Hidden mosaicism in patients with Klinefelter’s syndrome: implications for genetic reproductive counselling†

L. Garcia-Quevedo 1, J. Blanco 1, Z. Sarrate 1, V. Català 2, L. Bassas 3, and F. Vidal 1,*

1Unitat de Biologia Cel·lular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain 2Prenatal Genetics, SL, 08036 Barcelona, Spain 3Laboratori de Seminologia i Embriologia, Servei d’Andrologia, Fundació Puigvert, 08025 Barcelona, Spain

*Correspondence address. Tel: +34-93-581-27-81; Fax: 34-93-581-22-95; E-mail: francesca.vidal@uab.cat

Submitted on June 22, 2011; resubmitted on August 23, 2011; accepted on September 26, 2011

BACKGROUND: Most individuals with Klinefelter’s syndrome (KS) are azoospermic but residual foci of spermatogenesis have been observed in some patients. However, no consistent predictive factors for testicular sperm extraction success have been established and mosaicism could be a factor to investigate. In this study, we have assessed the degree of mosaicism in somatic and germinal tissues in KS, the meiotic competence of 47,XXY germ cells and the aneuploidy rate of post-reductional cells.

METHODS: Five patients with KS previously diagnosed as pure 47,XXY have been studied. Samples from four donors were processed as controls. The chromosome constitution of lymphocytes, buccal mucosa and testicular tissue was assessed by interphase fluorescence in situ hybridization for chromosomes X, Y and 18. In meiotic figures, sex chromosome number and pairing was confirmed.

RESULTS: 46,XY cell lines were observed in all patients and tissues analysed. The degree of mosaicism (mean ± SD) differed among tissues (lowest in lymphocytes: 4.8 ± 2.5%; highest in Sertoli cells: 42.3 ± 11.1%). Meiotic figures were found in three cases (KS1, KS2 and KS5), all of them showed an XY complement. Hyperhaploid post-reductional cells were found in all patients (range: 3.3–36.4%) and increased rates versus controls (P < 0.05) were observed.

CONCLUSIONS: Diagnosis of homogeneous KS based on lymphocyte karyotyping should be contrasted in other tissues. Mucosa cells could help to better approximate the degree of germ cell mosaicism. Our results indicate that 47,XXY germ cells are not meiotically competent. Increased post-reductional aneuploidy rate is related to meiotic errors in 46,XY cells. Appropriate genetic counselling is recommended in KS.

Key words: aneuploidy / cytogenetics / Klinefelter’s syndrome / meiosis / mosaicism

Introduction

Klinefelter’s syndrome (KS) is the most common chromosome abnormality, with an incidence of 3% in infertile males, and it represents 9–11% of azoospermic patients (De Braekeleer and Dao, 1991; Mau-Holzmann, 2005). Genetic diagnosis is usually ascertained by cytogenetic studies from blood lymphocytes through the evaluation of 20–50 metaphases (Hook, 1977). More than two-thirds of all cases are reported as homogeneous (47,XXX) and the remaining as mosaic (46,XY/47,XXX) or higher grade chromosome aneuploidies (reviewed by Lanfranco et al., 2004).

As mosaicism can be the source of the well-known clinical variations reported in KS individuals, and as karyotype analysis can overlook low mosaicism degree, several authors recommend incorporating interphase fluorescence in situ hybridization (FISH) analysis as a complement to conventional cytogenetics for a more specific and sensitive detection of mosaicism (Okada et al., 2001; Abdelmoula et al., 2004; Lenz et al., 2005). Moreover, the study of mucosa cells has also been proposed as a quick and reliable screening test for a better ascertainment of the chromosome constitution of patients with KS (Kruse et al., 1998; Westlander et al., 2001).

Individuals with KS are traditionally described as being infertile, however, residual foci of spermatogenesis have been observed in both non-mosaic and mosaic individuals (reviewed by Lanfranco et al., 2004). The reported presence of spermatozoa in the testicular tissue of patients with KS has led to offering testicular sperm...
Mosaicism in Klinefelter's syndrome

Materials and Methods

Study populations

Five azoospermic individuals (KS1–KS5) aged between 29 and 35 years, diagnosed as homogeneous 47,XXY by standard cytogenetic procedures (analysis of 20 G-banded metaphases from a peripheral blood lymphocyte culture) were the subjects of our study. Plasma hormone levels were as follows: 13–47.7 IU/l for FSH, 6.7–32.8 IU/l for LH and 4.5–16.2 nmol/l for testosterone. All patients had small testis volume (5–10 ml) and were enrolled as candidates for TESE. For every patient, a new sample of peripheral blood, a buccal swab and a sample of the testicular tissue obtained for sperm retrieval were analysed. Four cell types have been assessed: lymphocytes from peripheral blood, epithelial cells from buccal mucosa and Sertoli and germ cells (pre- and post-reductional cells and meiotic figures) obtained from testicular biopsies.

Four testicular samples from adult organ donors aged between 20 and 33 years have been used as the control population. Furthermore, control samples from buccal mucosa and lymphocytes were obtained from four healthy men (mean age: 31 ± 9.5 years).

Informed consents were obtained from participants and protocols were approved by the ethics committees of the centres involved.

Lymphocyte and buccal mucosa cells

Heparinized peripheral blood from patients KS1 to KS4 was obtained and cultured in 4% phytohaemagglutinin-supplemented medium (PHA M; GibCo, Invitrogen; Paisley, UK) at 37°C for up to 72 h. Cytogenetic preparations were obtained following standard procedures after 25 min incubation in Colcemid (0.16 μg/ml; GibCo, Invitrogen), hypotonic treatment (0.075M KCl) for 20 min at 37°C and fixation in Carnoy’s solution.

Oral smears were obtained by scraping the inner cheek and were processed for cytogenetic analysis. Buccal mucosa cells were incubated with hypotonic solution (0.035 M KCl) for 30 min at 37°C. Cells were washed twice with Carnoy’s fixative solution before spreading. Slides were treated with acetic acid solution (50% in H2O) for at least 30 min at 40°C to permeabilize the cells prior to FISH processing.

A triple-colour FISH was performed in both lymphocytes and buccal mucosa cells using centromeric DNA probes for chromosomes X, Y and 18 (CEP Y, Spectrum Orange; CEP X, Spectrum Green; CEP 18, Spectrum Aqua) as described by the manufacturer (Aneuvysy Assay Kit, Abbott Molecular, Abbott Park, IL, USA).

Control samples were processed following the same protocols described for KS individuals.

Testicular tissue

Testicular biopsies were obtained under local anaesthesia with the aim of freezing tissue for future ICSI. All testicular samples were sent to the laboratory at 4°C in isotonic solution and were processed for cytogenetic studies with the conventional method of Evans et al. (1964).

Before FISH, samples were stained with Leishman (20%) for 8–10 min. Prophase I, metaphase I and metaphase II figures were captured and coordinates were recorded to facilitate location and analysis after FISH. Preparations were de-stained in an ethanol series (70, 80 and 90%, each for 1 min) before FISH. The chromosome constitution of Sertoli cells, pre- and post-reductional germ cells and meiotic figures were analysed for chromosomes X, Y and 18 (Aneuvysy Assay Kit, Abbott Molecular).

A sequential FISH with Whole-Chromosome Painting probes (WCP) for the sex chromosomes (X-XP 23-FITC, Y-XP 24-TexasRed; Metha-Systems GmbH; Altltushein, Germany) was performed to evaluate the sex chromosome pairing at pachytene and metaphase I and to confirm the sex chromosome constitution of metaphases II.

Control testicular samples were processed following the same protocols described above.

Materials and Methods

Study populations

Five azoospermic individuals (KS1–KSS) aged between 29 and 35 years, diagnosed as homogeneous 47,XXY by standard cytogenetic procedures (reviewed by Radicioni et al., 2010). Successful spermatozoan recovery in adult KS men ranges from 16 to 60% (reviewed by Fullerton et al., 2010). Patient age seems to correlate with the presence of spermatозoa (Ferli et al., 2009; Ramasamy et al., 2009); however, no other consistent predictive factors of successful testicular sperm recovery have been reported (Radicioni et al., 2010).

Concerns and controversy over the safety of KS individuals using their own gametes for assisted reproduction have been raised, and several studies have focused on the analysis of the chromosome constitution of these patients’ germ cells (reviewed by Templado et al., 2011). All reports published to date are in agreement in describing increases in sex chromosome abnormalities and hyperhaploidies in the post-reductional germ cells from 47,XXY and 46,XY/47,XXY individuals (Estop et al., 1998; Foresta et al., 1998; Kruse et al., 1998; Foresta et al., 1999; Bielanska et al., 2000; Levron et al., 2000; Rives et al., 2000; Blanco et al., 2001; Hennebicq et al., 2001; Bergere et al., 2002; Yamamoto et al., 2002). The origin of these abnormalities has been related to the possible meiotic progress of the 47,XXY germ lines (Skakkebaek et al., 1969).

This hypothesis has been supported by some authors (Foresta et al., 1998; Ferlin et al., 2005) based upon the deviation in the X/Y sperm ratio (in favour of X-bearing spermatoozoa) and the presence of equivalent percentages of XX and XY sperm hyperhaploidies. However, most studies do not agree with these results but rather maintain that in KS individuals the only cells that progress through meiosis are 46,XY cells (Blanco et al., 2001; Bergere et al., 2002; Sciurano et al., 2009), thus indicating that patients with spermatogenic patches are individuals whose testicular tissue is mosaic. Therefore, the cytogenetic abnormalities observed in post-reductional germ cells must result from the abnormal meiotic progression of 46,XY spermatocytes in the compromised testicular environment, which is distinctive in patients with KS (Mroz et al., 1999).

The alteration of the testicular environment has been related to abnormal hormone levels and to the dysfunction of testicular somatic cells (reviewed by Radicioni et al., 2010). It has been suggested that the nursing function of XXY Sertoli cells may not be as effective as those of XY cells (Wikstrom et al., 2007; Sciurano et al., 2009). Thus, the mosaicism level in the testicular tissue, not only concerning germ cells but also for somatic cells, could be of relevance for the final outcome of spermatogenesis in KS and could have practical implications for the clinical management of these individuals.

The aims of the present study were: (i) to assess the occurrence of mosaicism in different tissues and cell types (somatic and germinal) in KS individuals previously diagnosed as being pure 47,XXY; (ii) to evaluate if any predictive value for successful TESE can be inferred from mosaicism data; (iii) to analyse the meiotic competence of 47,XXY spermatocytes and (iv) to evaluate the genetic reproductive risk in these patients.

Downloaded from https://academic.oup.com/humrep/article-abstract/26/12/3486/2915084 by guest on 10 November 2018
Microscope analysis and evaluation criteria

All evaluations were carried out using an Olympus BX-60 fluorescent microscope (Olympus Barcelona, Spain) equipped with specific filters for FITC, Cy3, Aqua and a multiband pass filter (DAPI/FITC/Texas Red).

A minimum of 500 lymphocytes (except for KS), 200 buccal mucosa cells and 1000 interphase nuclei (germ cells and Sertoli cells), and all of the meiotic figures observed were analysed for each individual.

FISH analysis in interphase cells was performed in accordance with the criteria described by Blanco et al. (2001). Briefly, germ cells were classified according to the number of sex chromosome hybridization signals and chromosome 18 was used as ploidy control. Strict scoring criteria for signal evaluation were used: signals must be of the same size and intensity and the distance between signals must be at least the same as the diameter of the signal. Overlapped nuclei were discarded from the analysis.

Sertoli cells exhibit a characteristic morphology, clearly distinct from interphage germ cells, showing a large fusiform nucleus with a prominent nucleolus and diffuse chromatin.

Meiotic figures were classified taking into account the distribution of hybridization signals from the two rounds of FISH. In pachytene cells, the presence of sex chromosome-specific centromeric signals plus one single WCP domain for the X and Y chromosomes (the sex vesicle), and a single centromeric signal for chromosome 18 (assuming pairing) was considered as being normal in 46,XY cells.

A cut-off level for false-negative FISH results was established by the analysis of 45,X and 45,Y interphase nuclei in testicular cells (Sertoli cells and pre-reductional cells), and all of the meiotic figures observed were analysed for each individual.

FISH analysis in interphase cells was performed in accordance with the criteria described by Blanco et al. (2001). Briefly, germ cells were classified according to the number of sex chromosome hybridization signals and chromosome 18 was used as ploidy control. Strict scoring criteria for signal evaluation were used: signals must be of the same size and intensity and the distance between signals must be at least the same as the diameter of the signal. Overlapped nuclei were discarded from the analysis.

Sertoli cells exhibit a characteristic morphology, clearly distinct from interphage germ cells, showing a large fusiform nucleus with a prominent nucleolus and diffuse chromatin.

Meiotic figures were classified taking into account the distribution of hybridization signals from the two rounds of FISH. In pachytene cells, the presence of sex chromosome-specific centromeric signals plus one single WCP domain for the X and Y chromosomes (the sex vesicle), and a single centromeric signal for chromosome 18 (assuming pairing) was considered as being normal in 46,XY cells.

A cut-off level for false-negative FISH results was established by the analysis of 45,X and 45,Y interphase nuclei in testicular cells (Sertoli cells and pre-reductional cells), and all of the meiotic figures observed were analysed for each individual.

A cut-off level for false-negative FISH results was established by the analysis of 45,X and 45,Y interphase nuclei in testicular cells (Sertoli cells and pre-reductional cells), and all of the meiotic figures observed were analysed for each individual.

Statistical analysis

The X-bearing/Y-bearing ratio was performed with McNemar test (Statistical Package for the Social Sciences 15.0.1 for windows). All other comparisons were performed with Fisher’s exact test (GraphPad InStat, version 3.05, 32bit for Windows 95/NT). Statistical significance was set at $P < 0.05$. Data are presented as mean ± SD values.

Results

Successful FISH results were obtained in the three tissues analysed (Fig. 1). Control preparations were used to establish cut-off levels for possible FISH false negatives in the different cell types analysed. Values for 1712 lymphocyte nuclei, 663 buccal mucosa cells, 620

Discussion

Karyotyping from lymphocyte culture of peripheral blood is the standard diagnostic technique used to determine chromosomal anomalies.
However, there is a high heterogeneity in the methods used to search for, establish and confirm mosaicism. Although the study of 50 metaphases is recommended to exclude <10% of mosaicism with a 0.99 confidence level (Hook, 1977) most routine karyotyping is based on the study of 20 metaphases. The patients analysed in our study would be a clear example of undetected mosaicism, since all have

**Figure 2** Triple colour-FISH images of Sertoli cell nuclei and germ cell nuclei in patient with KS. (A) Top left: Sertoli cell with an XY complement; bottom left: Y-bearing secondary spermatocyte; top right: X-bearing round spermatid. (B) Bottom left: XXY pre-reductional germ cell; top right: XXY Sertoli cell. Scale bar represents 10 μm.

**Figure 3** Percentage of 46,XY cells in five patients with KS.

**Table 1** Percentage of 46,XY cells in the tissues analysed in patients with KS.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age (years)</th>
<th>Blood</th>
<th>Buccal mucosa</th>
<th>Testis Pre-reductional cells</th>
<th>Sertoli cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS1</td>
<td>31</td>
<td>14/527 (2.7%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37/203 (18.2%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95/210 (45.2%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/11 (45.5%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KS2</td>
<td>29</td>
<td>16/530 (3%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44/132 (33.3%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>355/836 (42.5%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74/137 (54%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KS3</td>
<td>35</td>
<td>41/510 (8%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97/292 (33.2%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>287/714 (40.2%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58/239 (24.3%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>KS4</td>
<td>31</td>
<td>28/509 (5.5%)&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>31/192 (16.1%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>225/866 (26%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24/58 (41.4%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KS5</td>
<td>34</td>
<td>18/206 (8.7%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>235/834 (28.2%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40/86 (46.5%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>32 ± 2.4</td>
<td>4.8% ± 2.5%</td>
<td>21.9% ± 10.9%</td>
<td>36.4% ± 8.7%</td>
<td>42.3% ± 11.1%</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Indicate significant differences within columns (Fisher’s exact test; P < 0.05).
previously been diagnosed as pure 47,XXY. However, upon amplifying the study by interphase FISH, the presence of XY lymphocytes was seen with variable percentages (2.7–8%). These figures are clearly over the cut-off level for false-negative FISH results established in our laboratory for control individuals (0.5\(\pm\)0.9%). Thus, for the diagnosis of ‘pure KS’, it is advisable to increase the number of cells analysed, either by karyotyping or by FISH in interphase nuclei.

The observation of different percentages of 46,XY cells in the analysed tissues indicates that the degree of mosaicism is not uniform. Our data demonstrate that even in cases of a low percentage of mosaicism in peripheral blood (<10%), the patients present degrees of germinal mosaicism >36% (Table I). Our results confirm that the analysis of lymphocytes is not a good indicator of the testicular status and are in good agreement with what other authors suggest (Bielanska et al., 2000; Lanfranco et al., 2004). It has been described that the analysis of buccal mucosa is a rapid method to confirm and increase the accuracy of the diagnosis of patients with KS (Kamischke et al., 2003). In our study, the percentages of observed buccal mucosa 46,XY cells (21.9\(\pm\)10.9%) are closer to the mosaicism observed in the germinal line (36.4\(\pm\)8.7%), although its predictive value for

<table>
<thead>
<tr>
<th>Spermatogonial metaphases</th>
<th>Prophases</th>
<th>Leptotene</th>
<th>Zygote</th>
<th>Pachytene</th>
<th>Metaphase I</th>
<th>Metaphase II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS1</td>
<td>48</td>
<td>32</td>
<td>27</td>
<td></td>
<td>107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KS2</td>
<td>5</td>
<td>60</td>
<td>188</td>
<td>166</td>
<td>7</td>
<td>10</td>
<td>436</td>
</tr>
<tr>
<td>KS5</td>
<td>3</td>
<td>29</td>
<td>122</td>
<td>86</td>
<td>2</td>
<td>1</td>
<td>243</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>137</td>
<td>342</td>
<td>279</td>
<td>9</td>
<td>11</td>
<td>786</td>
</tr>
</tbody>
</table>

Table II Mitotic and meiotic figures analysed in patients with KS.

![Sequential cytogenetic analysis in pachytene-stage spermatocyte in patient with KS.](https://academic.oup.com/humrep/article-abstract/26/12/3486/2915084/4).

**Figure 4** Sequential cytogenetic analysis in pachytene-stage spermatocyte in patient with KS. (A) Leishman stain. Arrows point to the sex vesicle. (B) Triple-colour FISH with centromeric DNA probes for chromosomes X (green), Y (red) and 18 (blue). A single signal for each sex chromosome (XY) is identified inside the sex vesicle. (C) WCP for the sex chromosomes (X-green and Y-red) confirming the presence of a single X chromosome plus a Y chromosome forming the sex vesicle. Scale bar represents 10 \(\mu m\).

<table>
<thead>
<tr>
<th>23,X</th>
<th>23,Y</th>
<th>24,XY</th>
<th>25,XXY</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS1</td>
<td>6/14 (42.9%)</td>
<td>4/14 (28.6%)</td>
<td>4/14 (28.6%)*</td>
<td></td>
</tr>
<tr>
<td>KS2</td>
<td>14/34 (41.2%)</td>
<td>9/34 (26.5%)</td>
<td>7/34 (20.6%)*</td>
<td>4/34 (11.8%)</td>
</tr>
<tr>
<td>KS3</td>
<td>12/38 (31.6%)</td>
<td>12/38 (31.6%)</td>
<td>11/38 (28.9%)*</td>
<td>3/38 (7.9%)*</td>
</tr>
<tr>
<td>KS4</td>
<td>9/33 (27.3%)</td>
<td>5/33 (15.2%)</td>
<td>12/33 (36.4%)*</td>
<td>7/33 (21.2%)*</td>
</tr>
<tr>
<td>KS5</td>
<td>27/61 (44.3%)</td>
<td>27/61 (44.3%)</td>
<td>5/61 (8.2%)</td>
<td>2/61 (3.3%)*</td>
</tr>
<tr>
<td>Control mean (n = 4)</td>
<td>667/1454 (45.9%)</td>
<td>687/1454 (47.3%)</td>
<td>87/1454 (6%)</td>
<td>1/1454 (0.1%)</td>
</tr>
</tbody>
</table>

Table III Chromosome constitution of post-reductional cells in patients with KS and in controls.

No significant differences 23,X versus 23,Y in the five patients (McNemar test; \(P > 0.05\)). Others group includes: 22,Ø; 24,YY; 24,XX; 26,XXYY.

*Significant differences versus controls (Fisher’s exact test; \(P < 0.05\)).
successful sperm recovery could not be confirmed owing to the fact that spermatozoa were not observed in any of the five patients with KS.

Although the clinical significance of the mosaicism level in KS remains to be elucidated, the knowledge of the degree of mosaicism can help us to understand the reason for the variable response that some patients with KS show when faced with different therapies (treatments with aromatase inhibitors, hCG or clomiphene citrate). It has been reported that 77% of the patients who show blood concentrations ≥250 ng/dl testosterone after treatment have successful sperm retrieval (Ramasamy et al., 2009). It could be hypothesized that the Sertoli 46,XY cells would be those which would respond to the testosterone levels, recovering the functions of nursing. In fact, in our series, the two patients observed who do not show meiotic phases (KS3 and KS4) are those who have the lowest percentages of Sertoli 46,XY cells. Furthermore, patient KS5 with a high percentage of euploid Sertoli cells (46.5%), and despite having a low percentage of pre-reductional XY cells (28.2%), has meiotic phases. Thus, taken together, results suggest that the progression of spermatogenesis would depend on the percentage of Sertoli XY cells which colonize the seminiferous tubules. However, further studies would be of interest to evaluate the value of mosaicism in predicting successful sperm retrieval when pharmacologic therapeutic treatments are used.

The origin of mosaicism in germinal cells has been attributed to the occurrence of ‘correcting mitotic errors’ associated with the mitotic proliferation of the primordial germinal cells in the fetal testicle (Levron et al., 2000) and also of the spermatogonia in adult tissue (Sciurano et al., 2009), giving rise to isolated zones of euploid cells. These corrective processes would also have occurred in the other cell types, causing different degrees of mosaicism in each one. On the other hand, it has been described that the lack of inactivation of genes of the X supernumerary affects the germinal cells as well as somatic cells at different levels (Aksgaard et al., 2006). In the mouse model, Sertoli XXY cells show low levels of expression of androgen receptor (AR) (Lue et al., 2005). In human males, a delay in disappearance of anti-Müllerian hormone expression coupled with the up-regulation of AR has been described, and it is known that both are required for the last step of Sertoli maturation during puberty (Wikstrom et al., 2007). This, together with an altered activation pattern of apoptosis because of the hypergonadotrophic hypogonadism (Aksgaard et al., 2006), suggests that the degeneration of the Sertoli cells would preferentially affect the XXY cells, raising the relative proportion of the XY cells. Thus, the maintenance of the mosaicism or degeneration of the aneuploid line would be determined by the presence of two functional X chromosomes.

Mosaicism has been observed at the testicular level in all of the patients studied (mean of 36.4 ± 8.7%). All prophases and metaphases I (Table I) show the XY complement, even in the patient where the aneuploid line represents almost 55% of the cells (KS2, Table I). These results agree with those previously reported by our group (Blanco et al., 2001) and with recent studies (reviewed by Tuttelmann and Gromoll, 2010) and confirm the meiotic incompetence of the 47,XXY spermatocytes. Furthermore, neither the deviation from the 1:1 ratio nor the equivalent proportions of hyperhaploidies (XX and XY) in post-reductional cells described by other authors (Forresta et al., 1999; Yamamoto et al., 2002; Ferlin et al., 2005) have been observed in our study. What is more, the few spermatogonia metaphases observed were also 46,XY, indicating that this line would be more proliferative than the 47,XXY germ line.

The meiotic incompetence of the 47,XXY line has been related, as in the mouse model (Hunt et al., 1998), to the presence of the second X chromosome and the insufficient inactivation of the genes associated with the extra chromosome, which could cause the degeneration of the 47,XXY germinal cells (Aksgaard et al., 2006). On the other hand, given that it has been reported that anomalies in the sex vesicle formation lead to apoptotic processes (Burgoyne et al., 2009), it can be inferred that the anomalies of synopsis of the XXY complement lead to cell degeneration of any 47,XXY spermatocytes. Thus, the results of our study are in agreement with the descriptions made in the mouse model (Hunt et al., 1998) and support the hypothesis that the spermatogenic patches are populated by 46,XY cells.

The occasional presence of spermatogonia in testicular tissue has been described in certain KS individuals (Forresta et al., 1999; Rives et al., 2000; Blanco et al., 2001; Bergere et al., 2002; Lanfranco et al., 2004; Sciurano et al., 2009; Fullerton et al., 2010) and confirms that the process of spermatogenesis can be accomplished in some of the spermatogenic patches.

Our findings, where a significant incidence of aneuploidies in post-reductional cells was observed, clearly give support to the hypothesis that an altered testicular environment compromises the meiotic progression of the 46,XY cells (Mroz et al., 1999). Furthermore, the evidence from this study and others (reviewed by Templado et al., 2011) of increased chromosomal abnormalities in mosaic, as well as non-mosaic, patients with KS, deserves to be taken into account when considering offering assisted reproduction techniques to these individuals. The offer of TESE and ICSI to KS men has resulted in the reported births of more than one hundred children (Fullerton et al., 2010) and has resulted in the placing of a ‘scientific question mark’ on the infertile definition classically attributed to KS individuals; nevertheless, the label of ‘patients at genetic risk’ still merits being maintained and highlighted.

In conclusion, homogeneous KS diagnosis based on lymphocyte karyotyping should be contrasted in other tissues. FISH analysis on mucosa cells could help to ascertain the degree of germ cell mosaicism, however, further studies would be of interest to evaluate the predictive value of the degree of mosaicism for successful sperm retrieval. Spermatogenesis foci are populated by 46,XY cells and our results support the hypothesis that 47,XXY germ cells are not meiotically competent. The post-reductional aneuploidy rate in KS is higher than in controls and is related to meiotic errors in 46,XY cell lines. KS individuals should be considered as being patients with a reproductive risk, and appropriate genetic counselling is recommended.

**Authors’ roles**

L.G.-Q. was involved in experimental procedures, data collection and assembly, data analysis and interpretation, manuscript writing and final approval. J.B. was involved in study conception and design, data analysis and interpretation, manuscript writing and final approval. Z.S. was involved in data analysis and interpretation, and final approval of the manuscript. V.C. was involved in data collection and final approval of the manuscript. L.B. was the consultant andrologist, contributed in clinical assessment, tissue sampling, data collection and final approval of the manuscript. F.V. was involved in study conception...
and design, data collection and assembly, data analysis and interpretation, manuscript writing and final approval.

**Acknowledgements**

The authors wish to thank Laboratori de Seminologia i Embriologia, Fundación Puigvert (Barcelona) and Prenatal Genetics, SL (Barcelona) for providing the biological samples. This manuscript has been proof-read by Mr. Chuck Simons, a native English-speaking university instructor of English.

**Funding**

This work was supported by Projects CF-180034 (Universitat Autònoma de Barcelona) and SGR2009-282 (Generalitat de Catalunya). L.-G.-Q. is the recipient of a grant from the Universitat Autònoma de Barcelona (project CF-180034).

**References**


Tourayeh H, Staessen C, Liebaers I, Van Assche E, Devroey P, Bonduille M, Van Steirteghem A. Testicular sperm recovery