Single-nucleotide polymorphism microarray-based preimplantation genetic diagnosis is likely to improve the clinical outcome for translocation carriers

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STUDY QUESTION: Is preimplantation genetic diagnosis (PGD) for translocation carriers more effective when done with a single-nucleotide polymorphism (SNP) array using trophectoderm (TE) biopsy and frozen embryo transfer (FET) compared with traditional PGD based on fluorescence in situ hybridization (FISH-PGD) using blastomere biopsy and fresh embryo transfer?

SUMMARY ANSWER: The procedure using the SNP array combined with TE biopsy and FET significantly improves the clinical pregnancy rate for translocation carriers. The miscarriage rate also slightly decreases.

WHAT IS KNOWN ALREADY: FISH-PGD has been widely used in translocation carriers but the clinical outcomes have not been ideal. SNP arrays can detect both chromosome segmental imbalances and aneuploidy, and may overcome the limitations of FISH in PGD for translocation carriers.

STUDY DESIGN, SIZE AND DURATION: This was a retrospective study of 575 couples with chromosomal translocations, including 169 couples treated by SNP-PGD between October 2011 and August 2012, and 406 couples treated by FISH-PGD between January 2005 and October 2011.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study was set in an IVF center at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. In total, 169 couples underwent SNP analysis, including 52 Robertsonian translocation carriers and 117 carriers of reciprocal translocations. Blastocysts (n = 773) were biopsied and FET was carried out on the balanced embryos. Four hundred and six couples underwent FISH-PGD, including 149 Robertsonian translocation carriers and 257 reciprocal translocation carriers. In total, 3968 embryos were biopsied and balanced embryos were transferred fresh. The SNP-PGD results and clinical outcomes were compared with those of FISH-PGD.

MAIN RESULTS AND THE ROLE OF CHANCE: Reliable SNP-PGD results were obtained for 717 out of 773 (92.8%) biopsied blastocysts. The proportions of normal/balanced embryos, embryos with translocation-related and translocation-unrelated abnormalities, the median number of embryos per patient, the ongoing pregnancy rate per embryo transfer and the miscarriage rate were 58, 23, 19, 2, 69 and 12%, respectively, for Robertsonian translocation carriers and 36, 52, 12, 1, 74 and 11%, respectively, in reciprocal translocation carriers. Reliable FISH-PGD results were obtained for 3452 out of 3968 (87.0%) biopsied embryos. The proportions of normal/balanced embryos, unbalanced embryos, the median number of embryos per patient, the ongoing pregnancy rate per transfer and the miscarriage rate were 36, 64, 3, 38 and 17%, respectively, for Robertsonian translocation carriers and 20, 80, 1, 39 and 16%, respectively, for reciprocal translocation carriers. Thus, SNP-PGD achieved a...
Introduction

Translocation is one of the more common structural rearrangements of chromosomes, with a prevalence of ~0.2% (Stern et al., 1999). The two most common types of chromosomal translocations, Robertsonian and reciprocal, usually result in no obvious phenotypic abnormalities when balanced. However, these are still associated with reproductive risks, such as infertility, spontaneous abortion and the delivery of babies with mental retardation or developmental delay (Scriven et al., 1998; Stern et al., 1999).

Preimplantation genetic diagnosis (PGD) based on fluorescence in situ hybridization (FISH-PGD) technology using appropriate probes has been used for decades to detect chromosomal abnormalities in embryos from translocation carriers. The transfer of chromosomally normal/balanced embryos following PGD is reported to significantly reduce the risk of an affected pregnancy and decrease the risk of miscarriage (Munné et al., 1998, 2000; Kyu et al., 2004; Verlinsky et al., 2005; Fischer et al., 2010). However, the clinical application of FISH-PGD for translocation carriers is impeded by its low clinical pregnancy rate and high transfer cancellation rate. According to a 10-year data set collected by theESHRE PGD Consortium, after PGD for chromosome abnormalities only 26% of successfully diagnosed embryos were suitable for transfer, and only 64% of the cycles achieved a transfer (Harper et al., 2012). The latest data show that the clinical pregnancy rates per embryo transfer were only 28–32% for embryos with a Robertsonian translocation and 24–25% for those with a reciprocal translocation (Goossens et al., 2012).

Embryo mosaicism and the limitations of the FISH technique are factors that are recognized to adversely affect the diagnostic accuracy of PGD. Cell loss and nuclear damage during embryo fixation and overlapping hybridization signals and background interference during FISH can lead to hybridization failure or unreliable results; the misdiagnosis rate can be as high as 10% (Munné et al., 2000; Velilla et al., 2002; Li et al., 2005; Wilton et al., 2009). Owing to the limited number of probes, FISH was originally used to detect translocation-related chromosomal abnormalities. With the growing knowledge that aneuploidy is very common in preimplantation embryos (Gianaroli et al., 2002; Pujol et al., 2006), a number of centers have included additional FISH probes for common aneuploidies in addition to chromosome translocations (Fischer et al., 2010; Keymolen et al., 2012).

In recent years, array-based whole-genome amplification (WGA) technologies, including microarray comparative genomic hybridization (array CGH; aCGH) and single-nucleotide polymorphism (SNP) microarrays, have enabled the screening of every chromosome for whole-chromosome aneuploidy and segmental imbalance. These techniques have been shown to have clinical application for translocation carriers (Treff et al., 2010; Fiorentino, 2012). Promising studies have indicated that array-based PGD of translocation carriers can lead to transfer pregnancy rates of 45–70% (Fiorentino et al., 2011; Treff et al., 2011a; Colls et al., 2012; van Uum et al., 2012).

In addition to genetic testing techniques, the embryo biopsy stage (polar body, cleavage embryo or blastocyst) and the mode of embryo transfer (fresh or frozen embryos) can affect the outcome of PGD. It is now generally recommended that blastomere biopsy should be replaced by blastocyst biopsy to avoid a high mosaic rate and biopsy-related damage to cleavage-stage embryos, which might affect embryo development. However, more clinical data are required to confirm that the technique of SNP array-based PGD (SNP-PGD) combined with trophectoderm (TE) biopsy and frozen embryo transfer (FET) is superior to traditional FISH-PGD combined with Day 3 (D3) blastomere biopsy and fresh embryo transfer.

Materials and Methods

Materials

In total, 575 couples received PGD treatment in the Reproductive and Genetic Hospital of CITIC·Xiangya as the result of a reproductive problem, including primary or secondary infertility, a history of recurrent spontaneous abortion or the previous delivery of babies with congenital birth defects. Among them, 169 couples underwent SNP-PGD therapy between October 2011 and August 2012, and 406 couples underwent FISH-PGD therapy between January 2005 and October 2011. These couples requested FISH-PGD or SNP-PGD therapy because one of the couples carried a Robertsonian or reciprocal translocation according to conventional karyotype analysis.
Among the 169 couples who received SNP-PGD therapy (SNP-PGD group), 52 were Robertsonian translocation carriers and 117 were reciprocal translocation carriers. The mean maternal age was 31.7 ± 5.02 years (range: 20–40 years old) for Robertsonian translocation carriers and 30.8 ± 4.74 years (range: 21–41 years old) for reciprocal translocation carriers. Basal follicle-stimulating hormone (FSH) levels were 6.2 ± 0.87 and 6.8 ± 2.11 mIU/ml for Robertsonian translocation carriers and reciprocal translocation carriers, respectively. Of the 406 couples subjected to FISH-PGD (FISH-PGD group), 149 were Robertsonian translocation carriers and 257 were reciprocal translocation carriers. The mean maternal age was 30.8 ± 3.97 years (range: 20–42 years old) for Robertsonian translocation carriers and 29.9 ± 3.67 years (range: 22–41 years old) for reciprocal translocation carriers, and basal FSH levels were 6.7 ± 2.01 and 6.8 ± 2.04 mIU/ml, respectively (Table I).

Before undergoing PGD cycles, all participants accepted genetic counseling, understood the genetic risk of chromosome translocation and the advantages and limitations of PGD therapy and provided written informed consent. The ethical committee of the Reproductive and Genetic Hospital of CITIC-Xianga approved the SNP-PGD program.

Controlled ovarian hyperstimulation and fertilization

Pituitary desensitization was performed using either a long luteal GnRH agonist protocol or an antagonist protocol. In the agonist protocol, 1.5 mg of the GnRH analog, triptorelin (Decapeptyl; Ferring, Malmo, Sweden), was administered in the mid-luteal phase of the menstrual cycle. After full desensitization was achieved, 112.5–375 IU recombinant FSH (Gonal-F; Merck-Serono, Geneva, Switzerland; Puregon, NV Organon, Oss, The Netherlands) and/or human menopausal gonadotrophins (hMG, Lizhu, China) were used daily until the day of HCG administration. In the antagonist protocol group, recombinant FSH or hMG with a fixed dose of 150–225 IU/day was used from Day 2–3. When the leading follicle reached a diameter of 12 mm, 0.25 mg of the GnRH antagonist (cetrodite; Merck-Serono, Geneva, Switzerland) was applied until HCG was administered. The starting dose of gonadotrophins was based on patient age, body weight, ovarian reserve test and/or previous response to ovarian stimulation. HCG (5000–10 000 IU, Pregnyl; Merck) was injected when at least three follicles reach the size of 17 mm. Oocyte retrieval (OR) was performed 34–36 h later under general anesthesia using intravenous propofol (AstraZeneca UK Ltd). All eggs were fertilized by ICSI 4–6 h after OR, and normal fertilization was identified 16–18 h after injection by the presence of two pronuclei and two polar bodies.

Embryo culture and biopsy

All embryos were cultured in sequential media (G1 and G2, Vitrolife, Goteborg, Sweden) to either Day 3 or blastocyst stage under 6% CO2, 5% O2 and 89% N2 in a COOK mini-incubator (Bloomington, IN, USA) for further manipulation. The Day 3 biopsy procedure for cleavage-stage embryos was performed as previously described (Cheng et al., 2012). Prior to 2008, one blastomere was removed if the embryo contained less than six cells and two blastomeres were biopsied if the embryo contained six cells or more. From 2008, we adopted a single-blastomere biopsy for all embryos unless the first biopsy was unsuccessful. The definition of a successful biopsy was the removal of a cell without lysis, in order that the cell could be used for fixation and analysis (Joris et al., 2003). The blastomeres were sent to FISH analysis and the biopsied embryos were cultured continuously to D5 for transfer.

For the TE biopsy, an 18-μm hole was made in the zona pellucida of all embryos on the morning of D4. On D5 or D6 after fertilization, blastocysts in which TE cells had herniated out of the zona pellucida were chosen for biopsy. Approximately 4–8 TE cells were aspirated with a biopsy pipette (internal diameter: 30 μm) and dissected with Zilos TK laser (Hamilton Thorne, MA, USA). Biopsied TE cells were washed three times in G-MOPS medium (Vitrolife, Goteborg, Sweden) and then either used directly for WGA or stored at −20°C for future WGA.

Blastocyst vitrification and thawing

Blastocysts were vitrified soon after the biopsy using a Kitazato vitrification kit (Kitazato Biopharma Co. Ltd, Shizouka, Japan) in combination with closed High Security Vitrification Straws (Cryo Bio System, France). Each blastocyst was stored in an individual straw. The vitrification and thawing procedure was carried out according to the protocol recommended by the Kitazato vitrification kit. For the FET cycle, we transferred no more than two blastocysts, and we recommended single-blastocyst transfer to each patient. According to the number of blastocysts to be thawed, we prioritized the blastocyst for thawing based on the best quality before biopsy. After thawing, blastocysts were transferred to blastocyst medium and cultured for 1–2 h. Only blastocysts that survived thawing and re-expanded were considered suitable for transfer.

Luteal support

In the FISH-PGD group, luteal support was provided by Crinone® (progesterone gel; Columbia Laboratories, Inc., Livingston, NJ, USA) supplementation from the OR day until the first pregnancy test and was continued in all cycles with a positive serum beta-HCG until the documentation of fetal cardiac activity. In the SNP-PGD group, luteal support was not applied in the OR cycle as all available embryos were frozen for future FET cycles. For FET cycles, thawed blastocysts were transferred either 5 days after ovulation with a natural cycle or 5 days after the initiation of progesterone with an endometrial preparation with estradiol valerate and progesterone. Briefly, 6 mg of estradiol valerate was started from Day 3 for 10–15 days and then luteal support was applied when a satisfactory endometrial development (thickness ≥8 mm) was confirmed with ultrasound.

FISH analysis

Three types of probes, including centromeric-specific, locus-specific (LSI) and subtelomere probes, were used for FISH-PGD. All probes were purchased from Abbott-Vysis, Inc. (Downers Grove, IL, USA). The designed probe set contained sufficient probes to detect all expected unbalanced forms of the chromosomal rearrangement according to the ESHRE PGD consortium best practice guidelines for FISH-based PGD (Harton et al., 2011). For the analysis of Robertsonian translocation carriers, two LSI probes or two subtelomere probes specific to translocated chromosomes were used; for reciprocal translocation carriers, the probe sets were designed to detect balanced/normal embryos according to the principle of meiosis division. Usually, a combination of a centromeric probe (labeled with Spectrums啶Aqua) and two sub-telomeric probes to translocated segments labeled in SpectrumGreen and SpectrumOrange, respectively, was used for one round of FISH in cases of reciprocal translocation (Lellé et al., 2010). If a centromeric probe labeled in SpectrumAqua was not available, two rounds of FISH with four sub-telomeric probes including two distal probes and two proximal probes were used.

The FISH procedure was performed as we have previously described (Cheng et al., 2012). Briefly, the biopsied blastomeres were exposed for 5 min to hypotonic solution (1% sodium citrate in 6 mg/ml bovine serum albumin) and transferred into a small drop of Tween 20 fixative (0.01 N HCl, 0.1% Tween 20) on a clean slide. Fixed cells were analyzed by one round of FISH for Robertsonian translocation, or reciprocal translocation when three probes with different colors are available, or two rounds of FISH for reciprocal translocation when three probes with different colors...
WGA products were then used for SNP array screening for chromosomal abnormalities, including aneuploidy and unbalanced segment anomalies, affecting all chromosomes except for the Y chromosome. Individual embryonic DNA samples were hybridized to a GeneChip Mapping Nsp I 262 K microarray (Affymetrix, Inc., Santa Clara, CA, USA). Approximately 260,000 SNP signal intensities for each test sample were computationally compared with averaged signals from reference database. In Stage 1, the HapMap database was used as an initial reference database. In Stage 2, the reference database initially consisted of the HapMap database and seven sets of normal female SNP data from Stage 1. The HapMap database was gradually substituted by the normal female data detected by later SNP array analysis. Copy number and loss of heterozygosity (LOH) were analyzed using the Gene Chip Genotyping Analysis Software (TYPE), using a smoothing size of 16 megabases (Mb) to eliminate background signals as previously recommended; the minimum size of imbalance was reported to be approximately 2.36 Mb (Treff et al., 2011a,b). Y chromosome-specific PCR was performed to distinguish the normal male karyotype from Y chromosome loss, as the SNP array is unable to detect Y chromosome.

**Statistical analysis**

Maternal age, basal FSH levels and the average number of retrieved oocytes were compared between the SNP-PGD and FISH-PGD groups using Student's t-test. The Mann–Whitney U-test was used for the statistical analysis of the average number of biopsied embryos and transferred embryos between SNP and FISH groups. The chromosomal abnormality, clinical

### Table 1 Chromosome abnormalities detected by single-nucleotide polymorphism microarray-based preimplantation genetic diagnosis (SNP-PGD) and fluorescence in situ hybridization-PGD (FISH-PGD).

<table>
<thead>
<tr>
<th>Biopsied cycles</th>
<th>SNP-PGD</th>
<th>FISH-PGD</th>
<th>P-value*</th>
<th>SNP-PGD</th>
<th>FISH-PGD</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± SD)</td>
<td>31.7 ± 5.02</td>
<td>30.8 ± 3.97</td>
<td>0.818</td>
<td>30.8 ± 4.74</td>
<td>29.9 ± 3.67</td>
<td>0.481</td>
</tr>
<tr>
<td>Basal FSH level (mean ± SD)</td>
<td>6.2 ± 0.87</td>
<td>6.7 ± 2.01</td>
<td>0.620</td>
<td>6.8 ± 2.11</td>
<td>6.8 ± 2.04</td>
<td>0.620</td>
</tr>
<tr>
<td>Embryology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successfully biopsied</td>
<td>237</td>
<td>1370</td>
<td></td>
<td>536</td>
<td>2598</td>
<td></td>
</tr>
<tr>
<td>Successfully tested</td>
<td>218 (92)</td>
<td>1204 (88)</td>
<td>0.068</td>
<td>499 (93)</td>
<td>2258 (87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tested failure</td>
<td>19 (8)</td>
<td>166 (12)</td>
<td>0.068</td>
<td>37 (7)</td>
<td>340 (13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrable</td>
<td>126 (58)</td>
<td>435 (36)</td>
<td>&lt;0.001</td>
<td>177 (36)</td>
<td>449 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrable embryos per patient^a (median (range))</td>
<td>2 (0–6)</td>
<td>3 (0–12)</td>
<td>0.192</td>
<td>1 (0–6)</td>
<td>1 (0–7)</td>
<td>0.129</td>
</tr>
<tr>
<td>Embryos with chromosomal imbalance</td>
<td>92 (42)</td>
<td>769 (64)</td>
<td>&lt;0.001</td>
<td>322 (65)</td>
<td>1809 (80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Translocation-related imbalance</td>
<td>51 (23)</td>
<td>769</td>
<td></td>
<td>261 (52)</td>
<td>1809</td>
<td></td>
</tr>
<tr>
<td>Unbalanced translocation</td>
<td>37 (17)</td>
<td>–</td>
<td>–</td>
<td>206 (41)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unbalanced translocation and de novo aneuploidy</td>
<td>8 (4)</td>
<td>–</td>
<td>–</td>
<td>38 (8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unbalanced translocation and de novo segmental abnormality</td>
<td>6 (3)</td>
<td>–</td>
<td>–</td>
<td>16 (3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Translocation-unrelated imbalance</td>
<td>41 (19)</td>
<td>–</td>
<td>–</td>
<td>62 (12)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>De novo aneuploidy</td>
<td>21 (10)</td>
<td>–</td>
<td>–</td>
<td>29 (6)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>De novo segmental abnormality</td>
<td>9 (4)</td>
<td>–</td>
<td>–</td>
<td>17 (3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>De novo aneuploidy and segmental abnormality</td>
<td>4 (2)</td>
<td>–</td>
<td>–</td>
<td>6 (1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Complex aneuploidy</td>
<td>7 (3)</td>
<td>–</td>
<td>–</td>
<td>10 (2)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are n (%) unless stated otherwise.

^aTransferrable embryos per patient also refer to median normal/balanced embryos per patients.

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are not available. The FISH signals were observed using an Olympus BX-51 fluorescence microscope. Images were captured using the VideoTesT-FISH 2.0 software (version number 5.0.74.4803, VideoTesT Ltd, Russia).

**SNP array analysis**

To establish SNP array analysis for TE cells, we designed the study in two stages. In Stage 1, we collected one cell and five cells from two human embryonic stem cell (hESC) lines with a normal female karyotype and a trisomy 21 female karyotype, and 15 blastocysts donated by 13 couples with Robertsonian or reciprocal translocation undergoing FISH-PGD (Table II). The donated blastocysts were either diagnosed as ‘abnormal’ or ‘normal’ but with poor morphology. TE was re-biopsied for two parts from donated blastocysts, one part was for WGA and SNP array analysis and the second part was used for later validation by FISH. In Stage 2, the established SNP array platform was used for SNP-PGD diagnosis.

Biopsied samples were first subjected to WGA using a WGA4 GenomePlex Single Cell Whole Genome Amplification Kit (Sigma-Aldrich, MO, USA) as previously described (Tan et al., 2010). Briefly, samples were incubated at 50°C for 1 h in single-cell lysis and fragment buffer, heated to 99°C and then universal oligonucleotide primers were used for PCR amplification (25 cycles) of DNA fragments, according to the manufacturer’s recommendations. The integrity of WGA products was assessed by 2% agarose gel electrophoresis and purified with a Wizard SV Gel and a PCR Clean-Up System PCR Purification kit (Promega, WI, USA).
### Table II: Single-nucleotide polymorphism microarray-based preimplantation genetic diagnosis (SNP-PGD) validation results.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Karyotype of the donor</th>
<th>D3 FISH results (probe signals)</th>
<th>QC call rate</th>
<th>Result of D5 SNP array</th>
<th>Consistency</th>
<th>TE cell FISH Validation by (probe signals/number of cells analyzed)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HESC</td>
<td>46,XX</td>
<td>NA</td>
<td>75.87</td>
<td>arr(1–22,X) × 2</td>
<td>NA</td>
<td>NA</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>2</td>
<td>HESC</td>
<td>46,XX</td>
<td>NA</td>
<td>78.59</td>
<td>arr(1–22,X) × 2</td>
<td>NA</td>
<td>NA</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>3</td>
<td>HESC</td>
<td>47,XX,+21</td>
<td>NA</td>
<td>73.21</td>
<td>arr(1–20,22,X) × 2,(21) × 3</td>
<td>NA</td>
<td>NA</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>4</td>
<td>HESC</td>
<td>47,XX,+21</td>
<td>NA</td>
<td>82.93</td>
<td>arr(1–20,22,X) × 2,(21) × 3</td>
<td>NA</td>
<td>NA</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>5</td>
<td>Blastocyst</td>
<td>46,XY,t(1;22)(q23;q11)</td>
<td>CEP11<em>2,TUPLE1</em>2, ARSA*2</td>
<td>72.83</td>
<td>arr(1–22,X) × 2</td>
<td>Yes</td>
<td>CEP11<em>2,TUPLE1</em>2,ARSA*2/15</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>6</td>
<td>Blastocyst</td>
<td>46,XY,t(10;15)(q22;q24)</td>
<td>CEP10<em>2, Tel15q</em>1, Tel10q*3</td>
<td>71.98</td>
<td>arr 10q22.2q26.3(76 596 709–135 272 495) × 3, 15q25.1q26.3 (77 250 927–100 192 115) × 1</td>
<td>Yes</td>
<td>Tel10q<em>3,Tel15q</em>1,Tel10q*3/13</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>7</td>
<td>Blastocyst</td>
<td>46,XY,t(2;6)(q35;q21)</td>
<td>CEP6<em>2, Tel2p</em>2, Tel2q<em>1, Tel6q</em>3</td>
<td>72.73</td>
<td>arr 2q36.3q37.3(229 751 746–242 650 581) × 1.6q21q27 (108 099 428–170 716 684) × 3</td>
<td>Yes</td>
<td>CEP6<em>2,Tel2p</em>2,Tel2q<em>1, Tel6q</em>3/17</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>8</td>
<td>Blastocyst</td>
<td>46,XY,der(14;21)(q10;q10)</td>
<td>Tel14q<em>2,Tel12q</em>2, Tel22q<em>1, Kal1, DXZ1</em>1, DYZ3*1</td>
<td>88.03</td>
<td>arr(1–22,X) × 2</td>
<td>Yes</td>
<td>Tel14q<em>2,Tel12q</em>2, Tel22q<em>1, Kal1, DXZ1</em>1, DYZ3*1/10</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>9</td>
<td>Blastocyst</td>
<td>46,XY,der(10;13)(q24;q22)</td>
<td>Tel10p<em>1, CEP18</em>2, LSI13q4*2</td>
<td>84.4</td>
<td>arr 8p23.3q11.23(180 586–39 112 762) × 1, 8q21.32q24.3(92 990 662–146 263 569) × 3, 18p11.22p11.23 (21 071–17 486 209) × 3, 18q13.12q23.1 (37 423 417–76 115 554) × 1</td>
<td>Yes</td>
<td>Tel8p<em>1, Tel8q</em>3, Tel18q<em>3, CEP18</em>2/12</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>10</td>
<td>Blastocyst</td>
<td>46,XY,der(10;13)(q24;q22)</td>
<td>Tel10p<em>1, CEP18</em>2, LSI13q4*2</td>
<td>85.78</td>
<td>arr 10p15.3q23.1(148 946–84 530 558) × 1, 13q21.2q21.1 (17 960 319–54 543 292) × 3, (7) × 1</td>
<td>No</td>
<td>Tel10p<em>1, CEP18</em>2, LSI13q4<em>3, Tel7p</em>1, Tel7q*1/14</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>11</td>
<td>Blastocyst</td>
<td>46,XY,der(10;13)(q24;q22)</td>
<td>Tel10p<em>1, CEP18</em>2, LSI13q4*2</td>
<td>84.69</td>
<td>arr(13) × 3</td>
<td>Yes</td>
<td>Tel13q<em>3, Tel14q</em>2, TelXp<em>1, TelYq</em>1/19</td>
<td>Support SNP array</td>
</tr>
<tr>
<td>12</td>
<td>Blastocyst</td>
<td>46,XY,der(10;13)(q24;q22)</td>
<td>Tel10p<em>1, CEP18</em>2, LSI13q4*2</td>
<td>73.63</td>
<td>arr(1–22,X) × 2</td>
<td>No</td>
<td>LSI 13q14<em>2, Tel14q</em>2, DXZ1*2/13</td>
<td>Support SNP array</td>
</tr>
</tbody>
</table>

Continued
pregnancy, embryo implantation and miscarriage rates between the two groups were analyzed using the \( \chi^2 \) test. Significant difference was defined as \( \alpha = 0.05 \).

**Results**

The establishment of the SNP array method

The single cell and 5 cells from two hESC lines and 15 biopsied TE cells were successfully amplified by WGA. The one cell and five cells from hESC lines generated similar SNP results that were consistent with the original karyotype (Table II and Fig. 1A–D). In 15 analyzed blastocysts, 9 had a consistent SNP array results compared with D3 FISH, which was also verified by later FISH analysis of corresponding TE (Fig. 1E–H). The other six blastocysts had an inconsistent result between SNP array and D3 FISH, of which, in four the SNP array data were verified to be correct by later TE FISH (Fig. 1I and J) and the D3 FISH data were misdiagnosed, and in the other two the SNP array data were found to be incorrect (Table I). Overall, through later TE validation and a comparison with original hESC karyotype, we found that SNP array analysis was accurate in a large majority of cases (89.5%, 17 out of 19). We also found that the two misdiagnosed samples had low quality control (QC) call rates for the SNP array analysis (50.01 and 61.14, respectively) compared with the QC scores (≥ 70) of the other 17 samples. Thereafter, we arbitrarily set the QC score > 70 as a standard for a successful SNP array diagnosis.

Comparison of chromosomal abnormalities in the SNP-PGD and FISH-PGD groups

In the SNP-PGD group, all 773 blastocysts were successfully biopsied and reliable results were obtained for 717 (92.8%); the other 56 blastocysts (7.2%) had no results due to the lack of amplification. For Robertsonian translocation carriers, tests of 218 embryos were successful and 19 failed: 126 embryos were normal/balanced (57.8%) and 92 were abnormal (42.2%). Among the 92 abnormal embryos, 51 had chromosomal translocations (23.4% overall; 51 out of 218), 41 were unrelated to chromosomal translocations (18.8% overall; 41 out of 218) and 7 were classified as complex aneuploidy abnormalities (aneuploidies involving three or more chromosomes) (3.2% overall; 7 out of 218). The median number of normal/balanced embryos per patient was 2 (range from 0 to 6) (Table I). For tests of reciprocal translocation carriers, 499 were successful and 37 failed: 177 embryos were normal/balanced (35.5%) and 322 were abnormal (64.5%). Among the 322 abnormal embryos, 260 contained translocation-related chromosomal abnormalities (52.1% overall; 260 out of 499), 62 were unrelated to translocations (12.4% overall; 62 out of 499) and 10 contained complex aneuploidy abnormalities (2.0% overall; 10 out of 499). The median number of normal/balanced embryos per patient was 1 (range: from 0 to 6) (Table I).

Figures 2 and 3 are two SNP-PGD examples for Robertsonian translocation and reciprocal translocation, respectively. The SNP array data of all the embryos are listed in Supplementary data, Tables SI and SII, where part of the raw SNP data were submitted to the Gene Expression Omnibus (GSE 44994).

The de novo abnormalities discovered by the SNP array include aneuploidy and segmental gain or loss, which occurred on all chromosomes. The size of segmental abnormalities we detected ranged from 3.6 to...
The merits of SNP-PGD for translocation carriers
I47.7 Mb. We further analyzed the LOH using the GTYPE software. We found that it was more effective to analyze the LOH state of the X chromosome in male samples when using the HapMap database as a reference instead of using the SNP data of normal female embryos as the reference. Under these conditions, we did not detect whole-chromosomal or arm-specific uniparental disomy (UPD) among the 717 embryos with reliable SNP array results. However, when we used the current software and HapMap data as a reference, the segmental LOH was discovered in >50% of regions and was scattered through all chromosomes in all embryos. We further found that the average heterogeneity rate (AB call) of randomly selected 40 samples from the HapMap database was 22.3 ± 1.2%, but the heterogeneity rate of analyzed embryos (651 samples) was 11.1 ± 1.2%. We suggest that the WGA may lead to a decreased heterogeneity of almost half of the normal level, which may have impaired the accuracy of current method for analyzing the segmental UPD of WGA samples.

In the FISH-PGD group, all 3968 embryos were successfully biopsied and 3452 (87.0%) had reliable FISH-PGD results. The proportion of abnormal embryos in Robertsonian and reciprocal translocation carriers was 63.9% (769 out of 1204) and 80.1% (1809 out of 2258), respectively (Table II). Supplementary data, Figs S1–S3 present three examples of FISH-PGD for Robertsonian translocation and reciprocal translocation, respectively.

Comparison of pregnancy outcomes between SNP-PGD and FISH-PGD groups

There were no significant differences in the mean maternal age, basal FSH levels and the types of Robertsonian or reciprocal translocation (Table III) between the SNP-PGD and FISH-PGD groups. In addition, there were no significant differences in the average number of retrieved oocytes and good-quality D3 embryos before a biopsy between the SNP-PGD and FISH-PGD groups.

A detailed flowchart of the cases and their outcomes in each group are provided in Supplementary data, Fig. 54. In the 97 SNP-PGD FET cycles completed so far, we warmed 142 blastocysts, of which 140 survived, giving a survival rate of 98.6%. We performed 20 single-blastocyst transfer cycles and 16 two-blastocyst transfer cycles in Robertsonian translocation carriers, and 34 single-blastocyst transfer cycles and 27 two-blastocyst transfer cycles in reciprocal translocation carriers. The median number of biopsied embryos in the FISH-PGD group was higher than in the SNP-PGD group [Robertsonian translocation carriers: 9 (range: 1–24) versus 4 (range: 1–15); reciprocal translocation carriers: 10 (range: 1–30) versus 4 (range: 1–14)]. However, the clinical pregnancy rate per transfer cycle was lower in the FISH-PGD group than in the SNP-PGD group, at 38.4 versus 69.4% for Robertsonian translocation carriers; and 38.9 versus 73.8% for reciprocal translocation carriers. Similarly, the implantation rate was lower in the FISH-PGD group than in the SNP-PGD group, at 28.1 versus 51.9% for Robertsonian translocation carriers, and 32.2 versus 59.1% for reciprocal translocation carriers. Moreover, the miscarriage rate was relatively higher in the FISH-PGD group than in the SNP-PGD group (Robertsonian translocation carriers: 16.7 versus 12.0%; reciprocal translocation carriers: 16.2 versus 11.1%), and comparative genomic hybridization (CGH) analysis of the aborted tissue revealed unrelated chromosomal abnormalities in FISH-PGD miscarriages but not in SNP-PGD miscarriages. To date, all of the tracked prenatal diagnoses and delivered babies from both the SNP-PGD and FISH-PGD groups are normal and healthy.

Discussion

FISH-based PGD technology has been widely used for the detection of chromosomal abnormalities in preimplantation embryos for the last 10 years. However, FISH technology can detect only a limited range of translocation-related chromosomal abnormalities, due to limited availability of commercial probes. However, SNP array technology can detect aneuploidy that affects all chromosomes, as well as segmental abnormalities. It is possible that SNP array technology could replace the FISH in PGD clinical applications. In this study, we compared the advantages and disadvantages of these two schemes.

SNP-PGD is a more efficient method of detecting chromosomal abnormalities than FISH-PGD

Our results show that, compared with FISH, SNP-PGD could identify >15% more chromosomal abnormalities unrelated to translocation. Embryos with unbalanced chromosomes unrelated to translocation,
which cannot be detected by FISH, will result in a lower implantation rate and a higher abortion rate as shown in our study. Therefore, SNP-PGD embryo testing should lead to improved rates of clinical pregnancy and decreased numbers of miscarriages. It is inspiring that all delivered babies from FISH-PGD had normal/balanced karyotype as observed in SNP-PGD, which implies that the slightly increased miscarriage rate in FISH-PGD than in SNP-PGD may help to prevent abnormal fetuses being born, but this suggestion need larger sample size to prove.

We also noted the presence of some complex aneuploidy abnormalities (3.2% for Robertsonian translocation carriers and 2.0% for reciprocal translocation carriers), which may be related to the mitosis error during preimplantation development. However, more research is required to determine the cause.

Unlike FISH-PGD, SNP-PGD can detect all 24 chromosomes, including the Y chromosome. Therefore, SNP-PGD should theoretically decrease the number of transferable embryos. Although there was no significant difference in the average number of transferable embryos identified in the two groups, the proportion of analyzed embryos that was transferable was significantly higher in the SNP-PGD group than in the FISH-PGD group. It is possible that the SNP-PGD was more accurate than D3 FISH for the exclusion of false-positive diagnoses, which leads to a reduction of wasted embryos. However, we also cannot exclude the possibility that D3 biopsy dramatically impairs the blastocyst developmental potential.

The current SNP array is suggested for the analysis of LOH and UPD. However, in our study, we detected an abnormally high degree of LOH (34% region) in all chromosomes in each embryo, which is likely to be due to the WGA bias from a small amount of DNA. The amplification bias leads to the over- or under-amplification of both alleles of a certain region of the genome. Therefore, it is difficult to distinguish the true segmental LOH and amplification bias. It would be further interesting to determine and validate to what extend the amplification bias could lead to and the sensitivity of LOH detection under current conditions.

**TE biopsy has a reduced risk of embryo damage**

In the SNP-PGD group, the test material was TE cells biopsied from D5 or D6 blastocyst embryos, while in the FISH-PGD group, the test material was blastomeres biopsied from D3 embryos. Early studies found that biopsy at D3 has little detrimental effect on the developmental potential of embryos. However, more recent studies suggest that the clinical outcome of 1-cell biopsy was significantly better than that of a 2-cell
biopsy (De Vos et al., 2009; Kirkegaard et al., 2012). In this study, the majority of our D3 biopsies took only one blastomere for analysis, and the average numbers of retrieved oocytes and good-quality D3 embryos were comparable between the SNP-PGD and FISH-PGD groups. However, we observed that the average number of D5 blastocysts and the number of good-quality D5 blastocysts were significantly higher in the SNP-PGD group. Although this could be explained in part by the delayed blastocyst development in D3-biopsy embryos discovered by a recent time-lapse study (Kirkegaard et al., 2012), we still could not exclude the possibility that a D3 blastomere biopsy increases the risk of damaging the implantation potential of embryos. As the implantation rate is 2-fold higher in the SNP-PGD group than in the FISH-PGD group, which cannot only be explained by the fact the some transferrable blastocysts with extra chromosomal abnormalities unrelated to translocation were identified and excluded for transfer by SNP array. Therefore, a TE biopsy is recommended over a D3 blastomere biopsy.

In our study, 4–10 TE cells were usually biopsied from each blastocyst without obviously damaging their further implantation potential. This provides sufficient cells to ensure diagnostic accuracy and might be a more suitable method for genetic diagnosis and screening. This is also reflected by the lower test failure rate (8.0% for Robertsonian translocation and 6.9% for reciprocal translocation) in the SNP-PGD group.

Vitrification combined with thawed embryo transfer increases the clinical pregnancy rate

Thawed embryo transfer is a disadvantage of the SNP-PGD scheme in contrast to the use of fresh embryo transfer in the FISH-PGD scheme,
which increases the waiting time before transfer, the treatment cost and the risk of embryo damage due to freezing. However, vitrification combined with thawed embryo transfer provides sufficient time for genetic analysis and embryo transportation. In addition, embryo cryopreservation may be advantageous for avoiding embryo transfer under conditions of ovarian hyperstimulation syndrome and possible suboptimal endometrium. Our clinical outcomes showed that vitrification is associated with a high survival embryo rate, low embryo damage and a satisfactory clinical pregnancy rate. These observations indicate that the technique of vitrification and thawed embryo transfer combined with TE biopsy is suitable for clinical application.

In conclusion, our study showed that SNP-PGD combined with D5 biopsy and thawed embryo transfer has the potential to significantly improve the clinical outcomes compared with the traditional FISH-PGD scheme for chromosome translocation carriers.

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

Authors’ roles
G.-X.L. conceived the study, provided financial support and revised the manuscript. G.L. designed the study, analyzed the data and wrote the paper. Y.-Q.T. analyzed the data, signed the testing report of each patient and wrote the paper. Y.-Q.T. and K.T. established the SNP-PGD method. Y.-Q.T. and D.-H.C. established the FISH-PGD method. K.T. performed the SNP array experiments and interpreted the results. F.G. performed IVF clinic and embryo transfer. B.X. performed the SNP array experiments and interpreted the results. C.-F.L. performed embryo culture and intracytoplasmic sperm injection. K.-L.L. contributed to patient management. X.-C.T. contributed to embryology management.

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Conflict of interest
None declared.
References


