Preimplantation genetic diagnosis for couples at high risk of Down syndrome pregnancy owing to parental translocation or mosaicism

Clare M Conn, Jean Cozzi, Joyce C Harper, Robert M L Winston, Joy D A Delhanty

Abstract

The population risk for trisomy 21 is 1 in 700 births but some couples are at a much higher risk owing to parental translocation or mosaicism. We report on the first attempt to carry out preimplantation genetic diagnosis for two such couples using cleavage stage embryo biopsy and dual colour FISH analysis. Each couple underwent two treatment cycles. Couple 1 (suspected gonadal mosaicism for trisomy 21) had two embryos normal for chromosome 21 transferred, but no pregnancy resulted; 64% (7/11) unfertilised oocytes/embryos showed chromosome 21 aneuploidy. Couple 2 (46,XX,t(6;21)(q13;q22.3)) had a single embryo transferred resulting in a biochemical pregnancy; 91% (10/11) oocytes/embryos showed chromosome 21 imbalance, most resulting from 3:1 segregation of this translocation at gametogenesis. The opportunity to test embryos before implantation enables the outcome of female meiosis to be studied for the first time and the recurrence risk for a Down syndrome pregnancy to be assessed.

Keywords: preimplantation genetic diagnosis; Down syndrome; reciprocal translocation; gonadal mosaicism

The recurrence risk of a Down syndrome pregnancy after the birth of an affected child is approximately 1-2% and studies have shown that while the majority of second trisomy 21 pregnancies may be the result of chance alone, cytogenetic analysis is necessary to exclude parental translocation or mosaicism. In cases where such predisposing factors are present, parents can usually achieve a normal pregnancy with the help of prenatal diagnosis. However, a minority suffer repeated pregnancy termination following trisomy 21 conception and these couples are coming forward for genetic diagnosis before implantation to avoid later abortion.

Preimplantation genetic diagnosis (PGD) involves the screening of in vitro fertilisation (IVF) generated embryos by the genetic analysis of one or two biopsied blastomeres. Following the transfer of only those embryos that are diagnosed as unaffected, couples at high risk of transmitting single gene defects or chromosomal abnormalities can begin a pregnancy in the knowledge that it is not affected by the familial disorder. Fluorescent in situ hybridisation (FISH) is now the technique of choice for detecting the chromosome status in single cells for PGD and has been used successfully both for sexing embryos to avoid X linked disease and to screen embryos for age related aneuploidy for older women undergoing IVF.

In contrast, the aim of the treatment cycles presented here was to screen for specific imbalance of chromosome 21 from abnormal segregation of translocation chromosomes or mosaicism for individual patients. Although a wider selection of commercial probes is now becoming available, the development of suitable probe combinations is expensive and labour intensive. This means that, particularly for reciprocal translocation carriers, each case prepared for PGD requires individual research time dedicated to it. These factors probably account for the limited number of cycles which have been carried out to date, including those reported for carriers of Robertsonian translocations and chromosomal inversions.

Fluorochrome labelled α satellite probes give intense FISH signals and allow the use of a rapid two hour technique for PGD. Unfortunately, the lack of specific repeat probes for chromosomes 13, 14, 21, and 22, because of centromeric sequence homology, means that locus specific probes remain the only choice for accurately detecting the acrocentric chromosomes in interphase nuclei. For this reason, we have evaluated combinations of two differentially labelled YAC and cosmid probes specific for chromosome 21 in somatic cells and in spare IVF embryos donated for research. As has been shown in previous studies detecting trisomy 21 in uncultured amniocytes, this dual labelling of a single chromosome greatly increases the accuracy of diagnosis and this is a particularly important consideration for PGD based only on one or two biopsied cells. We present data on FISH analysis of chromosome 21 status in preimplantation embryos from four IVF cycles for two couples at high risk of a Down syndrome pregnancy owing to a balanced reciprocal translocation in one case and suspected gonadal mosaicism for trisomy 21 in the other.

Subjects, materials, and methods

ETHICAL APPROVAL

The preliminary study on surplus untransferred embryos and the clinical application of PGD were approved by the Human Fertilisation and Embryology Authority and the Research Ethics Committee of the Royal Postgraduate Medical School, Hammersmith Hospital. Informed written consent was obtained.
from patients for surplus embryos to be used for research purposes.

SPARE IVF EMBRYOS
Eighteen day 3 post-insemination embryos, surplus to IVF requirements and donated for research, were used in the preliminary work. All were normally fertilised (presence of two pronuclei 18 hours post-insemination) and scored as of good morphology (less than 25% blastomere fragmentation).

PATIENTS
Two couples at high risk of a Down syndrome pregnancy underwent a total of four PGD cycles (two standard IVF cycles and two intracytoplasmic sperm injection (ICSI) cycles). Both couples were counselled about the PGD procedure and the need to confirm diagnosis with prenatal diagnosis.

Couple 1
Although couple 1 have one normal child, they were referred for PGD after three trisomy 21 conceptions (two terminations of pregnancy and a Down syndrome child). DNA polymorphism analysis proved maternal origin of the extra chromosome in two cases and was compatible with maternal gonadal mosaicism. However, this could not be proven by somatic studies since the mosaicism appeared to have a mitotic origin (P Farndon, personal communication). FISH analysis did not detect a second trisomic cell line in lymphocytes of either partner. Maternal age was 36 years.

Couple 2
Couple 2 were referred for PGD owing to maternal balanced reciprocal translocation 46,XX,t(6;21)(q13;q22.3) detected after four early spontaneous abortions and the birth of a Down syndrome daughter (karyotype: 47,XX,t(6;21)(q13;q22.3),+21). Maternal age was 32 years.

PREIMPLANTATION GENETIC DIAGNOSIS (PGD)
Couples underwent routine IVF15 or ICSI16 and PGD was carried out as we have described previously. Briefly, patients underwent super-ovulation and oocyte retrieval was carried out by vaginal ultrasound guided aspiration. Oocytes were inseminated with a prepared sample of partner’s sperm or subjected to ICSI and assessed for normal fertilisation after 15 to 18 hours (day 1). Embryos were cultured in vitro until day 3 post-insemination when the majority had reached the 6-10 cell stage. At this time embryo biopsy was performed by gentle aspiration of one or two blastomeres through a hole made in the zona pellucida as described by Handside. Two blastomeres were only removed from embryos of six cells or more. After biopsy, embryos were immediately returned to normal culture conditions.

SLIDE PREPARATION
Biopsied blastomeres for PGD and whole day 3 post-insemination embryos for the preliminary work were spread using the same method described in detail previously. In brief, cells were transferred to poly-L-lysine slides in spreading solution (0.01 N HCl, 0.1% Tween 20) which was gently agitated until lysis occurred and all nuclei were clear of cytoplasm.

Unfertilised oocytes were analysed 48 hours after insemination and spread using the air drying method of Tarkowski. Control slides of metaphase and interphase nuclei were prepared from phytohaemagglutinin stimulated human peripheral lymphocytes using standard cytogenetic techniques.

DNA PROBES
Probes used in this study are listed in table 1. YAC probes were obtained from total genomic yeast DNA amplified by Alu-PCR as described by Lengauer et al. Cosmid probes were isolated from bacterial strains using a commercial maxiprep protocol (Wizard, Promega, USA). All probes were labelled via nick translation with biotin-14-dATP (Bionick labeling system, BRL, UK) or digoxigenin-11-dUTP (Nick translation system, Boehringer Mannheim, Germany) or both. Working concentrations of probe DNA were YACS 20 ng/µl and cosmids 15 ng/µl.

FLUORESCENT IN SITU HYBRIDISATION (FISH)
The FISH technique was carried out as we have previously described. Briefly, slides were pretreated by incubation in 100 µg/ml pepsin in 0.01 N HCl at 37°C for 20 minutes and fixation in 1% paraformaldehyde in PBS at 4°C for 10 minutes. Combinations of probes were applied to dehydrated slides in a final 10 µl volume under a coverslip, sealed with rubber cement, denatured simultaneously with nucleic DNA at 75°C for five minutes, and allowed to hybridise overnight (approximately 16 hours) at 37°C. Post-hybridisation washes were carried out at 45°C in 50% formamide/2× SSC followed by 2× SSC (3 × 3 minutes each). Signal detection and amplification were carried out via three sequential incubations: biotin labelled probes detected with FITC-avidin/biotinylated anti-avidin/FITC-avidin and digoxigenin labelled probes detected with mouse-anti-digoxin/TRITC-rabbit-anti-mouse/TRITC-goat-anti-rabbit IgG. Slides were then mounted in antifade medium (Vector Laboratories, USA) containing 1.25 µg/ml 4',6-diamidino 2 phenylindole (Sigma, UK) counterstain.

DIAGNOSIS
FISH signals were scored according to the criteria of Hopman et al using a Reichert Jung Polyvar fluorescence microscope. Image capture was carried out using a Zeiss Axioskop 20.
Table 2: Dual colour FISH analysis of chromosome 21 in spare IVF embryos for preliminary work

<table>
<thead>
<tr>
<th>Probes tested</th>
<th>Uniformly normal</th>
<th>Diploid mosaic</th>
<th>Aneuploid mosaic</th>
<th>Chaotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr 21 YACS</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chr 21 cosmids</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total 18 (100%)</td>
<td>13 (72%)</td>
<td>3 (17%)</td>
<td>1 (5.5%)</td>
<td>1 (5.5%)</td>
</tr>
</tbody>
</table>

Table 3: Couple 1. Gonadal mosaic for trisomy 21: FISH analysis of embryos from two PGD cycles

<table>
<thead>
<tr>
<th>Embryo</th>
<th>Biopsied cells</th>
<th>Cells in remainder of embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 trisomy 21</td>
<td>7 trisomy 21: 2 disomy 21</td>
</tr>
<tr>
<td>2</td>
<td>2 trisomy 21</td>
<td>3 trisomy 21: 1 tetrasomy 21</td>
</tr>
<tr>
<td>Cycle 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 normal</td>
<td>Transferred</td>
</tr>
<tr>
<td>4</td>
<td>2 normal</td>
<td>Transferred</td>
</tr>
<tr>
<td>5</td>
<td>2 trisomy 21</td>
<td>3 trisomy 21: 1 disomy 21: 1 monosomy 21</td>
</tr>
<tr>
<td>6</td>
<td>Not biopsied</td>
<td>5 trisomy 21</td>
</tr>
<tr>
<td>7</td>
<td>Not biopsied</td>
<td>2 tetraploid (arrested)</td>
</tr>
</tbody>
</table>

RESULTS OF PRELIMINARY WORK

Two combinations of two differentially labelled probes (one labelled with biotin and the other digoxigenin) were evaluated to detect chromosome 21 in interphase nuclei, one YAC probe combination (y940 and yGART2) and one cosmid contig probe combination (cCMP21.a and c242c) (table 1). Following this dual labelling strategy, normal nuclei exhibit four signals, two red and two green. Lymphocyte controls showed that both combinations gave strong discrete signals in interphase nuclei showing the expected number of four signals in 92% nuclei (n=100) for the two YAC probes and 88% nuclei (n=100) for the two cosmid probes. The slightly lower efficiency of the cosmid probes reflects the smaller signal size compared to the large YAC probe signals.

Two probe pair combinations were then applied to 18 normally fertilised spare embryos (118 blastomeres) of good morphology at the 6-9 cell stage on day 3 post-insemination. Ten embryos were hybridised with the YAC probe combination and eight embryos with the cosmid contig combination. Of these, 13 (72%) embryos were uniformly normal for chromosome 21 with each cell showing two signals per probe. Four embryos were mosaic with one or two cells differing from the remainder of the embryo and one was highly mosaic and scored as chaotic (table 2). Embryonic nuclei spread in this study by the HCl/Tween method are smaller and more compact than those spread with conventional methanol/acetic acid as a hypotonic stage is avoided. For this reason, these cells show highly discrete FISH probe signals (compared to lymphocyte nuclei) and this was found to be advantageous for diagnostic purposes where split signals from locus specific probes can lead to inaccuracies.

RESULTS OF PGD CYCLES

Couple 1. Suspected maternal gonadal mosaicism for trisomy 21

Two PGD cycles were carried out to exclude trisomic embryos using dual colour FISH with the two chromosome 21 specific YACS (y940 and yGART2). The fertilisation rate was particularly low in the first standard IVF cycle with just 31% (4/13) oocytes fertilised; this increased to 50% (9/18) fertilised in the second cycle from ICSI. In total, 31 oocytes were collected, 13 fertilised, five embryos were biopsied, and one double embryo transfer was carried out (cycle 2), but no pregnancy resulted. Two embryos (embryos 6 and 7) had fewer than six cells on day 3 post-insemination and so were not biopsied.

Five untransferred embryos were analysed; of these four showed trisomy 21 and one was tetraploid but arrested at the 2 cell stage. Only embryo 6 was completely trisomic for chromosome 21; the remaining three embryos were mosaic (the majority of cells trisomy 21 but one or two cells differing). All embryos were later reprobed for chromosome 9 to check ploidy and all cells were diploid except the one tetraploid embryo (embryo 7) (table 3). Of four unfertilised oocytes analysed, three were hyperhaploid for chromosome 21 and only one was normal haploid. In total, 7/11 (64%) oocytes/embryos analysed showed chromosome 21 aneuploidy (fig 1A-C).

Couple 2. Reciprocal translocation carrier 46,XX,i(6;21)(q13;q22.3)

Two PGD cycles were carried out to exclude chromosome 21 imbalance (and also any chromosome 6 imbalance associated with derivative chromosomes) using dual colour FISH with the two chromosome 21 specific probes (y940
and c242c which flank the breakpoint on chromosome 21 (21q22.3): y940 (21q22.2) mapping to the derivative chromosome 21 and c242c (21q22.3) mapping to the derivative chromosome 6. As these two differentially labelled chromosome 21 probes map closely on 21q, they occasionally appear as an overlapping orange signal when combined in interphase nuclei. For this reason the chromosome 6 α satellite probe (also detected as an orange signal) was excluded from the diagnosis to avoid ambiguous results but was used for reprobing after the diagnosis to provide more information on the segregation of the derivative chromosomes at maternal gametogenesis. Standard IVF was carried out for the first treatment cycle with a fertilisation rate of 47%. ICSI was used for the second cycle but only 2/13 (15%) oocytes fertilised and both resulting embryos were retarded on day 3 and so were not biopsied for diagnosis but spread whole for analysis. In total, 32 oocytes were collected, 11 fertilised, four embryos were biopsied, and one embryo scored as normal for chromosome 21. All cycles resulted in very few embryos and both resulting embryos from the first cycle were normal for chromosome 21. Two unfertilised oocytes were available for analysis and both showed an unbalanced chromosome complement: oocyte 1 with a normal chromosome 21 and a derivative chromosome 6 from adjacent 1 segregation and oocyte 2 with a derivative chromosome 6 only from 3:1 segregation. None were in fact balanced although the transferred embryo from the first cycle was normal for chromosome 21. Two embryos from the second cycle (embryos 8 and 9) were highly mosaic and the original zygote chromosome constitution was difficult to determine. Of the nine embryos, three showed chromosome constitutions compatible with 2:2 segregation and six with 3:1 segregation. None were in fact balanced although the transferred embryo from the first cycle was normal for chromosome 21. Two embryos for couple 2). Although severe chromosomal imbalance may have com-

<table>
<thead>
<tr>
<th>Embryo</th>
<th>Biopsied cell</th>
<th>Remainder of embryo</th>
<th>Probable segregation (maternal contribution for translocation chromosome)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 Normal for chr 21</td>
<td>Transferred</td>
<td>3:1 interchange aneuploidy † (21)</td>
</tr>
<tr>
<td>2</td>
<td>2 Trisomy 21q22.3→qter</td>
<td>Trisomy 21q22.3→qter (mosaic)</td>
<td>Adjacent 1 (der 6; 21)‡</td>
</tr>
<tr>
<td>3</td>
<td>1 Monosomy 21q22.3→qter</td>
<td>Monosomy 21q22.3→qter (mosaic)</td>
<td>Adjacent 1 (der 6; 21)</td>
</tr>
<tr>
<td>4</td>
<td>1 Trisomy 21q22.3→qter</td>
<td>Trisomy 21q22.3→qter</td>
<td>Adjacent 1 (der 6; 21)‡</td>
</tr>
<tr>
<td>5</td>
<td>Not biopsied</td>
<td>Monosomy 21pter→q22.3</td>
<td>Adjacent 2 (6; der 6)</td>
</tr>
<tr>
<td>6</td>
<td>Not biopsied</td>
<td>Normal/trisomy 21q22.3→qter</td>
<td>3:1 tertiary aneuploidy (6; der 6; 21)</td>
</tr>
<tr>
<td>7</td>
<td>Not biopsied</td>
<td>Trisomy 21</td>
<td>3:1 interchange aneuploidy (6; der 6; 21)</td>
</tr>
<tr>
<td>Cycle 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Not biopsied</td>
<td>Trisomy 21q22.3→qter/monosomy 21pter→q22.3</td>
<td>3:1 tertiary aneuploidy (6; der 6; 21)</td>
</tr>
<tr>
<td>9</td>
<td>Not biopsied</td>
<td>Trisomy 21q22.3→qter/monosomy 21q22.3→qter</td>
<td>3:1 interchange aneuploidy (6; der 6; 21)</td>
</tr>
</tbody>
</table>

*Most likely segregation to produce embryo outcome deduced from PGD results and reprobing data using chromosome 6 α satellite probe.
†Based on reprobing data from one biopsied cell.
‡Der = derivative chromosome.
promised embryo development, there is no obvious explanation for the rather low fertilisation rate since it is not thought to be affected by the chromosome constitution. In both cases the majority of embryos analysed were abnormal for chromosome 21, categorised as aneuploid or aneuploid mosaic. In total, of 16 embryos analysed for both couples, only three were normal for chromosome 21 and transferred, but no clinical pregnancies were achieved (table 5).

Low level mosaicism was seen frequently in untransferred embryos on day 4 from both couples with 61% (8/13) embryos showing one or two cells differing from the remainder of the embryo. The use of two differentially labelled probes to detect a single chromosome was seen greatly to increase the accuracy of detection by reducing scoring errors and has confirmed that the mosaicism observed in previous preimplantation diagnosis cycles is not a FISH artefact owing to hybridisation failure or overlapping signals, even when just one cell differs and no cell resulting from a reciprocal non-disjunction event is found. As PGD for specific chromosome abnormalities is still at the research stage, it is important to document such levels of mosaicism found in these cases. This was the first occasion that we have observed two disomic cells in an otherwise trisomy 21 embryo (couple 1, embryo 1), posing a risk of misdiagnosis by inadvertent sampling of the minor cell line only. However, it must be emphasised that while biopsy and diagnosis takes place on day 3, the remainder of untransferred embryos are analysed on day 4 (after embryo transfer), hence further cell division has occurred and so mosaicism levels could be higher. Also it would be unlikely that both normal cells were biopsied in a trisomic mosaic embryo and it seems reasonable to continue with transfer on the basis of two independently biopsied cells, diagnosed normal for the tested chromosome, as long as the risk of non-representative biopsy is acknowledged. Mosaicism has been well documented in human preimplantation embryos both from routine IVF and PGD cycles. While embryos showing highly abnormal chromosome constitutions (chaotic embryos) are unlikely to develop further, it is unclear how the low level mosaicism observed here would affect further development, as selective mechanisms to rescue less abnormal embryos may operate. It is possible that the presence of even a few chromosomally normal cells at such early cleavage stages may give rise to a normal fetus. This may follow either preferential distribution of aneuploid/polyplid cells to extraembryonic tissues, as seen in 2n/4n chimaeric mouse embryos, or somatic loss of supernumerary chromosomes, as seen in the majority of disomy/trisomy mosaics and uniparental disomy cases which originate as trisomic fertilisations.

Although interphase FISH analysis of sperm allows segregation patterns to be followed in male translocation carriers, results from couples 2 represent the first observations of reciprocal translocation segregation in preimplantation embryos from a female carrier. Interestingly, a variety of combinations of maternal chromosomes were observed, showing that translocation heterozygotes may often produce gametes from most of the possible chromosome segregations, although the vast majority will not be viable. The most common segregation patterns of the 46,XX t(6;21) observed were 3:1 with tertiary aneuploidy (three embryos) and 3:1 with interchange aneuploidy (three embryos). Following Jalbert et al, the quadrivalent formed at pachytene of meiosis I is asymmetrical with the large chromosome 6 and small chromosome 21 translocated segments and predicts, of 16 theoretical segregations, 3:1 segregation as the most likely outcome of segregation. In total, 64% embryos/oocytes analysed resulted from 3:1 segregation of this translocation at gametogenesis.

Analysis of unfertilised oocytes was carried out to complement the findings of the embryo analysis. All oocytes are retained for as long as possible to allow for late fertilisation, particularly when few embryos are available for diagnosis, and this is reflected in the small numbers of analysable oocyte metaphases that were available. For couple 1, four of five untransferred embryos showed trisomy 21 in the majority of cells and, although these results are compatible with maternal gonadal mosaicism, analysis of hyperhaploid unfertilised oocytes was needed to prove this diagnosis, by showing the existence of trisomic primary oocytes in this patient (J Cozzi, unpublished observations). Both oocytes analysed from couple 2 were unbalanced for the translocation chromosomes and if fertilised would have given rise to embryos with chromosome constitutions monosomy 6q13→qter/trisomy 21q22.3→qter (as in embryos 2 and 4) and monosomy 6q13→qter/monosomy 21pter→q22.3.

Although two IVF cycles have been carried out for both couples, each had only one cycle which resulted in embryo transfer, and given the low numbers of embryos available for diagnosis it is likely that multiple treatment cycles would be necessary to increase the chance of obtaining a normal pregnancy. However, our results show that 64% of oocytes/embryos for couple 1 and 91% oocytes/embryos for couple 2 were aneuploid for chromosome 21. This would predict a very high recurrence risk of a Down syndrome conception in both cases, as the majority of gametes produced must be chromosomally unbalanced. These results highlight how genetic analysis of embryos for

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Table 5  Summary. Analysis of chromosome 21 in preimplantation embryos from two couples at high risk of Down syndrome pregnancy undergoing PGD

<table>
<thead>
<tr>
<th>Parental karyotype</th>
<th>Diploid mosaic</th>
<th>Aneuploid mosaic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle 1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cycle 1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Combined totals</td>
<td>3 (19%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td></td>
<td>12 (75%)</td>
<td></td>
</tr>
</tbody>
</table>

*Normal for chromosome 21.
PGD enables the levels of normal gametes produced by individual patients to be assessed. This information not only allows couples to make an informed decision concerning future assisted reproduction, but is also of value to investigate patterns of meiotic segregation.

In conclusion, although standard prenatal diagnosis remains the method of choice for age related aneuploidy, PGD may offer an alternative treatment option for translocation carriers and gonadal mosaics experiencing repeated chromosomally abnormal pregnancies, as testified by the increasing number of referrals to our Centre. However, although large numbers of embryos can be screened simultaneously using this approach, where the majority of embryos are chromosomally abnormal, as found in both patients’ cycles detailed here, the chances of a continuing pregnancy will still be low.

We would like to thank all the staff of the Hammersmith Hospital IVF unit, especially Asanga Ao and Debbie Taylor for embryo manipulation and Dr Amir Lass and Dr Jonathon Skull for patient management. We also thank the following for providing suitable probes for this study: Matteo Adinolfi and Jon Sherlock (The Galton Laboratory, UCL, London, UK), Angela Davies (UMDS Bridge, UK), and Andreas Gnirke (Washington University School of Medicine, MO, USA). This work was supported by grants from The Wellcome Trust (JH) and The Royal Society CC is in receipt of a Medical Research Council (UK) studentship.

References

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