefore, gonadotrophin levels may not reflect the ovarian reserve before the onset of puberty.

In the past, we and others have shown that the serum anti-Müllerian hormone (AMH) level is a good marker for the size of the growing follicle pool, and, indirectly, also of the primordial follicle pool, reflecting the ovarian reserve (de Vet *et al.*, 2002; van Rooij *et al.*, 2002; van Rooij *et al.*, 2005; Pigny *et al.*, 2006; Visser *et al.* 2006). AMH is specifically expressed in granulosa cells of growing non-selected follicles. In women of reproductive age, serum AMH levels correlate strongly with AFC and levels decline over time to become undetectable at menopause. Recent studies in healthy normo-ovulatory adult women confirmed this decline in serum AMH with increasing age (Kelsey *et al.*, 2011; Lie Fong *et al.*, 2012). Interestingly, this decline in serum AMH levels precedes the changes in traditional markers for ovarian reserve, such as FSH, inhibin B and E2. Hence, it is believed that serum AMH levels constitute an ovarian reserve marker in adult women, independent from the HPG axis.

Serum AMH levels have been assessed in healthy females throughout life span and have been studied in two independent groups of patients with TS (Borgstrom *et al.*, 2009; Hagen *et al.*, 2010a). During childhood AMH levels increase slightly from birth onward and plateau during adolescence, suggesting that follicle dynamics in children may differ from that in adults. Not until the age of 25, the decline in serum AMH with increasing age is observed (Hagen *et al.*, 2010a; Kelsey *et al.*, 2011; Lie Fong *et al.* 2012). Nevertheless, assessment of serum AMH, also at a young age, is indicative of ongoing follicular development (Visser *et al.* 2012). In this European Society of Pediatric Endocrinology collaborative study, we measured serum AMH levels in 270 TS girls and correlated the values to age, karyotypes, pubertal development and to biochemical markers of gonadal function (serum LH, FSH, inhibin B and E2). In addition, we addressed the question whether recombinant human growth hormone (rhGH) therapy, which is recommended in TS to improve growth, may influence serum AMH levels in TS.

Methods

Patients and ethical approval

TS subjects were recruited for the study at three European pediatric endocrine units in Bern (Switzerland), Rotterdam (The Netherlands) and Tübingen (Germany). Most patients' data were available from registries: TS registry (Tübingen; $n = 109$; inclusion rate 109/117) and a national growth hormone (GH) registry (Rotterdam; $n = 140$; inclusion rate 140/142). In Bern, TS patients ($n = 21$; inclusion rate 21/21) were recruited during their regular medical follow-up visits between 2009 and 2010. Small amounts of properly stored serum were available for laboratory studies. The study was approved by the local ethical authorities of all three study centers. All patient and parents gave informed consent. The primary inclusion criteria for the study consisted of the diagnosis of TS based on a state-of-the art karyotype, clinical data (for details see Table I) and a serum sample for the determination of hormone measurements.

Criteria and definitions for grouping and analyses of the data were as follows: patients were divided into three groups according to their karyotype group: 45,X, 45,X/46,XX or other. Among the group 'other karyotype' additional subgroups were formed according to geneticists' recommendation shown in Table II. Analyses for spontaneous puberty and spontaneous menarche (yes/no) included only TS subjects 12 years and older who had no sex hormone replacement (estrogen) therapy. Breast development Tanner stage B2 or more was taken as definition for spontaneous pubertal development (Tanner and Whitehouse, 1976). As a control population, we used healthy girls and adolescents aged 0–20 years ($n = 252$) of our nomogram study (Lie Fong *et al.*, 2012). Detailed information on recruitment strategy and study populations are available in the original paper (Lie Fong *et al.*, 2012).

Laboratory methods

All patients provided a document of the exact G-band karyotype of their blood lymphocytes performed at their treatment center which generally included at least counting of 30 metaphases. LH and FSH were measured by immunochemiluminometric assays (Roche Modular E170, Hoffmann-La Roche Ltd., Basel, Switzerland) at each center and results were provided as IU/l. E2 was determined by a radioimmunoassay in pmol/l (Siemens Global Healthcare Diagnostics, Inc., former Diagnostic Products Corporation). Serum insulin-like growth factor-1 (IGF-1) was also determined by a commercially available kit (Nichols Institute Diagnostics, Bad Vilbel, Germany). For AMH and inhibin measurements, all serum samples were

Table I Characteristics of studied patients.

	ALL, *n* (%)	No GH, *n* (%)	GH Tx, *n* (%)	*P*-value[a]
Number	270	152	118	
Age >12 years, *n*	120 (44.4)	40 (26.3)	80 (67.8)	<0.001
Karyotype				0.89
45,X	136 (50.4)	75 (49.3)	61 (51.7)	
45,X/46,XX	22 (8.2)	12 (7.9)	10 (8.5)	
Other	112 (41.5)	65 (42.8)	47 (39.8)	
Age, years (mean/SD)	10.8 ± 4.8	8.7 ± 4.2	13.4 ± 4.1	<0.001
Age at start of GH, years (mean/SD)			7.4 ± 3.1	
Spontaneous puberty, *n* (of 41[c])[b]	22 (53.7)	11 (37.9)	11 (91.7)	0.002
Spontaneous menarche, *n* (of 47[c])[b]	8 (17.0)	1 (2.9)	7 (58.3)	<0.001
Measurable AMH, *n*	59 (21.9)	24 (15.8)	35 (29.7)	0.006
AMH level if measurable, ng/ml (mean/SD)	1.86 ± 1.75	2.13 ± 1.95	1.67 ± 1.61	0.33

[a] χ^2 test for categorical and *t*-test for continuous variables.
[b] >12 years, no HRT.
[c] Data set not available for all subjects.

Table II Detailed characterization of observed karyotypes and AMH levels.

Categories	Subcategories	*n* (%)	Measurable AMH [*n* (%)]	OR[2] (95% CI[2])	AMH [ng/ml (median/range)]
45,X		136 (50.4)	14 (10.3)	1 (reference)	0.53 (0.12–2.10)
45,X/46,XX		22 (8.1)	17 (77.3)	37.0 (11.2–122.3)	2.26 (0.24–6.37)
Others		112 (41.5)	28 (25)	3.4 (1.6–7.2)	1.06 (0.04–7.78)
	46,X,i(Xq)	40 (14.8)	5 (12.5)		
	46,X,i(Xp)	4 (1.5)	0		
	46,X,del(X)	15 (5.6)	8 (53.3)		
	46,X,r(X)	13 (4.8)	2 (15.4)		
	45,X/47,XXX	19 (7.0)	8 (42.1)		
	45,X/46,XY	9 (3.3)	2 (22.2)		
	'others'[1]	12 (4.4)	3 (25)		

Notes: Except for 45,X and 45,X/46,XX, the karyotypes include mosaicism of the karyotypes.
(1) 'Others' include marker chromosomes (of unknown origin; $n = 6$), derivatives of X chromosomes (2), translocations involving the X chromosome (2) and complex mixed mosaicisms (2).
(2) Data are adjusted for GH therapy and linear and quadratic term of age, and relate to measurable versus non-measurable AMH levels.

frozen and stored under standardized conditions before they were collectively sent to the laboratory of JAV in Rotterdam. Inhibin B (pg/ml) was determined in all serum samples with measurable AMH by an enzyme immunoassay (Oxford Bio-Innovation Ltd, Oxfordshire, UK). AMH (ng/ml) was determined in Rotterdam by an in-house developed AMH-ELISA (enzyme-linked immunosorbent assay; commercially available as the GenII Beckmann Coulter, Beckman Coulter, Inc.) (Kevenaar *et al.*, 2006). Previously, we have shown that AMH immunoreactivity in serum is stable during storage and after repeated freeze–thaw cycles (Al-Qahtani *et al.*, 2005; Kevenaar *et al.*, 2006), thus neither storage nor transport should affect measured AMH concentrations. This highly sensitive ELISA uses AMH standards in the range of 5–0.037 ng/ml. Of note, serum AMH measurement of the control population was performed with the same assay. Intra- and inter-assay coefficients of variation were <3 and 8% for FSH, 5 and 6% for LH, 5 and 7% for E2, 9 and 15% for inhibin B, 5 and 9% for IGF-1, and 5 and 10% for AMH.

Statistical analysis

Associations between AMH, age (linear and quadratic terms), karyotype and GH therapy were assessed in a multivariable logistic model with measurable AMH as outcome; therefore, all pairwise associations are adjusted for the other co-factors. Correlations between measurable AMH and spontaneous puberty in TS girls aged 12 years or older were calculated from logistic models with spontaneous puberty as outcome. The association with breast development was adjusted for age (linear term only), additional adjustment for karyotype was not possible. Adjustment for the association of AMH with menarche was not possible and is thus shown crude. The relationships between AMH and other hormones (FSH, LH, E2 and inhibin B) were each assessed in a logistic model with measurable AMH as outcome and the respective log-transformed hormone variable as independent factor, adjusting for karyotype and age (linear and quadratic terms). The correlation between measurable AMH

and standardized IGF-1 was also calculated from a logistic model adjusting for the same co-factors.

All *P*-values and 95% confidence intervals (CI) are two-sided. *P*-values <0.05 were considered significant. All analyses were done using Stata 11 (Stata Corporation, College Station, Texas).

Results

AMH levels in TS 0–20 years of age and correlation with karyotype

We measured AMH in 270 patients with karyotype proven diagnosis of TS. Patients' characteristics are summarized in Table I. The overall AMH was only measurable in 21.9% of TS subjects. A scatter plot of the AMH levels in TS is depicted in Fig. 1A, which shows that compared with healthy controls (Lie Fong *et al.*, 2012), AMH levels of subjects with TS were lower but some overlapped with controls. Similar to healthy controls, there is a quadratic relationship between age and measurable serum AMH in TS girls as shown in a logistic model; however, the association is non-significant (adjusted $P = 0.07$). The odds ratios (OR, adjusted for karyotype and GH therapy) for the linear and quadratic terms of age were 1.32 (95% CI 0.90–1.94; $P = 0.15$) and 0.98 (95% CI 0.97–1.00; $P = 0.07$), respectively, i.e. the odds of having a measurable AMH level increased with age when girls were young, but decreased at an older age.

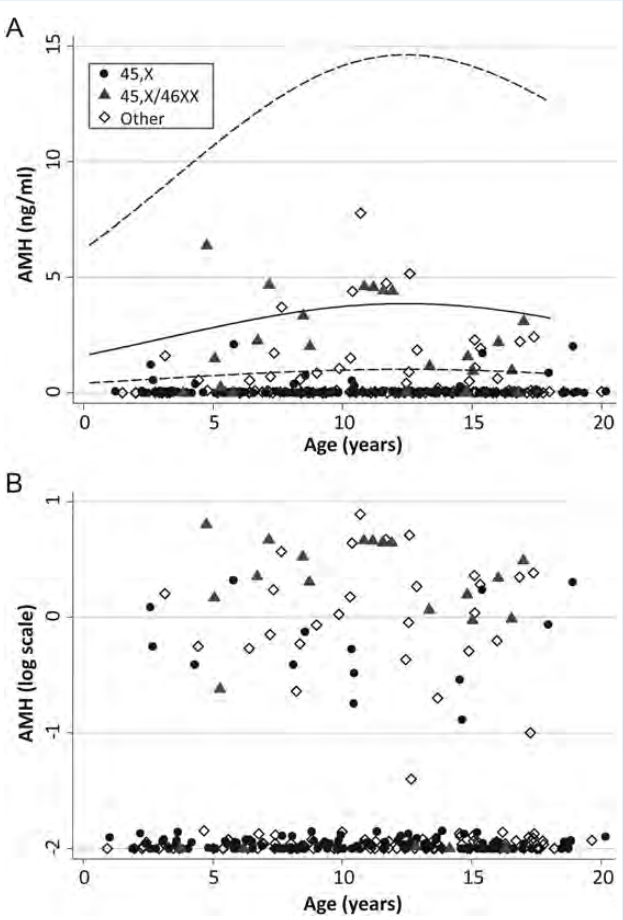

Figure 1 Serum AMH levels in Turner syndrome (TS) girls and adolescents with various karyotypes. (**A**) Absolute serum concentrations of AMH (ng/ml) of 270 subjects with TS are plotted versus age (years), stratified by karyotype. For comparison, the quadratic regression line for 252 healthy controls with a 90% prediction interval is shown (dashed lines). (**B**) Log AMH values versus age, stratified by karyotype. Non-measurable observations were assigned values of −2 on the log scale. Observations are jittered in order to better visualize the distribution. AMH correlates strongly with karyotype (adjusted $P < 0.001$).

Measurable AMH levels were found in 10% of TS with the karyotype 45,X (which comprises 50% of all TS subjects (Table II). In contrast, 77% of TS subjects with the karyotype 45,X/46,XX and 25% of TS subjects with other karyotypes had measurable AMH levels. Accordingly, serum AMH levels of TS subjects were strongly correlated with karyotype. The adjusted odds of having a measurable serum AMH with the karyotype 45,X/46,XX was 37 times higher when compared with the karyotype 45,X (adjusted OR 37.0; 95% CI 11.2–122.3; $P < 0.001$). The adjusted odds of a measurable serum AMH was 3.4 times higher with 'other' karyotypes compared with 45,X (adjusted OR 3.4; 95% CI 1.6–7.2; $P = 0.001$).

AMH and spontaneous puberty in TS without E2 therapy

About 54% of TS subjects older than 12 years had spontaneous breast development and ~17% had a history of spontaneous menarche (Table I). Figure 2A shows AMH levels of all TS subjects without estrogen replacement therapy stratified by breast development. There was a significant relationship between measurable serum AMH and spontaneous breast development. Breast development was found in 91.7% of TS girls older than 12 years with measurable serum AMH but only in 37.9% with undetectable serum AMH (Fig. 2B). As shown in an adjusted logistic model, TS girls aged 12 years and older had a 19.3 times increase in the odds of developing breasts with measurable serum AMH levels (adjusted OR 19.3; 95% CI 2.1–175.6; $P = 0.009$). Spontaneous menarche was reported in 58.3 and 2.9% of TS girls with and without measurable serum AMH, respectively (Fig. 2C). The odds ratio of menarche in TS was 47.6 times higher with measurable AMH (crude OR 47.6; 95% CI 4.8–472.9; $P = 0.001$).

Serum AMH in relation to FSH, LH, E2 and inhibin B in TS without E2 therapy

The relationship between AMH and other hormones reflecting gonadal function was studied in TS girls without estrogen replacement therapy. For FSH and AMH a strong negative association was found (Fig. 3A). The odds of a measurable AMH level was 19 times lower if the FSH level was rising by a factor of 10 (adjusted OR 0.053; 95% CI 0.015–0.190; $P < 0.001$). For the subgroup of girls younger than 12 years, the adjusted OR was comparable (0.042; 95% CI 0.007–0.240; $P < 0.001$). For the subgroup of girls older than 12 years, an FSH <10 U/l predicted a measurable AMH perfectly (therefore a logistic regression is not estimable). Similarly, we found a negative association between LH and AMH (Fig. 3B). The odds of a measurable AMH level was three times lower, if the LH level was rising by a factor of 10 (adjusted OR 0.33; 95% CI 0.15–0.71; $P =$

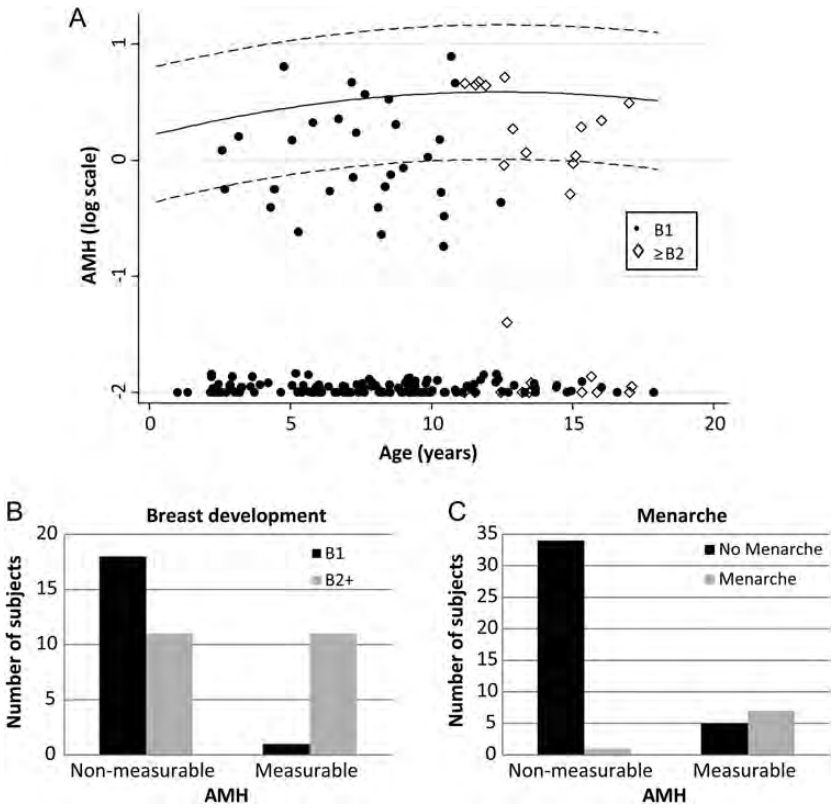

Figure 2 Serum AMH and puberty in TS without E2 therapy. (**A**) A scatter plot of AMH versus age and spontaneous breast development in TS. For comparison, the quadratic regression line for healthy controls with a 90% prediction interval is shown (dashed lines). The odds of spontaneous breast development are 19 times higher in TS girls older than 12 years if AMH is measurable (adjusted $P < 0.009$). (**B**) Spontaneous breast development in 41 TS subjects older than 12 years with or without measurable AMH. (**C**) Spontaneous menarche in 47 TS subjects older than 12 years with or without measurable AMH. AMH, anti-Müllerian hormone; TS, Turner syndrome).

0.005). For the subgroup of girls younger than 12 years, the adjusted OR was again comparable but non-significant (0.48; 95% CI 0.19–1.23; $P = 0.13$), while it was comparable and significant for the subgroup of TS girls older than 12 years (adjustment not possible, crude OR 0.08; 95% CI 0.01–0.46; $P = 0.005$). For both serum E2 and inhibin B levels no significant relationship with AMH levels was found (Fig. 3C and D). This was also true for both the subgroup of girls younger and older than 12 years.

Serum AMH levels and growth hormone treatment

Of the TS subjects, 43.7% were receiving daily recombinant human growth hormone (rhGH) treatment when they were studied for their AMH levels (Table I). While most TS patients from Bern and Tübingen were under GH therapy at the time they were studied, TS patients from Rotterdam were predominantly studied before GH treatment starts. Thus, we tested whether GH therapy may have an effect on serum AMH levels in TS subjects. Serum AMH levels versus age, stratified by GH treatment are shown in Fig. 4. Without GH therapy, 16% of TS subjects had a measurable AMH. In contrast, with GH therapy the number of subjects with measurable serum AMH was 30%. Accordingly, a strong relationship was found between GH therapy and measurable AMH when adjusted for age and karyotype in a logistic model. The odds of a measurable serum AMH were 4.1 times higher for TS with GH therapy when compared with TS without GH therapy (OR 4.1; 95% CI 1.9–8.8; $P < 0.001$). Similarly, a weak, although non-significant, relationship was found between measurable serum AMH and serum IGF-1 levels (taken as age-, sex and pubertal-related standard deviation scores) when corrected for karyotype and age in a subgroup of 73 GH-treated TS subjects from Bern and Tübingen (adjusted OR 1.63; 95% CI 0.95–2.80; $P = 0.08$).

Discussion

Most females with TS have gonadal dysgenesis with reduced ovarian reserve resulting in delayed or missing pubertal development, sub- or infertility and early menopause. Quality of life is influenced by this fact and early diagnosis and information may help to cope with it. Cryopreservation of ovarian tissue at an early age may be considered in TS patients to preserve their own follicles for later reproduction. Serum AMH has been shown to reflect the ovarian reserve in adult cycling women, and has been used as a marker for ongoing follicular development in women at risk for POI (Visser *et al.*, 2012). Indeed, a serum AMH level in the upper normal range seems predictive for finding remaining follicles in ovarian biopsies of TS girls

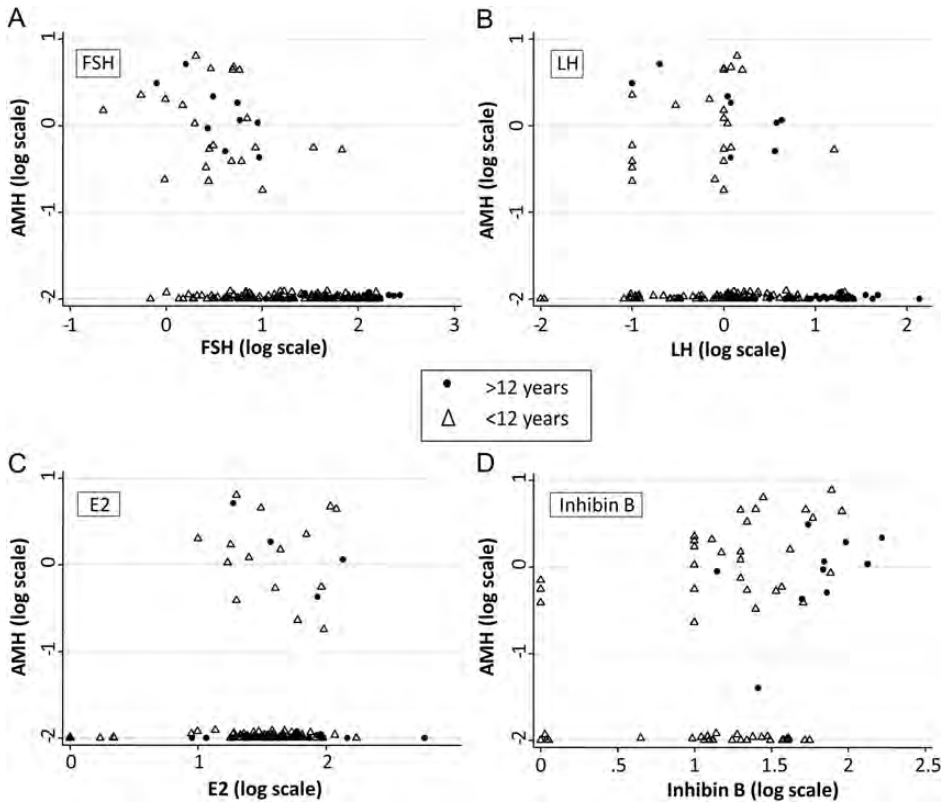

Figure 3 Serum AMH levels in relation to other hormonal values in TS without E2 therapy. (**A**) AMH versus FSH levels stratified by age. There is a strong negative relationship between measurable AMH and log-transformed FSH as shown by a logistic model (adjusted $P < 0.001$). (**B**) Studies of AMH versus LH levels shown as in (A). There is also a negative relationship between AMH and log-transformed LH (adjusted $P = 0.005$). (**C**) AMH and E2 levels. No relationship was found, but too many E2 values were missing (74 out of 196). (**D**) AMH and inhibin B. No significant relationship was found when adjusted for age and karyotype; however, limitations apply due to small observation numbers (72/196). AMH, anti-Müllerian hormone; TS, Turner syndrome.

(Borgstrom *et al.*, 2009). Other predictive factors for ongoing ovarian function in TS patients are signs of spontaneous puberty, mosaic karyotype and low serum FSH (Borgstrom *et al.*, 2009). In our 270 TS girls and adolescents, we found detectable serum AMH levels in 22%, which correlated strongly with the karyotypes. Having a mosaic 45,X/46,XX karyotype increased the odds of having a measurable serum AMH by 37 times compared with having a 45,X karyotype. A positive correlation was also found between serum AMH levels and clinical signs of pubertal development (breast, menarche). Serum AMH was negatively related to FSH in TS girls both younger and older than 12 years, and was negatively related to LH in girls older than 12 years indicating that there might be a pituitary–ovarian set point even before puberty. In contrast, no such relationship was found between serum AMH and E2 or inhibin B in TS girls without estrogen replacement therapy. Overall, our results are in line with two previous studies. The strength of Borgström's study (Borgstrom *et al.*, 2009) lies in the fact that serum AMH levels were directly correlated with the number of ovarian follicles assessed by histological analysis of 47 biopsies. Hagen's study (Hagen *et al.*, 2010a) consisted of AMH measurements in 926 healthy and 172 TS subjects aged 0–69 years; among them, 73 TS subjects were in the pediatric and adolescent cohort. For TS patients, serum AMH levels correlated with karyotype and ovarian function. We now present similar results of the largest cohort of pediatric patients with TS so far studied for AMH levels. Taken together all three studies suggest that serum AMH is an excellent marker of ovarian function in girls and adolescents with TS. The weakness of our current and published studies lies in the cross-sectional design which does not account for the possible dynamic changes in AMH levels of individuals over time. For instance, we chose to group girls into prepubertal and pubertal categories using a cut-off age of 12 years when ~75–90% of normal girls (Tanner and Whitehouse, 1976) and girls with TS (Schweizer *et al.*, 2000; Massa *et al.*, 2003; Martin *et al.*, 2004; Ranke *et al.*, 2007) would have started puberty. With this cut-off, there is a small false-negative number of TS girls entering puberty spontaneously later. On the other hand, more than 50% of TS girls with spontaneous puberty onset (B ≥ 2) will not be able to complete puberty (Reindollar, 2011) and might be 'falsely' included in the pubertal group in a cross-sectional study, thereby overestimating the number of subjects with possible future fertility. Such shortcomings can only be overcome in the future by performing a longitudinal study for AMH in TS from birth to menopause.

AMH plays an important role in ovarian folliculogenesis where it is produced in granulosa cells from birth to menopause (Visser *et al.*,

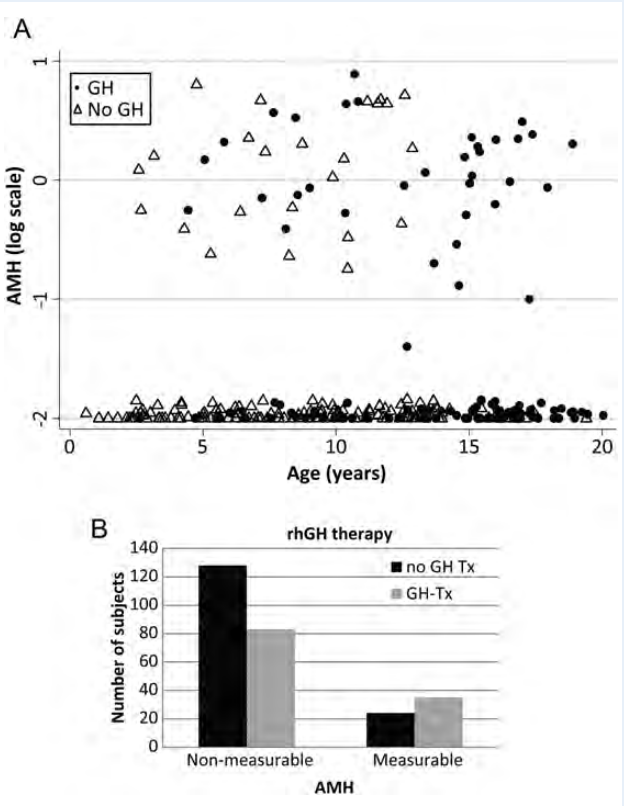

Figure 4 Serum AMH levels in TS with or without rhGH therapy. (**A**) A scatter plot of AMH versus age stratified for hrGH therapy. There is a clear relationship between GH and AMH when adjusted for age and karyotype. The odds of a measurable AMH is 4.1 times higher in the group with GH therapy (adjusted $P < 0.001$). (**B**) Measurable or non-measurable AMH in TS subjects (absolute numbers) with or without rhGH treatment. AMH, anti-Müllerian hormone; TS, Turner syndrome.

2006). In humans, AMH expression can first be observed in granulosa cells of primary follicles, and expression is strongest in pre-antral and small antral follicles ($\leq$4 mm) (Weenen *et al.*, 2004). AMH expression disappears in follicles of increasing size and is lost in follicles $>$8 mm (Weenen *et al.*, 2004). Serum AMH concentrations decrease over time to become undetectable at menopause, correlating with the decline in AFC with increasing age (de Vet *et al.*, 2002; van Rooij *et al.*, 2002, 2004, 2005). In contrast, serum AMH levels increase slightly during mini-puberty and remain constant during adolescence, regulated through a yet unexplained mechanism (Hagen *et al.*, 2012; Lie Fong *et al.*, 2012). Also in our cohort of TS subjects with measurable AMH, we observed a borderline quadratic relationship between AMH and age with a rise during early childhood and a starting decrease in the older age group. This may suggest that follicle dynamics in TS patients with measurable AMH is similar to that in healthy controls. Recently, a prospective study in prepubertal and pubertal girls treated for cancer demonstrated the decline in serum AMH reflecting the gonadotoxic effect of the cancer treatment (Brougham *et al.*, 2012). Combined with that study, our results suggest that AMH seems to be an equally good marker of ongoing follicular development in girls, and that it therefore reflects the number of remaining primordial follicles also in girls and adolescents with TS.

Finally, our study shows for the first time a relationship between rhGH therapy and serum AMH levels. AMH was detectable in 30% of GH-treated TS girls compared with 16% without GH. Similarly, we found a weak correlation between serum IGF-I levels and measurable AMH in GH-treated TS patients, although this correlation failed to reach significance. Several studies have suggested an important role for the GH/IGF system in ovarian follicular development. For instance, IGF-I and FSH can act synergistically to enhance follicular development (Silva *et al.*, 2009). *In vitro* studies of mouse and goat follicles suggest that GH enhances progression of primordial to primary follicles and stimulates follicle growth and viability (Liu *et al.*, 1998; Martins *et al.*, 2010). In mice lacking GH action, the number of healthy follicles was strongly reduced (Bachelot *et al.*, 2002). Thus, it is possible that GH treatment in TS patients results in increased follicle viability, which would explain the increased percentage of TS girls with measurable AMH upon GH treatment. However, GH-treated TS patients also more frequently displayed spontaneous puberty, which is indicative of ongoing ovarian function. With our current study design it is not possible to make a distinction between these possible explanations. Thus, longitudinal follow-up studies are needed to determine whether GH treatment in patients with TS is beneficial for ovarian function. Our results do suggest that GH does not directly affect AMH levels, since there was no difference in serum AMH levels in TS patients with or without GH treatment. Similarly, in short prepubertal girls born small for gestational age serum AMH levels were also not affected upon GH treatment and were similar to those observed in control girls (Lem *et al.*, 2011).

In summary, we suggest that puberty and fertility issues in patients with TS are addressed in childhood to improve the quality of care and to make reproductive interventions an option. Serum AMH correlates nicely with karyotype, signs of pubertal development, LH/FSH (this study) and ovarian follicle count (Borgstrom *et al.*, 2009). Longitudinal studies are needed to solve the questions whether AMH levels at a young age are predictive, or whether multiple measurements are needed over time; and how GH treatment influences AMH levels. However, based on our and other studies, we suggest that serum AMH may serve as a marker of ongoing follicular development in prepuberty, helping the pediatrician to deliver best possible information on future pubertal development and fertility to TS girls and their parents.

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Authors' roles

J.A.V., M.B.R. and C.E.F. contributed to idea and study design. J.A.V., A.C.S.H.K., G.R.J.Z., M.B.R., C.E.F. contributed to data collection. J.A.V., A.L., M.B.R., C.E.F. contributed to data analysis and statistics. J.A.V., A.L., M.B.R. and C.E.F. contributed to manuscript preparation.

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Conflict of interest

None declared.

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