Preimplantation genetic diagnosis for X;autosome translocations: lessons from a case of misdiagnosis

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Preimplantation genetic diagnosis (PGD) is offered to couples carrying a reciprocal translocation in an attempt to increase their chance of phenotypically normal offspring. For the selection of embryos that are balanced for the translocation chromosomes, it is critical to use a combination of DNA probes that can take account of all the segregation patterns of the particular translocation. The frequency of the different segregation types differs depending on the chromosomes involved, the location of the breakpoints and the number of chiasmata and the sex of the carrier. We report on a case of misdiagnosis after PGD–fluorescence in situ hybridization in a female translocation 46,X,t(X;5)(q13;p14) carrier. Transfer of two embryos diagnosed as balanced for the translocation chromosomes resulted in a singleton pregnancy that miscarried at 8 weeks’ gestational age. The unbalanced karyotype of the fetus was consistent with 3:1 segregation resulting in tertiary trisomy for the derivative chromosome 5: 47,XX,+der(5)t(X;5)(q13;p14)mat. Based on additional molecular cytogenetic studies of fetal tissue and the initially investigated blastomeres, we concluded that the misdiagnosis was most probably due to a technical error, i.e. a partial hybridization failure or co-localization of the Xq/Yq subtelomere probe signals. No evidence for a normal cell line (mosaicism) was found in the fetus, which could have explained the discrepancy. This case demonstrates the importance of using two diagnostic probes or testing 2 cells to detect translocation products with potentially viable imbalance. X;autosome translocations are a special case due to the added complication of X chromosome inactivation and particular caution is advised when designing a PGD strategy.

**Key words:** PGD / misdiagnosis / reciprocal translocation / X-autosome translocation / segregation types

**TRIAL REGISTRATION NUMBER:** not applicable.

Introduction

Reciprocal translocations are the most common form of chromosomal abnormality and, occur in ~1 in 600 live births (Jacobs et al., 1992); however, X;autosome translocations are very rare. Carriers of a balanced reciprocal translocation have a high risk of producing a genetically abnormal conceptus due to chromosomal imbalances arising during meiosis, leading to recurrent abortions or, in the minority, phenotypically abnormal offspring. Translocation carriers may increase their chances of an ongoing pregnancy, and decrease the risk of abnormal offspring, by IVF with preimplantation genetic diagnosis (PGD). When performing PGD to assess the chromosomal constitution of embryos from translocation carriers, it is recommended to use a combination of DNA probes that can take account of all the expected unbalanced forms of the particular translocation (Harton et al., 2011). Efficiency and specificity of a given probe combination should be tested prior to any PGD–fluorescence in situ hybridization (FISH) analysis (Wilton et al., 2009).

The homologous segments of the chromosomes involved in a reciprocal translocation pair together to form a quadrivalent during meiosis. Different modes of meiotic segregation may occur, but only the alternate segregation results in a normal chromosomal pattern or a balanced rearrangement. The segregation types which lead to chromosomal imbalances are adjacent-1, adjacent-2, 3:1 and 4:0 (Scriven et al., 1998). The frequency of each segregation type differs depending on the chromosomes involved, the location of the breakpoints, the number of chiasmata and the sex of the carrier (Jalbert et al., 1980; Midro et al., 1992). It has been postulated that the predominant segregation mode associated with viable imbalance follows the longest axis of the quadrivalent (Jalbert et al., 1980). Based on data from fetuses and newborns, the alternate and adjacent-1 modes of segregation seem to be the most prevalent

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segregation patterns in reciprocal translocations. The possibility of a 3:1 segregation depends on the make-up of the meiotic quadrivalent: a 3:1 segregation is more likely to occur if the chromosomes involved in the translocation differ significantly in size (Jalbert et al., 1980). Furthermore, the frequency of 3:1 segregation products in embryos was found to be higher in female carriers than in male carriers (Ko et al., 2010; Lledó et al., 2010).

In addition to the high risk at conception of chromosome imbalance common to all reciprocal translocations, X;autosome translocations are complicated by X inactivation. The long arm of the X chromosome contains the inactivation centre (XIC), which includes the X inactivation-specific transcript gene (XIST, located in band Xq13.2). In females with a normal sex chromosome complement (46,XX), the XIC is responsible for the inactivation of one of the X chromosomes, which is expected to be random. However, in the situation of a balanced X;autosome translocation, there is a risk of accompanying functional monosomy of some of the autosomal material if the derivative chromosome containing the XIC is inactivated and not the intact (normal) X chromosome. Female balanced translocation carriers with a normal phenotype are typically found to have highly skewed inactivation of the intact X chromosome (Mattei et al., 1982). However, PGD techniques that detect chromosome segment copy number cannot ascertain whether the intact X has been inactivated nor the degree of skewing and therefore there will be a degree of uncertainty whether a female embryo with a balanced translocation will result in a child with a phenotype at least similar to the mother, more severe, or perhaps even non-viable resulting in miscarriage. In addition, X;autosome translocations are often associated with premature ovarian failure in females, especially if the X chromosome breakpoint is in the Xq13–q26 critical region (Rizzolio et al., 2006), and infertility in males (Mattei et al., 1982). The potential viability and phenotype associated with an unbalanced product of the translocation will depend on the sex of the conceptus and the chromosome regions of imbalance; the severity can be mitigated where there is accompanying inactivation of a trisomic region of the autosome and this needs to be kept in mind when assessing X;autosome translocations for PGD and advising couples.

We describe a PGD procedure for a maternal X;5 reciprocal translocation in which an embryo, being diagnosed as normal/balanced for the translocation based on cleavage stage blastomere FISH analysis, gave rise to a fetus with an unbalanced karyotype. The 3:1 segregation resulted in a tertiary trisomy in the embryo that unexpectedly survived till almost 8 weeks gestational age. The most probable cause for the misdiagnosis appeared to be an incomplete hybridization of one of the probes or signal co-localization.

### Case Report

In a couple with a history of three spontaneous abortions (none is known to have been karyotyped) at ~6, 8 and 9 weeks gestational age, respectively, a balanced translocation 46,X,t(X;5)(q13;p14) (Fig. 1A) was detected in the 33-year-old female partner (the proband). FISH analysis showed that the XIST gene was located on the translocated segment of the X chromosome on the derivative chromosome 5 (BAC clone RP11-13M9, sized 154.7 kb, and located on chrXq13.2:72,900,958-73,055,695, according to NCBI36/hg18, in-house labelled with biotin and streptavidin-conjugated TexasRed). The same translocation appeared to be present in her mother and a younger brother, but not in her sister. Her mother had experienced one spontaneous abortion. Her brother had a severe oligozoosperma. The 33-year-old male partner of the proband showed a normal 46,XY karyotype.

The couple opted for PGD and after genetic counselling, which included the explanation and discussion of all their reproductive options, possible cytogenetic outcomes and consequences of the translocation in male and female offspring and the possibility of miscarriage after PGD. Following pre-IVF investigation, including hormonal analysis to exclude premature ovarian failure (FSH 5.0 IE/l, estradiol 0.14 nmol/l) and semen analysis (normospermia), probes were developed for PGD–FISH. According to the PGD guideline applicable at the time of PGD analysis (Thornhill et al., 2005), the probe mixture comprised a probe representative of 5p15 (RP11-20K9, clone set from Sanger Institute, Cambridge, UK) labelled with FITC, the centromeric region of chromosome X (Vysis CepX, Abbott Molecular Inc., Des Plaines, IL, USA) labelled with SpectrumAqua, and the subtelomeric region of Xq/Yq (Vysis TelVysis XqYq, Abbott Molecular Inc.) labelled with SpectrumOrange. Evaluation of all possible segregation options (Supplementary data, Table S1) revealed that the probe scheme would not detect two zygote complements consistent with 2:2 adjacent-2 segregation: the X,5,5,der(5) karyotype (Supplementary data, Table S1A; line 6), which would result in the same signal pattern as a normal or balanced male complement, and the X,der(X),Y,5 karyotype (Supplementary data, Table S1B; line 5), which would result in the same signal pattern as a normal or balanced female complement. However, we regarded both zygote complements as lethal and unable to implant. Inactivation of the der(5) in the X;5,5,der(5) karyotype could in theory produce a Turner variant, but the chance of a full inactivation of the der(5) was assumed to be very small and the resulting genotype would probably be lethal, by the combination of Turner syndrome and partial trisomy of chromosome 5.

All the probes were tested individually and as a combination to ascertain their suitability and to exclude polymorphisms. Lymphocyte metaphase spreads (10) and interphase nuclei (100) from cultured peripheral blood were examined from both partners and a known 46,XY control individual (Thornhill et al., 2005; Harton et al., 2011). All the probes hybridized as expected and individual probes indicated normal copy number in at least 95% of diploid interphase nuclei.

For IVF–PGD, a long agonist down-regulation protocol was used in combination with recombinant FSH as described earlier (Dumoulin et al., 2010), Laser-assisted biopsy of embryos was performed on Day 3 after ovum pick-up (Zilos, Hamilton Thorne Biosciences Inc., Beverly, MA, USA). Biopsy of one blastomere was performed in embryos consisting of 4–7 cells and 2 cells were biopsied from embryos containing 8 or more cells. In case the biopsied blastomere(s) was (were) unsatisfactory (e.g. no nucleus) additional blastomeres were removed for analysis. Each individual blastomere was fixed on a coated glass slide according to Coonen et al. (1994a), FISH analysis was performed according to Coonen et al. (1994b). Embryos with an unbalanced or inconclusive FISH result were processed for confirmation of diagnosis by spreading the whole of the remaining embryo. Only embryos with a balanced FISH result for all cells analysed were eligible for transfer (Supplementary data, Table S2).

The first two IVF–PGD cycles of the couple were cancelled because of a high risk of ovarian hyperstimulation syndrome. In the third stimulation cycle, an ovum pick-up was performed, but none of the nine embryos biopsied was suitable for transfer because of unbalanced test results. In the fourth cycle, 11 embryos were biopsied. Two embryos gave a normal/
balanced result, 7 an unbalanced result and 2 embryos gave an inconclusive result. The two embryos, one male and one female, which were designated as having a normal/balanced complement for the chromosomes involved in the translocation, were transferred on Day 4 after ovum pick-up. Fifteen days after embryo transfer the female partner had a positive urinary hCG test. Ultrasound evaluation at 6 weeks and 6 days gestational age demonstrated a single fetus with heart beat and a crown-rump length of 6.6 mm, corresponding with the gestational age. Two weeks later, at a gestational age of 8 weeks and 6 days, no heart beat could be detected and the crown-rump length of the fetus was 12.5 mm, corresponding with a gestational age of 7 weeks and 5 days. Five days later the non-viable pregnancy was evacuated by dilatation and curettage, and the tissue was isolated for genetic analysis.

Analysis of embryonic tissue was performed using QF-PCR using the Aneufast Multiplex QF-PCR kit for rapid diagnosis of trisomy 21, 18, 13 and sex chromosomal aneuploidies according to the manufacturer’s instructions (Aneufast, Genomed-Ltd, Kent, UK). Analysis of the X chromosomes short tandem repeat (STR) markers indicated three copies of the loci tested, at Xq21.33 (DXS6809), Xq26.2 (HPRT) and Xq28 (DXS8377). Karyotyping of cultured cells from chorionic villi and umbilical cord tissue showed an unbalanced product of the maternal translocation; the loci tested, at Xq21.33 (DXS6809), Xq26.2 (HPRT) and Xq28 (DXS8377). Karyotyping of cultured cells from chorionic villi and umbilical cord tissue showed an unbalanced product of the maternal translocation.

The characteristic of a viable 3:1 tertiary segregation of the maternal translocation resulting in trisomy for the derivative chromosome 5.

According to Jalbert et al. (1980), the most likely viable unbalanced segregations are adjacent-1, and 3:1 tertiary mode. The characteristic of adjacent-1 is that the sum of the centric segments (Xpter → q13 + 5pter → p14) is larger than the sum of the translocated segments (Xpter → q13 + 5pter → p14). The characteristic of a viable 3:1 tertiary segregation mode is that the shortest centric segment (Xpter → q13) is attached to the shortest translocated segment (5pter → p14), which is not the case in the t(X;5). Nonetheless, we found a 3:1 tertiary trisomy in tissue of a fetus surviving till the eighth gestational week (Fig. 1B). An explanation for this unexpected finding might be that the characteristics of the different segregation types as described by Jalbert et al. (1980) were/are based on abnormal living probands from autosomal reciprocal translocation carriers. Many unfavourable segregations of autosomal translocations may only occur at conception, while the large autosomal imbalance (duplication of 5p14 → qter) in our proband most likely was partly inactivated due to the presence of XIST on the derivative chromosome 5 and thus resulted in a relatively long viability, including a positive heart beat at almost 7 weeks. Unfortunately, in our case the X inactivation studies of cultured metaphase spreads from fetal tissue were not successful due to insufficient cell growth.

Thus, X;autosome translocations pose a particular challenge due to X
chromosome inactivation and the potential for the inactivation process to spread into the autosome (Giorda et al., 2008). Therefore these translocations do not follow the usual rules and a well-recognized consequence of inactivation is that some unbalanced products with an otherwise lethal amount of autosomal imbalance can have the potential to be viable (Gardner et al., 2012).

Segregation studies on embryos of autosomal translocation carriers show that the frequency of abnormal embryos does not differ between male and female translocation carriers. However, the frequency of 3:1 segregation was reported to be higher in female carriers than in male carriers (Ko et al., 2010; Lledo et al., 2010). Ko et al. (2010) differentiated between 3:1 tertiary mode and interchange mode, but there were no large differences in frequencies of the tertiary monosomy, tertiary trisomy, the interchange monosomy and the interchange trisomy. The female fetus reported here showed a trisomy for the derivative chromosome 5, caused by 3:1 tertiary segregation of the maternal translocation. The additional Xq material on the derivative chromosome 5 was shown to contain the XIST gene by FISH analysis of maternal metaphases. Thus, inactivation of that Xq region with inactivation spreading over the centric fragment of chromosome 5 can be expected. However, inactivation of the extra chromosome 5 material is unlikely to be complete, resulting in a functional duplication of a large proportion of chromosome 5, explaining the fetal demise.

Based on additional molecular cytogenetic studies of fetal tissue and the initially investigated blastomeres (Fig. 1C and D), we concluded that the misdiagnosis was most probably due to a technical error, i.e. a partial hybridization failure or co-localization of the Xq/Yq subtelomere probe signals. No evidence for a normal cell line (mosaicism) was found in the fetus, which could have explained the discrepancy. The segregation pattern that resulted in the unbalanced karyotype of the embryo was unexpected, but nonetheless should have been picked up by the FISH probes that were selected and tested during the pre-IVF–PGD work-up. The probes were chosen based on the scheme described by Scriven et al. (1998): one subtelomeric probe for each of the chromosome segments involved in the translocation and a probe indicative of one of the centric segments. Ideally, all segregation products that are likely to give rise to viable unbalanced rearrangements in a recognizable pregnancy should be identified by at least two probes or concordant results should be obtained from two blastomeres per embryo, in order to obtain a reliable diagnosis (Harton et al., 2011). We significantly underestimated the survival potential for the unbalanced product of this translocation found in the fetus.

If a misdiagnosis in PGD for a reciprocal translocation occurs, this can be due to a PGD-unrelated pregnancy, mislabelling of the slide or transfer of a wrong embryo (human errors), chromosomal mosaicism (intrinsic, embryonic error), probe failure or contamination (extrinsic, technical errors) and wrong segregation analysis (human error) (Wilton et al., 2009). Based on the data collected by the PGD consortium of the European Society of Human Reproduction and Embryology (ESHRE), the percentage of clinically significant misdiagnosis in PGD for translocations is ~0.4% (3 out of 746 clinical pregnancies; Goossens et al., 2012). However, this figure is likely to be underestimated due to under-reporting of misdiagnosis and the impracticability of confirming the karyotype of every failed pregnancy following PGD. In the present case, careful re-evaluation led to the conclusion that partial hybridization failure of the probe representing Xq/Yq or signal co-localization was the cause of the misdiagnosis. It is well known that no FISH probe has 100% hybridization efficiency and in the Dutch PGD consortium, couples are always counselled that the reliability of FISH analysis for reciprocal translocations at a single cell level is estimated to be 95%. The most likely effect of a technical misdiagnosis is that a diploid embryo will be diagnosed as aneuploid or inconclusive. The incorrect diagnosis, due to hybridization failure or signal co-localization, that an unbalanced embryo is normal/balanced can be minimized by using probes for both centric segments and both translocated segments. Where this is not possible, careful assessment of the translocation is necessary to ensure that there are two diagnostic probes for every unbalanced product that has potential to be viable or result in a recognizable pregnancy. Furthermore, in X;autosome translocations, the relatively frequent occurrence of gonosomal numerical aneuploidies should be taken into account.

In conclusion, every reciprocal translocation segregation product that is likely to give rise to viable imbalance should have two diagnostic probes or concordant results should be obtained from two blastomeres per embryo (Harton et al., 2011). However X;autosome translocations are of a specific nature due to the added complication of X chromosome inactivation and particular caution is advised when designing a PGD test.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

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J.V.E.-A.: generated data and wrote the manuscript; E.C., E.C.D., J.A.L.: evaluated and edited the manuscript; B.R., R.F.S. generated data; C.M.A.v.R.-A.: generated clinical data, evaluated and edited the manuscript.

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Conflict of Interest
None of the authors has any conflict of interest to declare.

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