CLINICAL STUDY

45,X/46,XX mosaicism below 30% of aneuploidy: clinical implications in adult women from a reproductive medicine unit

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Abstract

Objective: Turner’s syndrome (TS) is well known, but prognosis for 45,X/46,XX mosaicism below 30% of aneuploidy has not been established. We evaluated differences in clinical features and biological parameters between patients with numerical sex chromosome mosaicism diagnosed incidentally and control women.

Design: Retrospective observational study of clinical features and biological parameters.

Methods: Standard endocrinological and gynecological examination was done and early-follicular-phase blood values were collected from the medical records of women aged 21–43, who were referred to our ward from 1996 to 2006 because of infertility and were karyotyped. Seventy-one women with sex chromosome mosaicism (45,X/46,XX) ranging from 4 to 28% were assigned a chromosomally normal woman (46,XX) matched according to age (n=71).

Results: In group 45,X/46,XX, 8% or more of aneuploidy accounted for a smaller height compared to controls (P<0.01). Body mass index was increased from 6% of aneuploidy (P=0.02) and was positively correlated to the percentage of 45,X cells (P<0.0001); menarche occurred earlier from 10% of aneuploidy (P=0.01) and was inversely correlated to the percentage of 45,X cells (P=0.045). No difference was found between the groups for FSH, LH, estradiol, inhibin B, and TSH values. Spontaneous abortions were more frequent in case of mosaicism (P=0.01), and recurrence was positively correlated to the percentage of aneuploidy (P=0.008).

Conclusion: Sex chromosome mosaicism is responsible for clinical changes from 6% of aneuploidy, corresponding to the main phenotypical features of TS.

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Introduction

Turner’s syndrome (TS) is a dysmorphic syndrome which affects 1 in 2500 to 1 in 3000 liveborn girls. Monosomy X (45,X) represents half of the karyotype spectrum of this syndrome, and the rest includes structural X chromosome abnormalities or mosaicism (1). The main features in adolescence are short stature and primary or secondary amenorrhea because of gonadal dysgenesis. Other impairments consist of heart defect, kidney malformations, overweight, hypothyroidism, ophthalmological and otological defects, gastrointestinal disorders, dermatological pathologies, and neoplasia.

Anyone in whom TS has been established after birth is usually identified by phenotypical features suggestive of this syndrome. Nevertheless, 45,X/46,XX mosaicism could be diagnosed incidentally among common people without noticeable features, or prenatally as a result of amniocentesis in advanced maternal age. Indeed, the percentage of 45,X/46,XX mosaicism in the general female population was estimated as 3.1% by Guttenbach et al. and as 3.5% by Peschka et al. (2, 3). If prognosis of 45,X monosomy is well known, there is a lack of knowledge concerning mosaic forms, particularly for mosaicism below 30% of aneuploidy. However, correlation between mosaic and phenotypical or biological parameters has been investigated: follicular counts were increased in individuals with the lowest degree of mosaicism (4); Fechner et al. have illustrated a link between FSH value and 45,X/46,XX mosaicism at an early age (5), and a highest frequency of spontaneous menarche was found among women with mosaicism versus nonmosaic TS patients (6).

In addition, the meaning of ‘low level’ mosaicism for a numerical gonosome anomaly is still being debated. One difficulty comes from the fact that there is no consensus on the definition of low level mosaicism,
which has been considered by some authors as the presence of <10% of abnormal cells (7–10) and by others as the presence of <6% of abnormal cells (3).

Having noticed a significant frequency of 45,X/46,XX mosaicism in patients referred to our unit of reproductive medicine, we wondered whether this could account for the phenotypical or biological changes related to TS. Insomuch as phenotypical manifestations of TS could be due to either haploinsufficiency of genes in the pseudoautosomal regions of the X chromosome or aneuploidy itself, we also assumed that clinical features were proportional to the percentage of 45,X cells.

Thus, the aim of our study was to assess differences in clinical features and biological parameters between women with numerical sex chromosome mosaicism (45,X/46,XX) diagnosed incidentally and control women (46,XX), and also to see if phenotypical signs of TS were correlated to the percentage of aneuploidy among 45,X/46,XX women. At last, comparisons were carried out with successive thresholds of mosaicism to assess if thresholds chosen by cytogeneticists have a clinical significance.

Materials and methods

Study population

Women were retrospectively recruited at the Reproductive Medicine Unit of Brest University Hospital from among a population who had been karyotyped from 1996 to 2006 prior to assisted reproductive technologies (ARTs). Investigation of the female partner of infertile patients was done because it was recommended before ARTs (7, 11). Indications for karyotypes were unknown at the time of recruitment. Women with sex chromosome aneuploidy were collected in group 1 (n = 71); aneuploidy concerned only numerical sex chromosome mosaicism (45,X/46,XX) and ranged from 4 to 28% of 45,X cells (Fig. 1).

For randomization purposes, a chronological patient list was used. Each woman from group 45,X/46,XX was assigned a control and was matched according to age by picking the next chromosomally normal woman (46,XX) on this list. These control women constituted group 2 or 46,XX (n = 71).

All clinical or biological information that was needed was retrospectively collected from medical reports. All 142 women had undergone a routine diagnostic evaluation including a standard endocrinological and gynecological examination: clinical history was taken for menarche, menses, height, body mass index (BMI), and also for spontaneous fertility prior to recruitment.

Laboratory analysis

Chromosome analysis was carried out on phytohemagglutinin-stimulated peripheral lymphocytes cultured for 72 h. Chromosomes were harvested according to standard procedures, and the R-banded karyotypes were described according to the recommendations of the international system for human cytogenetic nomenclature (12). Sixteen cells were karyotyped. However, if at least one of these 16 cells showed a loss or a gain of a gonosome, the number of analyzed metaphases was increased to 25. If a second abnormal cell was observed, the analysis was considered to be completed; if not, the number of metaphases was increased to 50 (13).

Early-follicular-phase blood values of FSH, LH, inhibin B, estradiol (E2), and TSH were measured in our laboratory at day 2 or 3 of menses. In this part of the study, women with premature ovarian failure (POF) were excluded in each group for FSH, LH, inhibin B, and...
Table 2 Demographic data and main features of groups 45,X/46,XX (n=71) and 46,XX (n=71).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>45,X/46,XX (n=71)</th>
<th>46,XX (n=71)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.9 ± 4.3</td>
<td>33.4 ± 3.9</td>
<td>0.31</td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>25.8% (15/58)</td>
<td>33.3% (22/66)</td>
<td>0.36</td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>52.1% (37/71)</td>
<td>40.8% (29/71)</td>
<td>0.13</td>
</tr>
<tr>
<td>Smoking</td>
<td>33.8% (24/71)</td>
<td>38.0% (27/71)</td>
<td>0.36</td>
</tr>
<tr>
<td>Menarche (years)</td>
<td>13.2 ± 1.3</td>
<td>13.0 ± 1.3</td>
<td>0.53</td>
</tr>
<tr>
<td>Menses (days)</td>
<td>29.6 ± 5.1</td>
<td>30.0 ± 6.7</td>
<td>0.72</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 4.3</td>
<td>23.6 ± 4.5</td>
<td>0.92</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 6</td>
<td>164 ± 6</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± 1 S.D. or percentage (%). The two groups did not differ in age at recruitment, either in main phenotypical features of Turner's syndrome (menarche, menses, body mass index, or height) or for parameters that influence spontaneous fertility (menses, BMI, dysmenorrhea, or smoking). Data were missing for dysmenorrhea in each group. BMI, body mass index.

E₂ assessments (n = 3 in group 45,X/46,XX; n = 2 in group 46,XX). POF was defined by a 6-month period of amenorrhea and a FSH level higher than 40 UI/l.

Table 3 Clinical features according to karyotype. 45,X/46,XX women are sub-sampled according to the percentage of aneuploidy: 4–6% vs 8–28%. Data are expressed in mean ± 1 S.D.

<table>
<thead>
<tr>
<th>Aneuploidy Range</th>
<th>45,X/46,XX (n=28)</th>
<th>46,XX (n=43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.9 ± 4.0</td>
<td>33.2 ± 4.8</td>
<td>0.20</td>
</tr>
<tr>
<td>Menarche (years)</td>
<td>13.5 ± 1.2</td>
<td>12.8 ± 1.3</td>
<td>0.24</td>
</tr>
<tr>
<td>Menses (days)</td>
<td>28.6 ± 3.9</td>
<td>29.7 ± 5.3</td>
<td>0.93</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 ± 6*</td>
<td>160 ± 6 1*</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.9 ± 4.1†</td>
<td>24.7 ± 4.2†</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Comparisons were made between group 46,XX and sub-samples of group 45,X/46,XX according to the percentage of aneuploidy (4–6% vs 8–28%). Women with aneuploidy above or equal to 8% were shorter than both controls (P<0.02) and women with aneuploidy below or equal to 6% (P=0.02), and also had higher BMI than women with aneuploidy below or equal to 6% (P=0.01).

Results

Demographic data

No TS was diagnosed in patients of any group. Figure 1 represents 45,X/46,XX women distributed according to their percentage of aneuploidy. Most of them had sex chromosome mosaicism equal to or lower than 12% of aneuploidy: two women had 16% of aneuploidy and one woman had 28% of aneuploidy.

No difference was found between the groups as to why couples referred to our unit of reproductive medicine. These indications were those habitually observed for ARTs: tubal factor, endometriosis, dys ovulation, diminished ovarian reserve, recurrent spontaneous abortion (RSA), and male factor, mixed or idiopathic.

Indications for karyotypes did not differ between groups, except for recurrent pregnancy loss, which was seen more often in group 45,X/46,XX (Table 1).

Clinical status

Menarche, menses, height, and BMI did not differ between the two groups (Table 2); nevertheless, differences were found after sampling 45,X/46,XX women according to their percentage of aneuploidy. First, we observed that aneuploidy above or equal to 8% accounted for a smaller height compared to both controls and women with aneuploidy below or equal to 6% (160 ± 6 vs 164 ± 6 cm; P=0.02; Table 3); 45,X/46,XX women were also shorter than controls when groups were restricted to women recruited for male infertility only (160 ± 6 vs 165 ± 6 cm; P=0.02). Secondly, BMI was significantly higher from 6% of aneuploidy among 45,X/46,XX women (24.4 ± 4.4 vs 21.6 ± 4.1 kg/m²²; P=0.01), and overweight independent groups. GraphPad Prism software (version 4.0; GraphPad Software, San Diego, CA, USA) was used to perform Spearman’s rank correlation. Spearman’s rank correlation was used to test the direction and strength of the relationship between the percentage of aneuploidy and menarche, height, BMI, and miscarriage recurrence. 

P<0.05 was considered significant.
occurred from 10% of aneuploidy (25.7 ± 4.1 kg/m²; Tables 3 and 4). Furthermore, BMI was positively correlated to the percentage of 45,X cells (P=0.0001). Lastly, no woman had primary amenorrhea, and all women had spontaneous menarche in both groups. However, menarche occurred earlier in group 45,X/46,XX when the percentage of aneuploidy reached 10% or more than in women with aneuploidy below or equal to 8% (12.3 ± 1.0 vs 13.5 ± 1.3 years; P=0.01; Table 4). Moreover, age of menarche was inversely correlated to the percentage of 45,X cells in the group 45,X/46,XX (P=0.045).

### Biological changes

No difference was found between the two groups for early-follicular-phase blood values of FSH (8.41 ± 2.6 vs 8.28 ± 4.0 UI/l; P=0.85), LH (4.99 ± 3.4 vs 5.04 ± 2.3; P=0.89), E₂ (50.6 ± 23.7 vs 51.7 ± 32.1 pg/ml; P=0.81), inhibin B (55.5 ± 36.5 vs 57.4 ± 33.7 pg/ml; P=0.80), or TSH (1.74 ± 0.8 vs 1.83 ± 1.1 µIU/ml; P=0.64). However, differences appeared after pooling 45,X/46,XX women depending on their percentage of aneuploidy: E₂ level was significantly increased in women with 6% or less of aneuploidy than in women with 8% or more of aneuploidy (58.1 ± 14.6 vs 51.6 ± 19.6 pg/ml; P=0.03). E₂ level was also significantly increased in women with 10% or less of aneuploidy than in women with 12% or more of aneuploidy (52.7 ± 19.2 vs 37.7 ± 17.9 pg/ml; P=0.02). Furthermore, E₂ level was inversely correlated to the percentage of aneuploidy in group 45,X/46,XX (P=0.02). No more difference was found for the other parameters after sub-sampling 45,X/46,XX women.

### Spontaneous fertility

Three and two women had POF diagnosed at the time of recruitment in groups 45,X/46,XX and 46,XX respectively. The frequencies of POF were 4.2 and 2.8% in these groups respectively (P=NS).

The mean number of first trimester pregnancy losses per patient was 2.0 ± 0.9 in group 45,X/46,XX and was 1.2 ± 0.4 in group 46,XX (P=0.01). Frequency of spontaneous pregnancy loss was significantly increased in group 45,X/46,XX than in group 46,XX (48.9 vs 24.1%; P=0.0026). There was also a positive correlation between the recurrence of spontaneous miscarriage and the percentage of 45,X cells in group 45,X/46,XX (P=0.026). Complementary investigations were carried out in group 45,X/46,XX, and they revealed two women with Hashimoto’s disease, one woman with heterozygous prothrombin mutation, and two women with a FSH level higher than 15 UI/l as a possible other etiology for RSA. Having excluded these women, positive correlation was still significant (P=0.0028).

### Discussion

Clinically, we confirmed that the main phenotypical features of TS are visible even in the case of mosaicism below 30% of 45,X cells. Moreover, it seems to appear as from 6% of aneuploidy, which has not been demonstrated yet. However, phenotypical expression level depends on the feature studied.

First, short stature is one of the cardinal symptoms of TS, and the mean final height of patients without recombinant human GH is habitually 20 cm shorter than the mean height of normal female population from the same country. Thereby, mean height of TS patients in France is 142 cm (14), whereas mean normal height is 163 cm (15). It is known that TS patients with mosaicism are often taller than 45,X monosomic patients (16), but are also shorter than normal women. Although it appears in our study that the heights of the two groups are not so different from the heights of the French normal population, mosaicism involves shorter height from 8% of aneuploidy. Genetically, short stature homebox gene located in the pseudoautosomal regions of X (Xp11–22) and Y (Yp11) is implicated in skeletal growth troubles by haploinsufficiency in TS patients (17). Women with this deletion have less important growth failure (−2 s.d.), than women with monosomy or important X short arm deletions (−3 s.d.) (18). Another gene located between regions Xp11.2 and Xp22.1 was implicated in height regulation (19): it seems that patients with this deletion had normal stature, but were nonetheless smaller than expected considering their parents’...
heights. In our study, X chromosome haploinsufficiency is involved in height regulation by a global gene haploinsufficiency, but mosaicism below 28% does not induce significant growth failure.

Secondly, TS is associated with overweight after the age of 5 (20). In our study, BMI was also influenced by mosaicism with a positive correlation between BMI and the percentage of 45,X cells. However, the hypothesis of gene haploinsufficiency for overweight was not upheld by Ohman et al. because of a candidate locus for obesity located on X short arm Xp24 (21). Nevertheless, Bakalov et al. demonstrated an insulin secretion defect in TS patients compared with both 46,XX patients with diminished ovarian reserve and controls matched according to age (22); they illustrated that haploinsufficiency of genes located on X chromosome accounted for pancreatic β-cell impairment. This finding could partly explain the positive correlation demonstrated in our study, with proportional pancreatic cell impairment depending on the percentage of aneuploidy. However, this hypothesis was not tested in our study because of the use of a retrospective method.

The fact that BMI and height achieve statistical significance at a different cut point is probably a random variation in our study. Indeed, height is always negatively correlated to BMI, so as the group becomes more severely affected, height will decline and BMI will go up. However, it is possible that the lack of significance for the height of the patients from 6% of aneuploidy is linked to the inadequate size of the sample: small height variations will significantly affect BMI, as it is squared on the denominator, but the sample is probably too small (18 subjects with 4% of aneuploidy) to achieve significance between groups 4 and 6–28% women of 45,X cells (164 ± 6 vs 162 ± 6 cm; P = NS). More investigations are necessary to conclude on this point.

Thirdly, we demonstrated that all patients with sex chromosome mosaicism had spontaneous menarche. This notion was illustrated by Pasquino et al. for whom most TS patients with spontaneous menarche were those with mosaicism; indeed, in 69 patients over 12 and for whom TS was diagnosed, 63% had spontaneous pubertal development and 50% had spontaneous menarche at the mean ± s.e.m. age of 13.2 ± 1.5 (6). Nevertheless, the authors did not specify the percentage of aneuploidy in this group. The age of menarche in France is 12.6 (23), which is not different from that of our control group (13.0 ± 1.3). In our study, earlier menarche was found for women with mosaicism above or equal to 10%, as well as an inverse correlation between menarche and the percentage of 45,X cells. Thus, the mean age of menarche is earlier in sub-sample 45,X 10–28% (12.3 ± 1.0) than in both general population and sub-sample 45,X 4–8%. Nevertheless, age of menarche in sub-samples is in the normal range in France. Spontaneous menarche occurs only if follicles are present in the ovary. Hreinsson et al. have found follicles in eight ovarian cortical biopsies of TS women, and demonstrated that follicular density was inversely correlated to FSH level, but also that follicular counts were increased in individuals with the lowest degree of mosaicism (4), meaning that early follicle depletion due to X haploinsufficiency causes earlier menarche. This contradicts the data of Lachlan et al. who have demonstrated that menarche occurs later (14.2 ± 1.5 years) in case of deletions proximal to Xp22.1, and leads to more frequent POF (24). In our study, 45,X/46,XX patients with confirmed POF (n = 3) also had belated menarche (14.0 ± 0 years), but no other women with menarche after 14 had POF. On this ground, it has been demonstrated that age at menarche is not affected by menopausal age, even in case of POF (25). It would mean that the more the aneuploidy increases, the more the oocyte depletion increases, which leads to primary amenorrhea. Therefore, sex chromosome mosaicism below 30% of aneuploidy leads to earlier menarche, but does not involve more POF in our study.

As phenotypical consequences of mosaicism had been found, the same repercussions on spontaneous fertility were expected. Indeed, TS is one of the genetic etiologies of infertility and is associated with ovarian failure or recurrent pregnancy loss in cases of preserved fertility (1). We investigated two features indicating the ovarian reserve: early-follicular-phase blood values and spontaneous fertility. Surprisingly, the results proved conflicting: in our study, no obvious difference arose from usual tests of ovarian reserve between 45.X/46,XX women and controls, whereas mosaicism was associated with increased first trimester pregnancy terminations. Concerning biological values, differences were significant only among sub-samples in group 45.X/46,XX but never between 45.X/46,XX sub-samples and control women. However, the highest mean E2 levels did not reach that considered as poor prognostic value (> 80 pg/ml) by Smotrich et al. at day 2 or 3 of menses (26), which means the tests used in our study were not discriminating enough, or mosaicism had no real impact on ovarian reserve. Parameters with more sensitivity to answer this question may be lacking in our study because of our retrospective study range starting from 1996 to 2006, we had neither antral follicular count nor anti-Mullerian hormone assessments, which now seem to provide more discriminating acuteness in adulthood (27–29). However, these parameters would not have shown any difference between groups 45,X/46,XX and 46,XX since Sonntag et al. had found no difference in assisted reproductive technology results between the two corresponding groups, which is considered the best ovarian reserve test (10). Therefore, in our study, sex chromosome mosaicism below 30% has no impact on the ovarian reserve of females.

Although expected results were not found for the assessment of ovarian reserve in group 45.X/46,XX, important consequences for spontaneous fertility were shown, among which was the unfavorable prognosis...
in the first trimester of pregnancy. Nevertheless, the impact of sex chromosome mosaicism on RSA is disputed. The significant difference found for the mean number of first trimester abortions between groups 45.X/46.XX and 46.XX might be due to a recruitment bias: seven karyotypes were requested for RSA in group 1, but none in the control group (P=0.006). Yet, the significant correlation found between the recurrence of abortion and the percentage of 45.X cells was independent of any recruitment bias, all the more so because correlation was still significant after excluding women with other etiologies for RSA. These results are in contrast to those reported by Horsman et al., who illustrated in 1987 that mosaicism frequency was not higher in a population with RSA (1.5%) than in a control group without RSA (30); however, the percentage of mosaicism in their study group did not exceed 10% of aneuploidy, whereas the most numerous pregnancy losses concerned women with 12% aneuploidy in our study. On the other hand, RSA in 45.X mosaic patients could rather be the result of an increased rate of diminished ovarian reserve according to Kuo & Guo (31); this was not confirmed in our study either. On the contrary, these results suggest a conduction of the aneuploidy to the oocyte nucleus without repercussion on follicular atresia, thus confirming the known impact of parental chromosome aberrations on abortions (32). Therefore, could the observed changes possibly be the result of mosaicism limited to tissues? Two authors have demonstrated that aneuploidy can be found in different parts of the ovary: in cortical biopsies by de Grouchy et al. (33), and in granulosa cells among 16 patients of the study (F Gallon, F Morel, V Amice & M De Braekelee, unpublished observations) by us. It means that mosaicism below 30% of aneuploidy has physical repercussions but does not induce endocrine ovarian defect although it is transmitted to the ovary. It is known that only one X is required for ovarian differentiation (34), but two are required for ovarian maintenance (35). Several studies have shown that proximal Xq and proximal Xp contain regions of importance to ovarian maintenance, among them are Xq13 and Xp11. Deletions in these areas are responsible for POE. Nevertheless, some authors consider that impaired ovarian development is due to the length of the lacking region rather than to specific genes during meiosis (18). According to our study, it seems that 30% of aneuploidy does not negatively affect ovarian maintenance, but it could be transmitted to the oocyte.

**Conclusion**

We can conclude that sex chromosome mosaicism that is diagnosed incidentally has a clinical impact from 6% of aneuploidy in adulthood. This result corroborates data found in childhood, even if age-dependent loss of X chromosome is confirmed (18, 36). While no consensus has been established on cytogenetic grounds for the definition of low level sex chromosome mosaicism, we have demonstrated that the clinical threshold seems to be 6%. This result has an important significance particularly for the prenatal screening of such aneuploidy. Parents can be reassured about their descendants as far as phenotypical feature prognosis is concerned, as well as for spontaneous pubertal development. Nevertheless, patients should be informed about the likelihood of higher rates of first trimester miscarriage.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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