Inhibin A and B in adolescents and young adults with Turner’s syndrome and no sign of spontaneous puberty*

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BACKGROUND: The aim of this study was to assess levels of inhibin A and B, FSH and LH in Turner’s syndrome (TS) without signs of spontaneous ovarian activity. METHODS: Twenty-four girls with TS (median age, 14.7 years) without signs of spontaneous ovarian function were included in the study. Sixty prepubertal girls (PPG) (10.3 years) that had not yet experienced menarche (all Tanner stage 1), and 34 pubertal girls (PG) (13.8 years) (Tanner stage 3–4), who were regularly menstruating, served as controls. The levels of inhibin A and B, FSH, LH, and pubertal stage were determined. RESULTS: Inhibin A was not detected in females with TS, or in almost all PPG (59 of 60) (P not significant), and inhibin B in TS females, while most PPG produced inhibin B (53 of 60, P < 0.0005). FSH and LH were elevated in TS, but with overlapping values. In follow-up samples in TS, three of twenty-four females showed detectable levels of inhibin A and/or B. In one of these, 6 serial samples were available. At 20 years this patient had a high level of LH and FSH, which declined, and concurrently inhibin A and inhibin B rose, only later to decrease, when FSH and LH started to rise again. In comparison with PG baseline levels of inhibin A and B were lower in TS, with inhibin A detectable in 23 of 34, and inhibin B detectable in 32 of 34 PG. Levels of FSH and LH were also different, although with overlapping values. CONCLUSION: The result raises the possibility that functional or partly functional ovaries are present in some females with TS, without apparent menstrual cycling.

Key words: gonadotrophins/inhibin A/inhibin B/puberty/spontaneous pregnancy/Turner’s syndrome

Introduction
Females with Turner’s syndrome (TS) are characterized by short stature and gonadal insufficiency. The gonadal insufficiency is mirrored by high levels of FSH and LH in early childhood (2–5 years) and after the time of normal onset of puberty (11 years) (Conte et al., 1975). However, in the neonatal period and during late childhood the level of FSH and LH is comparable with levels in control girls (Heinrichs et al., 1994; Conte et al., 1975). In adulthood, as in other conditions of hypergonadotrophic hypogonadism, the level of FSH and LH is increased to menopausal levels. Inhibin A and B are produced by the ovaries; inhibin is a dimeric glycoprotein composed of an α-subunit and either a βA-subunit, called inhibin A, or a βB-subunit, called inhibin B (Burger, 1993), and has been shown to exert negative feedback on FSH secretion. The granulosa cells of the dominant follicle and the corpus luteum are the predominant sources of inhibin A (Muttukrishna et al., 1997), while there is evidence to suggest that inhibin B is secreted by the small developing follicles (Roberts et al. 1994). It has been suggested that inhibin A can be used as a marker of the quality of the mature follicle and that inhibin B reflects the number of follicles present (Hayes et al. 1998).

During early puberty and late childhood in girls with TS the question arises whether or not there is any evidence of endocrine function of the ovaries. Often the levels of FSH and LH do not give a clear picture. Therefore, a definite marker of ovarian function would be valuable in daily clinical practice. Thus, hypothetically inhibin A and B could be candidate markers for evaluating the function of the ovaries in TS patients. Presently inhibins have not been evaluated in TS. We examined 24 females with TS without any signs of ovarian function and a control group of 60 normal prepubertal females. The levels of inhibin A and B were determined; the levels of FSH, LH, pubertal stage and body composition variables were also determined.
Materials and methods

Patients
Twenty-four females with TS, median age 14.7 years (range 9.5–20.2) were recruited. Inclusion criteria were TS verified by chromosomal karyotyping (Table I). They were all without signs of spontaneous ovarian function as judged from physical examination, interview concerning menstrual status and subsequent FSH evaluation. Some of the included females had low levels of FSH, but were included because they otherwise did not show signs of ovarian function. All females with TS were receiving conventional growth hormone treatment during the study, and some were on very low dose estrogen therapy in order to induce puberty (n = 14). Ninety-four normal girls were included as controls. None of the control girls was receiving any medication. Seventy-eight of these were part of a larger study of normal females (Sehested et al., 2000), and 16 were recruited from local schools and were presumed to have normal karyotype. Sixty control girls, median age 10.3 years (range 9.1–12.3) were Tanner stage 1, and had not yet experienced menarche, while 34 control girls, median age 13.8 years (range 11.5–17.4) were menstruating (Tanner stage 3–5). There was no available information regarding length of menstrual cycles, time since menarche, or regularity of menses in the control girls. In the cycling pubertal girls (PG) no attempt was made to perform systematic sampling at any specific time point (i.e. follicular or luteal phase). In girls that have just experienced menarche this may in fact be difficult due to irregularity of menstrual cycling.

All subjects and parents received oral and written information concerning the study prior to giving written informed consent. The protocol was approved by the local Scientific Ethics Committee.

Design
A blood sample was drawn from all participants. From almost all TS patients (n = 23), 1–5 additional samples (median: 2 samples) were drawn with varying intervals (<1–4 years) and a detailed physical examination was performed with determination of Tanner stage; height and weight were measured and body mass index (BMI) calculated as weight (in kg) divided by height (in metres) squared.

Assays
Blood samples were drawn at random time points from an antecubital vein between 16.00 and 17.00 h in TS females, between 9.00 and 17.00 h in control females, and centrifuged. Although samples were obtained at different times of the day, there was no information on circadian periodicity of inhibin level in women and potential bias is difficult to assess. Serum was stored at −20°C until analysis. Samples were stored for 4–6 years before analysis. Recent data indicate that inhibin B is relatively stable in samples during long-term storing (Andersson et al., 1998). Serum inhibin A was measured in duplicate in a double antibody enzyme immunoassay using a monoclonal antibody raised against the inhibin βA-subunit in combination with a labelled antibody raised against the inhibin α-subunit, as previously described (Groome et al., 1994). Serum inhibin B was measured in duplicate in a double antibody enzyme immunoassay using a monoclonal antibody raised against the inhibin βB-subunit in combination with a labelled antibody raised against the inhibin α-subunit, previously described (Groome et al., 1996). In the inhibin A assay the detection limit was 7 pg/ml, and the intra- and inter-assay coefficient of variation (CV) were 15 and 18% respectively; in the inhibin B assay the detection limit was 20 pg/ml, and the intra- and inter-assay coefficient of variation were 15 and 18% respectively. The given intra- and inter-assay CVs of 15 and 18% respectively, represent the highest CVs observed at the very low range of the assay (close to the detection limit). At higher levels both the intra- and inter-assay CVs were lower. Since this study involves samples at the very low range, intra- and inter-assay CVs at this very low range are reported. Serum FSH and LH were measured by time-resolved immunofluorometric assay (DELFIA, Wallac, Turku, Finland), with detection limits of 0.06 and 0.05 IU/l, respectively. Intra- and inter-assay CVs were both below 8% in the FHS and LH assays.

Statistics
All statistics were performed in SPSS Windows version 8.0. Data were examined by the Mann–Whitney test. Results are expressed as median and range. All inhibin A, inhibin B, and LH values less than assay sensitivity were assigned a value below assay sensitivity (inhibin A: 6 pg/ml; inhibin- B: 19 pg/ml; LH: 0.04 IU/l) for statistical computations. This approach to values below assay sensitivity was chosen, because it gives the most conservative estimate of a difference between any two groups. Statistical significance was assumed where P < 0.05.

Results

TS versus prepubertal control group
Age, height, weight and BMI were higher in females with TS compared with prepubertal control females (Table II). At baseline there was no detectable inhibin A in females with TS and most prepubertal control females (59 of 60) [Turner (T) versus control (C): <7 versus <7 (<7–8) pg/ml, P = not significant]. Likewise we could not detect any inhibin B in females with TS, while 53 of 60 prepubertal control females had detectable levels of inhibin B [T versus C: <20 versus 37 (<20–180) pg/ml, P < 0.0005 (Figures 1C and 2C)]. FSH [T versus C: 57.5 (0.32–144) versus 2.13 (0.28–6.39) IU/l, P < 0.0005] and LH [T versus C: 15.3 (<0.05–45.2) versus 0.2 (<0.05–4.8) IU/l, P < 0.0005] were significantly increased in patients with TS compared with controls (Figures 1A, B and 2A, B). However, there was an overlap between the two groups, and for five females with TS the levels of both FSH and LH were indistinguishable from the levels of FSH and LH in the control females (Figures 1A and B). None of these females had detectable inhibin B at baseline. In three of these five females, one repeat sample still showed undetectable inhibins (follow-up: 3 month, age: 9.5, 9.5, and 10.5 years respectively), and unchanged gonadotrophins (FSH 9.6–6.1, 2.9–5.8, and 2.9–5.8; LH 0.1–0.1, 0.1–0.2 and 0.1–0.2). These three females did not receive estrogen during the time of study.

However, serial samples with different time intervals revealed inhibin A and/or inhibin B levels above the detection level in the two other adolescents with initial low levels of FSH and LH. Two measurements were available, and in both, inhibin B was
Table II. Distribution of age, height and weight in patients with Turner’s syndrome

<table>
<thead>
<tr>
<th></th>
<th>Turner’s syndrome</th>
<th>Prepubertal controls</th>
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<th>Pubertal controls</th>
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<tr>
<td>n</td>
<td>24</td>
<td>60</td>
<td></td>
<td></td>
<td>34</td>
<td></td>
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<tr>
<td>Age/years</td>
<td>14.7 (9.5–20.2)</td>
<td>10.3 (9.1–12.3)</td>
<td>&lt;0.0005</td>
<td>13.8 (11.5–17.4)</td>
<td>NS</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Height/cm</td>
<td>146.7 (124.0–155.0)</td>
<td>140.4 (129.3–158.3)</td>
<td>0.05</td>
<td>164.0 (150.6–172.3)</td>
<td></td>
<td>0.0005</td>
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<tr>
<td>Weight/kg</td>
<td>42.6 (24.8–66.6)</td>
<td>31.8 (25.3–48.6)</td>
<td>&lt;0.0005</td>
<td>49.2 (35.6–60.2)</td>
<td>0.04</td>
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<tr>
<td>BMI/kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>21.1 (15.8–28.3)</td>
<td>15.8 (13.2–23.6)</td>
<td>&lt;0.0005</td>
<td>18.4 (15.2–22.8)</td>
<td>0.005</td>
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*Mann–Whitney test, prepubertal or pubertal controls versus Turner’s syndrome. Data are median (range). NS = not significant; BMI = body mass index.

Figure 1. Scatter plots of (A) FSH; (B) LH; and (C) inhibin B as a function of age. Filled circles (●) indicate females with Turner’s syndrome, open circles (○) indicate prepubertal and dark grey triangles (▼) indicate pubertal control females.

Figure 2. Box plot with median, 25th, and 75th percentiles as vertical boxes with error bars indicating 10th, and 90th percentiles, and outliers indicated as individual points of (A) FSH; (B) LH; and (C) inhibin B in females with Turner’s syndrome and no evidence of ovarian function, in prepubertal and in pubertal control females. Please note that a sole horizontal bar indicates that all values were below the detection limit (inhibin B in Turner’s syndrome).
measurable in the second sample, and in one subject inhibin A was also measurable (Case 1: age 15.7 years, inhibin B 109 pg/ml; Case 2: age 15.3 years, inhibin A 46 pg/ml, inhibin B 37 pg/ml). Their karyotypes were 45,X, and 46,X, idic (X)(pter→p21:p21→qter) respectively. These girls received low dose estrogen for the induction of puberty during the time of study. In another girl, six serial samples were available. At 20 years of age, she had high levels of LH and FSH, which declined, and concurrently inhibin A and inhibin B rose, only later to decrease, when FSH and LH started to rise again (Figure 3). One blood sample (sample four), showed a high level of inhibin A, which could be suggestive of a luteal phase at the time of sampling. However, the female had never experienced spontaneous menstruation. Her karyotype was 45,X. She was receiving conventional low dose estrogen therapy to induce puberty, which was unchanged during the study. There was no correlation between age and inhibin A, FSH or LH at baseline in the females with TS (Figure 1).

**TS versus pubertal control group**

Weight was lower, but BMI higher and age similar in females with TS, in comparison with pubertal controls. Baseline levels of inhibin A [T versus C: <7 versus 9 (<7–69) pg/ml, \( P < 0.0005 \)] and inhibin B [T versus C: <20 versus 95 (<20–202) pg/ml, \( P < 0.0005 \)] were lower in TS, with inhibin A detectable in 23 of 34, and inhibin B detectable in 32 of 34 control females. Levels of FSH [T versus C: 57.5 (0.32–144) versus 4.87 (1.24–8.87) IU/l, \( P < 0.0005 \)], and LH [T versus C: 15.3 (<0.05–45.2) versus 3.42 (0.16–25.6) IU/l, \( P = 0.006 \)] were also different, although with overlapping values (Figure 2).

**Discussion**

Most females with TS are theoretically not expected to produce inhibin A and B, due to the early and accelerated destruction of follicles and almost complete absence of viable ova shortly after birth. Indeed, the main results of the present study support this hypothesis, and inhibin A and B are not detectable at baseline in adolescents with TS without signs of ovarian function. However, serial measurements of samples showed that a few of these females actually did produce either inhibin A, inhibin B or both at some point. Also, most normal prepubertal females have detectable levels of inhibin B. It was not possible to completely separate prepubertal control females from females with TS without signs of ovarian function using FSH and LH—the traditional markers of gonadal function, nor was it possible using inhibin A or B. Three of the females with TS and low overlapping levels of FSH and LH were the ones that did, on serial sampling, show measurable levels of inhibin B, or inhibin A and B. Corresponding estradiol values from the study subjects would have been of definite interest in order to assess the clinical significance of the results, but were not available.

During the normal menstrual cycle inhibin B concentrations increase in the early follicular phase in response to the rising FSH concentrations, exerting negative feedback on the pituitary (Groome et al., 1996; Sehested et al., 2000), and are primarily produced by the granulosa cells of the small developing follicles (Roberts et al., 1994). However, inhibin A concentrations increase prior to ovulation in parallel with the late follicular phase rise in estradiol and subsequently increase further after ovulation and reach peak values in the mid-luteal phase, being primarily a product of the granulosa cells of the dominant follicle and the corpus luteum (Groome et al., 1996; Muttukrishna et al., 1997).

Reproductive ageing is associated with decreased levels of inhibin A and B in the face of increasing levels of FSH, with inhibin B possibly being the earliest marker of a decline in reproductive potential (Klein et al., 1996; Welt et al., 1999). In older cycling women with a raised early follicular FSH, inhibin B is reduced (Muttukrishna et al., 2000), and in postmenopausal women inhibin B is not detectable, while inhibin A is still present, suggesting extragonadal sources of inhibin A (Ala-Fossi et al., 1998). Previously, it has been the general view that most females with TS never reproduce because of dysgenetic gonads as the germ cells degenerate during fetal life (Carr et al., 1968). However, 10–20% of
females with TS do cycle regularly for a shorter or longer period (Pasquino et al., 1997), and about 5% are able to become pregnant and give birth (Lippe, 1991). Thus, a thorough evaluation of reproductive capacity is relevant in pubertal females and young adolescents with TS.

Recently data have been presented suggesting that viable follicles are present in females with TS and the ‘classical’ karyotype, 45,X, even at the age of 12–19 years. Hreinsson et al. took ovarian biopsies from five females with TS (45,X), and found 1.5–128 follicles per mm³ of the ovarian cortical tissue (Hreinsson et al., 2001). The authors conclude that cryopreservation for later treatment of infertility might be an option in TS. The data from the latter study of and the present one suggest that there may indeed be viable follicles present in the ovaries of even females with classic TS (45,X), and this may explain why 30% or more of females with TS show at least some signs of puberty (Pasquino et al., 1997). A future scenario could be to offer the possibility of cryopreserving ovarian tissue from young girls/adolescents with TS, as a part of an IVF programme that would therefore have to be integrated with paediatric departments to extend the offer before final degeneration of follicles. Technically, it should be possible to extend such an offer (Hovatta, 1999), although ethical considerations may hinder such a development.

In the present study we used an assay for inhibin B with a detection limit of 20 pg/ml, and in the lower range of detection the CV was higher than in the mid-range. One must of course have to keep this level in mind when evaluating the results of the present study. The present detection level is clearly under the level for the entire follicular level of inhibin B in women. However, since we do not know where in a given cycle we sampled the cycling pubertal control females, this means that females that were actually sampled in the luteal phase, levels of circulating inhibin B would be undetectable in some. This may explain why some of the cycling females in the pubertal control group had undetectable levels of inhibin B (and inhibin A). In a recent study of more than 300 women (18–45 years), we found that 4% had undetectable levels of both inhibin A and B, probably because they were sampled during the late luteal and early follicular phase (unpublished results). Furthermore, since inhibin B shows pulsatility in cycling women during the day, with pulses of 60 min, one measurement does not entirely reflect inhibin B secretion (in the mid-follicular phase at least) (Lockwood et al., 1998). However, since most of the females examined in the present were not cycling, pulsatility may be of only minor importance.

In conclusion, the present study showed that inhibin A and B can be detected in some females with TS, but without signs of ovarian function. Thus viable follicles may be present in more females with TS than previously expected. It may be suggested that a prospective study with systematic blood-sampling and ovarian biopsies or imaging will shed more light on ovarian function in apparently non-cycling adolescents with TS.

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References