FSH, LH, inhibin B and estradiol levels in Turner syndrome depend on age and karyotype: longitudinal study of 70 Turner girls with or without spontaneous puberty

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BACKGROUND: Ovarian function in Turner syndrome (TS) patients depends on the specific karyotype. This retrospective clinical study evaluates the pituitary–gonadal axis during infancy, childhood and adolescence in TS patients according to karyotype and ovarian function.

METHODS: A cohort of 70 TS patients (0–16 years) followed at a tertiary referral centre for paediatric endocrinology were included. Longitudinal measurements of reproductive hormones (FSH, LH, inhibin B and estradiol) prior to hormonal replacement treatment in 66 patients related to karyotype (A, 45,X; or B, miscellaneous karyotypes) and ovarian function (spontaneous puberty or absent spontaneous puberty) were compared with an age-matched reference range of 2406 healthy Danish females.

RESULTS: The prevalence of spontaneous puberty was 6% for 45,X and 54% for miscellaneous karyotypes, P = 0.001. In all TS patients, gonadotrophins were higher during infancy and at expected puberty compared with levels at mid-childhood, where 21/25 and 23/27 had FSH and LH levels, respectively, within the reference range. In patients with absent spontaneous puberty, 10/12 had FSH in the reference range during the mid-childhood nadir. 45,X-TS patients had undetectable inhibin B at 0–16 years. Ovarian failure was predicted in 20/20 patients with exclusively undetectable inhibin B, while 9/10 with detectable inhibin B entered puberty spontaneously. Estradiol levels were elevated from 4 to 8 years.

CONCLUSIONS: Ovarian function in TS patients is associated with the specific karyotype, and multiple undetectable inhibin B values during mid-childhood may predict absence of spontaneous puberty, although the specificity of the test is low. The biphasic age pattern of gonadotrophins was preserved in all patients, and spontaneous gonadotrophins are not useful as a diagnostic marker for TS in girls aged 6–10 years.

Key words: Turner syndrome / FSH / LH / inhibin B / estradiol

Introduction

Girls with Turner syndrome (TS) have characteristic physical features as a result of complete or partial absence of the second X chromosome, with or without mosaicism. TS affects ~1/2000 live born females (Nielsen and Wohleter, 1991), and the TS phenotype varies to some degree with the specific karyotype. The classical X-monosomic TS (45,X) is usually associated with prenatal degeneration of ovarian follicles, resulting in streak gonads and absent pubertal development (Singh and Carr, 1966; Weiss, 1971; Reynaud et al., 2004), although a few cases of spontaneous puberty have been reported (Weiss, 1971; Pasquino et al., 1997; Hreinsson et al., 2002). The density of ovarian follicles in patients with mosaic TS is higher than in 45,X-TS (Hreinsson et al., 2002), and ~50% of TS girls with 45,X/46,XX have sufficient ovarian function to enter puberty spontaneously (Pasquino et al., 1997). The ovarian function of TS patients with miscellaneous karyotypes depends on the X chromosome aberration (Ogata et al., 2001).
In most prepubertal TS patients, ovarian failure and reduced ovarian feedback result in significantly elevated FSH and LH levels during early childhood (0–5 years) and adolescence (>10 years), whereas gonadotrophin levels are not significantly elevated at age 5–10 years compared with healthy girls (Conte et al., 1975; Heinrichs et al., 1994; Chrysis et al., 2006; Fechner et al., 2006).

Inhibin B is secreted by developing follicles, and serum levels are associated with total follicle count (Danforth et al., 1998). In healthy girls, serum inhibin B and estradiol levels express a biphasic age pattern with high levels at 3 months of age, low levels during the prepubertal period and subsequently rising serum levels marking the initiation of puberty (Sehested et al., 2000; Chellakooty et al., 2003; Akssglaede et al., 2009). In prepubertal TS patients, very low levels of serum estradiol and inhibin B have been demonstrated (Gravholt et al., 2002; Wilson et al., 2003a).

However, most available data on gonadotrophins, inhibin B and estradiol serum levels in TS girls are based on cross-sectional studies of TS girls in limited age periods, and the majority of studies do not evaluate the influence of specific TS karyotypes on reproductive hormone levels.

We therefore found it important to report our longitudinal data of circulating FSH, LH, inhibin B and estradiol levels including the entire age span from 0 to 16 years in a large group of TS patients with and without spontaneous pubertal development. We hypothesize that the remaining ovarian function is dependent on the specific karyotype in girls and adolescents with TS, and that reproductive hormones will be more severely affected in TS patients with a monosomic 45,X karyotype. We therefore evaluated the karyotype, FSH and inhibin B as markers of remaining ovarian function in girls with TS.

### Materials and Methods

#### Controls

A total of 2406 healthy girls participated as controls. They were recruited as part of population-based studies of healthy Danish girls, and included 583 infant girls from an ongoing longitudinal cohort of healthy pregnant women and their offspring, as well as from studies of healthy school children: The COPENHAGEN Puberty Study.

Reference ranges for reproductive hormones are cross-sectional, including one serum sample from each control. These data have previously been reported in age-specific intervals, but are now combined to include the entire paediatric age range, although values in the 1–5 years range are sparse (Sehested et al., 2000; Chellakooty et al., 2003; Akssglaede et al., 2009).

#### Patients

All girls classified as TS patients who were followed at the Department of Growth and Reproduction, Rigshospitalet, Denmark (1991–2009) were included. 71 patients were identified from the patient registry at our department based on International Classification of Diseases-10 codes (Q96–Q96.9). One patient with severe concomitant hepatic illness was excluded. Serum concentrations of FSH, LH, inhibin B and estradiol had previously been determined at standard clinical visits, and these data were compiled retrospectively. All patients were routinely examined by paediatric endocrinologists. Information concerning use of sex hormone treatment [hormone replacement therapy (HRT), oral contraceptives, GnRH agonist and oxandrolone] was retrieved from patient files. Only reproductive hormone levels measured prior to sex hormone treatment were included. Two patient files were unavailable. In these cases, only reproductive hormones before the age of 10 years were included.

#### Karyotypes

Diagnosis of TS was confirmed by karyotyping using routine G-banding, including counting of at least 10 metaphases, 3 of which were fully analysed. One clinical geneticist (S.K.) validated all karyotypes in the present study. Depending on their karyotype, the patients were divided into two groups: group A, 45,X (n = 29); and group B, miscellaneous karyotypes (n = 41).

#### Clinical examination

A thorough clinical examination was performed in all patients and controls, including pubertal staging. The patients were grouped according to their ovarian function. Group 1: absent spontaneous puberty in the case of induction of puberty by HRT, or prepubertal [breast stage 1, according to Tanners classification (Tanner, 1962)] at >12 years of age. Group 2: spontaneous puberty with pubertal arrest in the case of spontaneous puberty [presence of breast stage 2 or higher (palpation of glandular breast tissue) or menarche without previous HRT], subsequently treated with estrogen/progesterone due to lack of pubertal progression or secondary amenorrhoea. Group 3: spontaneous puberty with ongoing ovarian function in the case of spontaneous puberty and ongoing pubertal progression or regular spontaneous menstrual bleeding.

#### Reproductive hormone assays

Blood samples (0–16 years) were drawn from 66 to 70 TS patients. The number of samples listed below are the total number of patients in the karyotype group and the number of patients with longitudinal samples in parentheses. For serum FSH, 318 measurements were drawn from a total of 51 patients: group A, 19 (14); group B, 32 (26). For serum LH, 314 measurements were drawn from a total of 50 patients: group A, 19 (14); group B, 31 (25). For estradiol, 277 measurements were drawn from 51 patients: group A, 19 (14); group B, 32 (23). For inhibin B, 238 measurements of inhibin B were drawn from 49 patients: group A, 18 (13); group B, 31 (23). Inhibin B was withdrawn from 30 patients followed at time of pubertal onset.

All blood samples from patients and controls were analysed in the same laboratory blinded for the technician for age, pubertal staging and karyotype. Non-fasting blood samples were drawn between 8 a.m. and 5 p.m. from an antecubital vein, clotted and centrifuged, and serum was stored at −20°C until hormone analyses were performed. All samples were analysed longitudinally within a period of 3 weeks from blood sampling. Serum FSH and LH were measured by time-resolved immunofluorometric assays (Delfia; PerkinElmer, Boston, MA) with detection limits of 0.06 and 0.05 IU/l for FSH and LH, respectively. Intra- and inter-assay coefficients of variation (CVs) were <5% in both gonadotrophin assays. Estradiol was measured by radioimmunoassay [Pantex, Santa Monica, CA (before 1998 distributed by Immuno Diagnostic Systems, Bolton, UK)]. The detection limit was 18 pmol/l, and the intra- and inter-assay CVs...
were <8 and 13%, respectively. Inhibin A and B levels were measured by double-antibody immunometric assays (Beckman Coulter Ltd, UK and Serotec, UK, respectively). The inhibin B assay had a detection limit of 20 pg/ml, and the intra- and inter-assay CVs were 16%.

**Statistical analyses**

Fisher’s exact probability test was used to determine whether the prevalence of spontaneous puberty was significantly different between karyotype subgroups. The same test was used to determine whether significantly more patients with undetectable inhibin B experienced absence of spontaneous puberty compared with patients with at least one detectable inhibin B value. Reference curves for reproductive hormones as a function of age were obtained for girls using a local linear regression smoother. The data for estradiol were log-transformed, whereas inhibin B, LH and FSH measurements were square root-transformed to obtain normal distribution. The mean curve of variance was estimated by smoothing of the observations and residuals, respectively. After calculation of mean and SD on the transformed data, the data were transformed back, resulting in geometric means and 95% prediction interval (±2 SD).

**Ethical considerations**

The studies of healthy girls were approved by the local ethics committee (# KF 01 282214 and # V200.1996/90) and conducted in accordance with the second Declaration of Helsinki. All healthy girls and their parents gave informed consent. Blood samples from girls with TS were collected as part of their routine clinical follow-up and results were stored in a clinical database approved by the Danish Data Protection Agency.

**Results**

Descriptive characteristics of the patients according to karyotype and the prevalence of spontaneous pubertal onset are shown in Table I. In karyotype groups A and B, the median ages (range) at TS diagnosis (or at the first available blood sample) were 5.2 years (0.0–15.8) and 8.2 years (0.0–16.8), respectively.

Longitudinal FSH, LH, inhibin B and estradiol levels by age are shown in Figs 1–4. Eight girls with Y chromosome material in group B were gonadectomized to prevent gonadoblastoma. Reproductive hormone levels after gonadectomy are illustrated by orange lines.

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**Table I Description of the study population: TS karyotype subgroups and prevalence of spontaneous pubertal onset.**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>n</th>
<th>&gt;12 years</th>
<th>Spontaneous puberty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45,X (group A)</td>
<td>29</td>
<td>18</td>
<td>1 (6)(a)</td>
</tr>
<tr>
<td>Miscellaneous karyotypes (group B)</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous karyotypes (without Y material)</td>
<td>33</td>
<td>26</td>
<td>14 (54)(a)</td>
</tr>
<tr>
<td>45,X/46,XX</td>
<td>13</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>45,X/46,Xi(X)(q10)</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>46,X:i(X)(q10)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>45,X/46,X,r(X)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45,X/46,X,r(X),inv(4)(p11q11)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
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<tr>
<td>45,X/46,X,-der(X)t(X;X):p11.4;q13</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45,X,der(X)t(X;22):p11.22;q10;22</td>
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<td></td>
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<tr>
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<tr>
<td>Miscellaneous karyotypes (containing Y material)</td>
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</tr>
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<td>45,X/46,XY</td>
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<td>NA</td>
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<td></td>
<td>NA</td>
</tr>
<tr>
<td>46,X,+mar Y</td>
<td>1</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>44(b)</td>
<td>15 (34)</td>
</tr>
</tbody>
</table>

NA, not applicable due to bilateral gonadectomy; MF, information not available due to missing file.
\(a\)versus B (non-gonadectomized): \(P = 0.001\).
\(b\)No gonadectomized patients or MF.
Puberty

Spontaneous puberty occurred in 6 and 54% of patients in groups A and B, respectively (gonadectomized patients were excluded), \( P = 0.001 \) (Table I). In patients with 45,X/46,XX, the prevalence of spontaneous puberty was high (8/9). In the case of spontaneous pubertal onset, pubertal arrest was experienced in 1/1 (100%) and 5/14 (36%) of groups A and B, respectively.

FSH

Independent of karyotype, FSH levels showed a marked biphasic age pattern from 0 to 16 years (Fig. 1A and B). The biphasic age pattern was preserved in patients subjected to gonadectomy (Fig. 1B, orange lines) and in patients with absent spontaneous puberty (Fig. 1C). All monosomic patients demonstrated highly elevated FSH levels during infancy and early childhood (age 0–5 years) and at the time of expected puberty (>10 years), but they all (\( n = 12 \)) had at least one FSH value within the reference range during the mid-childhood nadir (5–10 years) (Fig. 1A). Of 13 patients in group B, 9 (including one gonadectomized patient) had FSH within the reference range at the mid-childhood nadir (Fig. 1B). The majority of patients with 45,X/46,XX (10/13) had no FSH measurements outside the reference range from 0 to 16 years. Of 12 patients with absent spontaneous puberty, 10 (83%) had FSH levels within the reference range during mid-childhood, but levels were highly elevated at the time of expected puberty (Fig. 1C). The majority of patients entering puberty spontaneously demonstrated FSH levels within the reference range. The patient with the most elevated FSH [45,X,der(X)t(X;22)(p11.22;q10)-22] had elevated LH, undetectable inhibin B and experienced pubertal arrest (Fig. 1D).

LH

Regardless of karyotype, LH demonstrated the same biphasic age pattern as FSH levels (Fig. 2A and B). The majority of patients demonstrated moderately elevated LH levels during infancy and early childhood (78% in group A and 75% in group B) and at the time of expected puberty (75% in group A and 56% in group B; Fig. 2A and B). During the mid-childhood nadir, 11/11 in group A and 12/16 in group B presented LH values within the reference range. Patients with absent spontaneous puberty had LH within the reference range or slightly elevated LH at the time of expected puberty. The majority of patients entering puberty spontaneously presented LH levels within the reference range (Fig. 2D).

Inhibin B

All X-monosomic TS patients demonstrated undetectable inhibin B levels at all times (Fig. 3A). In group B, 12/32 (38%) demonstrated detectable levels (Fig. 3B), but no patients subjected to gonadectomy had detectable

**Figure 1** Serum FSH levels (IU/l) in girls with TS (\( n = 51 \)) according to age, karyotype and spontaneous puberty onset compared with a reference range based on 2406 healthy Danish girls (grey dots). Lines represent geometric means and 95% prediction interval (± 2 SD). Girls with 45,X monosomy (red, A); miscellaneous TS karyotypes before (blue) and after gonadectomy (orange) (B); TS patients with absent spontaneous puberty (C) and spontaneous puberty (D) are shown according to karyotype. Age at spontaneous pubertal onset is illustrated by filled circles.
inhibin B (Fig. 3B, orange lines). All 45,X/46,XX patients with multiple measurement of inhibin B (n = 7) had at least one detectable value.

Inhibin B was recorded in sera from 30 patients with either spontaneous puberty (n = 10; Fig. 3D) or absent spontaneous puberty (n = 20; Fig. 3C). Of 20 patients with absent spontaneous puberty, 19 had exclusively undetectable inhibin B (one patient had detectable inhibin B at 9.0 and 9.2 years and eight other undetectable values from 8.2 until 12.1 years). Of 10 patients entering puberty spontaneously, 9 had at least one detectable inhibin B value (the one patient with exclusively undetectable inhibin B only had two measurements at 7.5 and 15.0 years). Of these patients, six had both detectable and undetectable inhibin B values. Only four of nine patients had inhibin B measured prior to pubertal onset. Exclusively undetectable inhibin B (number of measurements per patient: median 3, range 1–21) predicted absent spontaneous puberty in 19/20 patients, and the patient who entered puberty spontaneously experienced pubertal arrest at Tanner breast-stage B2. Thus, exclusively undetectable inhibin B levels during childhood predicted premature ovarian failure (absence of pubertal onset or pubertal arrest) in 20/20 patients.

Estradiol

X-monosomic TS patients (group A) demonstrated elevated prepubertal levels of circulating estradiol compared with healthy girls (Fig. 4A). At age 4–8 years, 4/7 patients had elevated levels, but estradiol declined to undetectable levels at the time of expected puberty. In group B, estradiol levels were not as high as in group A, but 8/12 demonstrated detectable levels at age 4–8 years (Fig. 4B). After spontaneous pubertal onset, the majority of patients had estradiol levels in the reference range (Fig. 4D).

Discussion

In this unique cohort of 70 TS girls, reproductive hormone levels were highly dependent on karyotype and chronological age. Independent of remaining ovarian function, gonadotrophin levels were within the reference ranges in TS patients during mid-childhood. Multiple undetectable inhibin B values during childhood were strongly associated with premature ovarian failure.

One previous report confirms our findings concerning elevated FSH levels in X-monosomic TS patients and miscellaneous karyotypes in early childhood (0–5 years), compared with 45,X/46,XX mosaicism (Fechner et al., 2006). The study was based on a growth hormone trial, and patients with Y chromosome material were only included if gonadectomized. Our longitudinal study provides additional information about patterns of gonadotrophin levels in TS girls over a large age range, including Y chromosome-positive patients before and after gonadectomy.
In healthy girls, the pituitary–gonadal axis is activated post-natally, resulting in peak FSH and LH levels at ≈3 months of age, which subsequently decrease to minimal levels at mid-childhood. At pubertal onset, the pituitary–gonadal axis is reactivated (Sehested et al., 2000; Chada et al., 2003; Chellakooty et al., 2003; Aksglaede et al., 2009). We revealed the same biphasic age pattern for gonadotrophins in all TS patients independent of karyotype, which is consistent with previous findings (Conte et al., 1975; Chrysis et al., 2006). In X-monosomic and gonadectomized patients who suffered the most severe ovarian failure, gonadotrophin levels were markedly elevated during infancy and at the time of expected puberty but not during mid-childhood. This indicates that the ovaries are not the main inhibitors of gonadotrophin levels during mid-childhood. In support of our present findings, identical biphasic gonadotrophin patterns were found in biologically intact and neonatally ovariectomized female rhesus monkeys, with amplification of elevated gonadotrophin levels during infancy and at the time of expected puberty but not during mid-childhood. This indicates that the ovaries are not the main inhibitors of gonadotrophin levels during mid-childhood. In support of our present findings, identical biphasic gonadotrophin patterns were found in biologically intact and neonatally ovariectomized female rhesus monkeys, with amplification of elevated gonadotrophin levels during infancy and at the time of expected puberty (de Roux et al., 2003; Seminara et al., 2003). Withdrawal of other hypothalamic stimulators or activation of inhibitory signals have also been suggested to block pulsatile GnRH release in childhood, but the exact mechanisms are still unknown (Mitsushima et al., 1994, 1996; El Majdoubi et al., 2000; Wilson et al., 2003b). Conclusively, spontaneous gonadotrophin values are not useful for the diagnosis of TS in girls age 6–10 years.

In contrast to previous findings (Wilson et al., 2003a), we observed intermittent measurable levels of serum estradiol in X-monosomic TS and in patients with miscellaneous karyotypes at age 4–8 years. This finding was unexpected, as these patients had signs of reduced ovarian function, indicated by high FSH and LH levels during adolescence, undetectable inhibin B levels and lack of spontaneous pubertal onset or pubertal arrest. The patient files did not indicate exposure to any external estrogen sources. Limited specificity and sensitivity for extremely low estradiol concentrations may in theory yield falsely elevated estradiol levels due to methodological problems. However, it is unlikely that such a phenomenon would only be present in sera from this specific age group.

An alternative explanation of the measurable estradiol levels could be extragonadal production of estradiol derived from adrenal androgens. Previous longitudinal data showed that primary ovarian failure in TS is associated with earlier onset of adrenarche and significantly higher serum dehydroepiandrosterone levels compared with healthy age-matched girls and TS patients with spontaneous puberty (Martin et al., 2004). Aromatase cytochrome P450 that catalyses the aromatization of testosterone to estradiol and androstenedione to estrone is up-regulated by FSH (Beitins and Padmanabhan, 1991). In theory, this could explain the elevated prepubertal estradiol values in TS patients.

**Figure 3** Serum inhibin B levels (in pg/ml) in girls with TS (n = 49) according to age, karyotype and spontaneous puberty onset compared with a reference range based on 2406 healthy Danish girls (grey dots). Lines represent geometric means and 95% prediction interval (±2 SD). Girls with 45,X monosomy (red, A); miscellaneous TS karyotypes before (blue) and after gonadectomy (orange) (B); TS patients with absent spontaneous puberty (C) and spontaneous puberty (D) are shown according to karyotype. Age at spontaneous pubertal onset is illustrated by filled circles.
However, if true, one would expect elevated estradiol in older patients as well. Unfortunately, we do not have sufficient data on adrenal androgen levels in our 4–8-year-old TS patients to clarify this further.

In our study, the specific TS karyotype seems to be highly predictive of the remaining ovarian function. Other studies have found fewer differences in ovarian function between TS-karyotype groups (Pasquino et al., 1997), and there are even cases of 45,X patients with preserved fertility (Mortensen et al., 2010). This stresses the need for a reliable biomarker of ovarian function during mid-childhood. In this study, solely undetectable inhibin B levels measured prior to pubertal onset were found in all patients with premature ovarian failure. Inhibin B has previously been proposed as a marker of ovarian function in TS girls in a smaller longitudinal study (Gravholt et al., 2002). The specificity of a random undetectable inhibin B value as a marker of ovarian failure is diminished by the fact that 37% of healthy girls (6–12 years of age) have undetectable inhibin B (Sehested et al., 2000). Although this fact restricts the specificity of undetectable inhibin B levels as a screening test of ovarian failure, consecutive measurement of fluctuating inhibin B in a given patient will increase the possibility of detecting remaining ovarian function. This is clearly demonstrated in our present study in which 9/10 patients entering puberty spontaneously had at least one detectable measurement of inhibin B. As the majority of gonadotrophins in TS girls during mid-childhood are within the reference range, FSH levels do not seem to add to the specificity of any test of remaining ovarian function during mid-childhood. Obviously, an elevated FSH at later ages, i.e during adolescence, is highly indicative of primary ovarian failure.

The TS patients in this study are included in a recent multicentre study in which we concluded that anti-Müllerian hormone (AMH) may be a sensitive and specific screening marker of ovarian function in healthy girls and TS patients (Hagen et al., 2010). Until confirmation of these findings, or if analysis of AMH is not available, we recommend that clinical guidelines concerning evaluation of the residual ovarian function during mid-childhood in girls with TS should rely on the specific karyotype preferably supported by longitudinal evaluation of inhibin B.

**Conclusion**

In this longitudinal study, we found significant correlation between specific TS karyotype and remaining ovarian function. Furthermore, we demonstrated a preserved but amplified biphasic age pattern of gonadotrophins in TS patients. We demonstrated considerable differences in reproductive hormone levels among TS girls, depending on their specific karyotype, chronological age and residual ovarian function. However, the mid-childhood decrease of gonadotrophin secretion was independent of karyotype and ovarian reserve, which illustrates that normal gonadotrophin levels during mid-childhood do not exclude TS. Multiple undetectable levels of inhibin B during

**Figure 4** Serum estradiol levels (in pmol/l) in girls with TS (n = 51) according to age, karyotype and spontaneous puberty onset compared with a reference range based on 2406 healthy Danish girls (grey dots). Lines represent geometric means and 95% prediction interval (+2 SD). Girls with 45,X monosomy (red, A); miscellaneous TS karyotypes before (blue) and after gonadectomy (orange) (B); TS patients with absent spontaneous puberty (C) and spontaneous puberty (D) are shown according to karyotype. Age at spontaneous pubertal onset is illustrated by filled circles.
childhood were highly predictive of ovarian failure and lack of spontaneous pubertal onset.

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