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Data in Brief

Using SNP array to identify aneuploidy and segmental imbalance in translocation carriers

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ABSTRACT

Translocation is one of the more common structural rearrangements of chromosomes, with a prevalence of 0.2%. 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.

In recent years, array-based whole-genome amplification (WGA) technologies, including microarray comparative genomic hybridization (array CGH; aCGH) and single-nucleotide polymorphism (SNP) micro-arrays, 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. Promising studies have indicated that array-based PGD of translocation carriers can lead to transfer pregnancy rates of 45–70% [2]. 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.

Specifications	
Organism/cell line/tissue	*Homo sapiens*
Sex	Both male and female
Sequencer or array type	Affymetrix GeneChip Nsp 262K
Data format	Raw data (CEL files)
Experimental factors	Single nucleotide polymorphism (SNP), aneuploidy, and chromosome imbalance
Experimental features	Copy number analysis of 717 blastocysts from chromosomal translocation carriers, Robertsonian and reciprocal.
Consent	All patients consented before starting the study
Sample source location	Reproductive and Genetic Hospital of Citic-Xiangya

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Direct link to deposited data

Deposited data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44994

Experimental design, materials and methods

Study design and clinical information

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. The donated blastocysts were either diagnosed as 'abnormal' or 'normal' but with poor morphology. TE was re-biopsied for two parts

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from donated blastocysts, the first part was for WGA and SNP array analysis, the second part was used for later validation by FISH.

In Stage 2, the established SNP array platform was used for SNP-PGD diagnosis. In total, 169 couples received PGD treatment between October 2011 and August 2012 in the Reproductive and Genetic Hospital of Citic-Xiangya, because either the husband or the wife of each couple carried a Robertsonian or reciprocal translocation according to conventional karyotype analysis. The clinical manifestation of these couples included primary or secondary infertility, a history of recurrent spontaneous abortion or a previous delivery of babies with congenital birth defects. Among the 169 couples who received SNP-PGD therapy, 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. The clinical information of SNP-PGD patients is seen in Table 1.

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-Xiangya approved the SNP-PGD program.

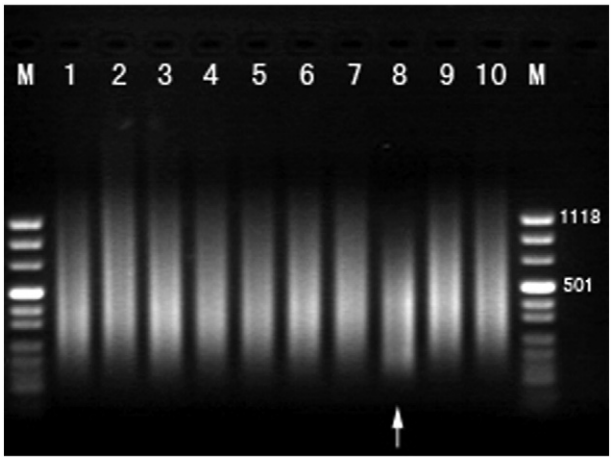

Fig. 1. The validation of whole genome amplification (WGA). The quality of the WGA DNA can be qualitatively determined by loading 2.5μL of the final products onto a 1.5% agarose gel. The DNA with good quality showed a size range of 100–1000 bp, with a mean size of ~400 bp. The poor quality DNA (lane 8, with arrow) has a size range lower than average, from 50 to 800 bp, such WGA DNA cannot pass the quality control of SNP-array, and may lead to failure of analysis.

Blastocyst vitrification and thawing

Blastocysts were vitrified soon after the biopsy using a Kitazato vitrification kit (Kitazato Biopharma Co. Ltd, Shizuoka, 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 a blastocyst medium and cultured for 1–2 h. Only blastocysts that survived thawing and re-expanded were considered suitable for transfer.

Embryo culture and biopsy

Pituitary desensitization followed by controlled ovarian hyperstimulation was performed on these patients to achieve multiple follicle growths. Oocytes were retrieved and fertilized by intracytoplasmic sperm injection. The resulted embryos were further cultured in sequential media (G1 and G2, Vitrolife, Goteborg, Sweden) under 6% CO_2, 5% O_2 and 89% N_2 in a COOK mini-incubator (Bloomington, IN, USA) for further manipulation.

For the TE biopsy, an 18-mm hole was made in the zona pellucida of all embryos. 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 mm) and dissected with Zilos TK laser (Hamilton Thorne, MA, USA). Biopsied TE cells were washed three times in a G-MOPS medium (Vitrolife,Goteborg, Sweden) and then either used directly for WGA or stored at −20 °C for future WGA.

Whole genome amplification

Biopsied samples were first subjected to WGA using a WGA4 Genome-Plex Single Cell Whole Genome Amplification kit (Sigma-Aldrich, MO, USA) as previously described [1]. Briefly, samples were incubated at 50 °C for 1 h in a 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 quality of the WGA DNA can be qualitatively determined by loading 5–10% (4–8 μL) of the final reaction onto a 1.5% agarose gel. The DNA size should range from 100 to 1000 bp, with a mean size of ~400 bp. WGA DNA was purified using the Wizard SV Gel and PCR Clean-Up System PCR Purification kit (Promega, WI, USA). Fig. 1 shows the validation of WGA.

SNP array analysis

Two hundred and fifty nanograms of purified 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 262K

Table 1
The clinical information of SNP-PGD patients.

	Robertsonian translocation	Reciprocal translocation
Biopsied cycles	52	117
Maternal age (mean ± SD)[a]	31.7 ± 5.02	30.8 ± 4.74
No. of male carrier	32 (62%)	55 (47%)
No. of female carrier	20 (38%)	62 (53%)
Oocyte number	14.8 ± 6.44	14.9 ± 7.60
Blastocyst number on D5	4.9 ± 3.46	5.6 ± 3.38
Number of good blastocysts on D5	2.3 ± 2.52	2.7 ± 2.35
Biopsied embryos (median (range))	4 (1–15)	4 (1–14)
Transferrable embryos per patient [b] (median (range))	2 (0–6)	1 (0–6)
Cycles with all biopsied embryos diagnosed abnormal	5 (10%)	35 (30%)

[a] Data are mean ± SD or n (%) unless stated otherwise.
[b] Transferrable embryos per patient also refer to median normal/balanced embryos per patients.

Table 2
Result of SNP-PGD in chromosomal translocation carriers.

Embryology	Robertsonian translocation	Reciprocal translocation
Successfully biopsied	237	536
Successfully tested	218 (92%)	499 (93%)
Tested failure	19 (8%)	37 (7%)
Transferrable	126 (58%)	177 (36%)
Embryos with chromosomal imbalance	92 (42%)	322 (65%)
Translocation-related imbalance	51 (23%)	261 (52%)
Unbalanced translocation	37 (17%)	206 (41%)
Unbalanced translocation and de novo aneuploidy	8 (4%)	38 (8%)
Unbalanced translocation and de novo segmental abnormality	6 (3%)	16 (3%)
Translocation-unrelated imbalance	41 (19%)	62 (12%)
De novo aneuploidy	21 (10%)	29 (6%)
De novo segmental	9 (4%)	17 (3%)
De novo aneuploidy and segmental abnormality	4 (2%)	6 (1%)
Complex aneuploidy	7 (3%)	10 (2%)

microarray (Affymetrix, Inc., Santa Clara, CA, USA). Approximately 260,000 SNP signal intensities for each test sample were computationally compared with averaged signals from the reference database. For SNP-typing, we analyzed probe intensities by the 'GeneChip Genotyping Analysis Software (GTYPE) version 4.1 (Affymetrix)' using the dynamic model, and adjusted the 'Parameter Value' for 'Homozygote Call Threshold' as well as for 'Heterozygote Call Threshold' to 0.33.

For SNP-copy number analysis, we used the 'Copy Number Analysis Tool' (CNAT4.0.1, Affymetrix), and set smoothing size to 16 megabases (Mb) to eliminate background signals as previously recommended; the minimum size of imbalance was reported to be 2.36 Mb [2,3]. The copy number state of each chromosome was interpreted by a five-state Hidden Markov model with prior probabilities set equal for all five states. Standard deviation settings were 0.11 for hidden-states zero, one, three and four, while 0.08 for hidden-state 2. We set the transition decay at 4000 Mb, readjusted outliers using a threshold of 1000 bp and allowed outliers to be readjusted.

In Stage 1, we selected 44 normal female SNP data from the HapMap database to generate reference database. In Stage 2, the reference database initially consisted of the above HapMap data and seven normal female SNP data from Stage 1. Then, the reference database was gradually supplemented with new SNP data from late diagnosed normal female by SNP array analysis. Copy number and loss of heterozygosity (LOH) were analyzed by using the GeneChip Genotyping Analysis Software (GTYPE). 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. The results of SNP-PGD were summarized in Table 2. The SNP array results of blastocysts from a Robertsonian translocation carrier and a reciprocal translocation carrier were displayed in Figs. 2 and 3.

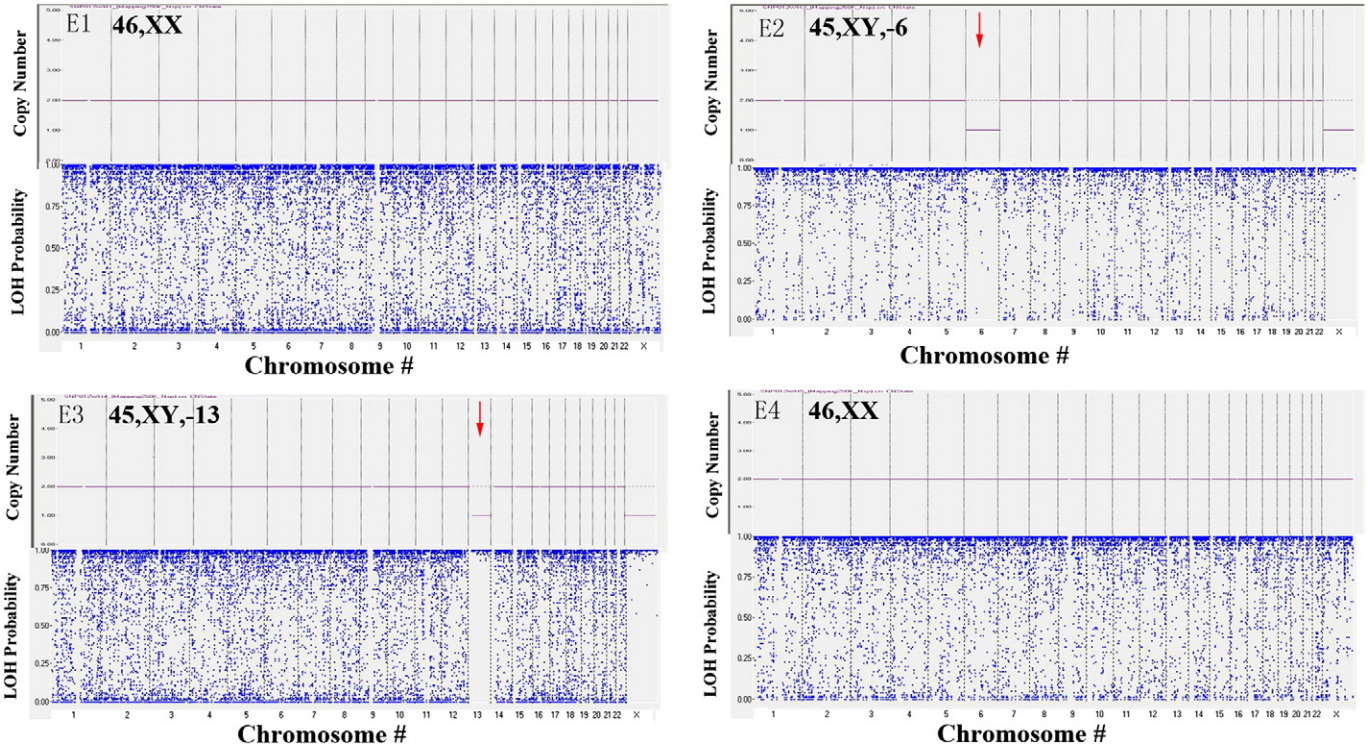

Fig. 2. An example of SNP-PGD for a single Robertsonian translocation carrier with a karyotype of 45,XX,der(13;14)(q10;q10). The upper part of each graph indicates the SNP copy number assignments (0, 1, 2, 3, or 4) on the y-axis and the arrows indicate the abnormal chromosomes, the lower part indicates the SNP LOH probability (0–1) on the y-axis and the arrows indicate the chromosomes with unique LOH probability distributions. The chromosome number is indicated for both graphs on the x-axis. E1–E4 represent 4 blastocysts from the patient. E1 and E4 were normal/balanced embryos. E2 and E3 were abnormal embryos. E2 was a monosomy 6 (arrow) unrelated to Robertsonian translocation. The SNP array result was arr6p25.3q27 (119,769-170,716,684) × 1. E3 was a monosomy 13 (arrow). The SNP array result was arr13q12.11q34 (17,960,319-114,092,980) × 1. The monosomy could be reflected by a shift in the LOH probability of the SNPs toward 1 on the graph (i.e., complete LOH).

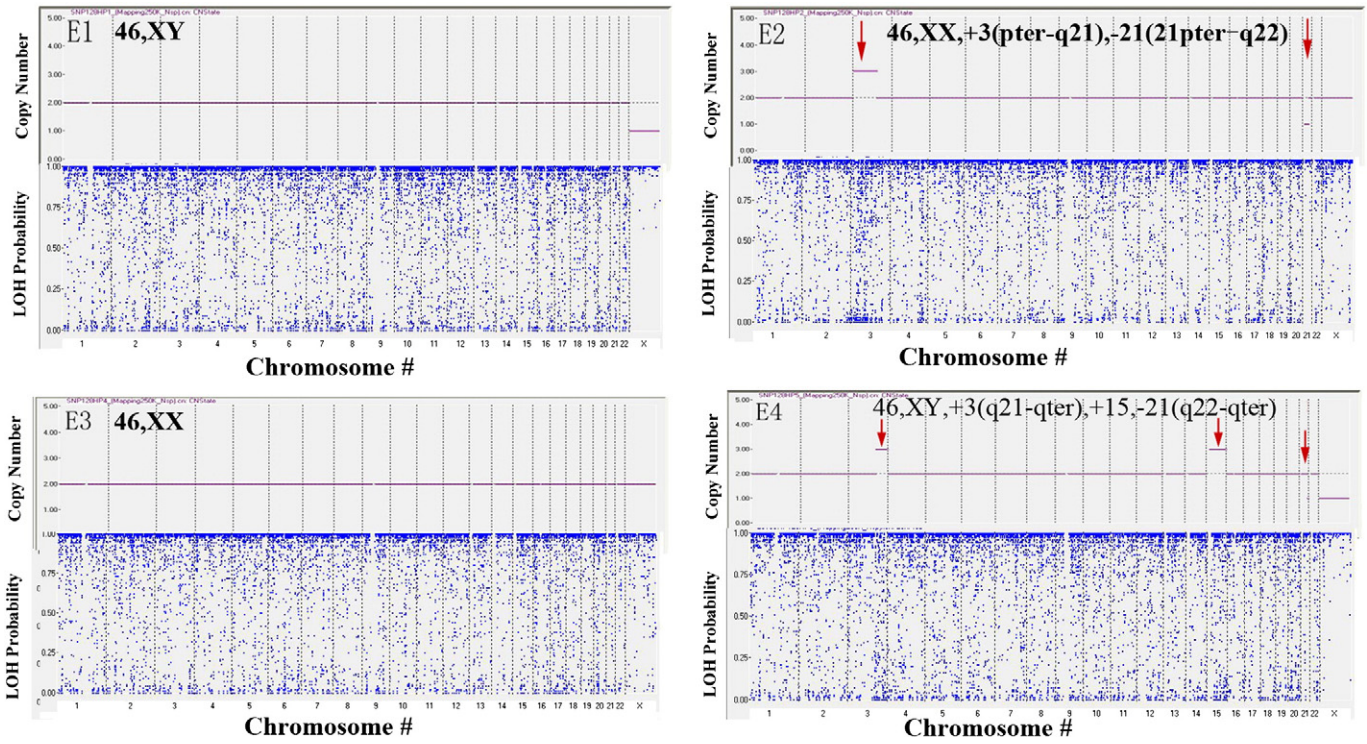

Fig. 3. An example of SNP-PGD for one reciprocal translocation carrier with a karyotype of 46,XY,t(3;21)(q21;q22). E1 and E3 were normal/balanced embryos. E2 was a partial trisomy 3p26.3q21 and partial monosomy 21p11.2q22 (arrow). The SNP result of E2 was arr3p26.3q21 (48,602-121,264,951) × 1,21q10q22.11 (13,298,815-31,355,081) × 1. E4 carried abnormalities including partial trisomy 3q21q29, partial monosomy 21q22.12q22.3 and unrelated trisomy 15 (arrow). The SNP result of E4 was arr3q22.3q29 (138,633,205-199,318,809) × 3,(15) × 3,21q22.12q22.3(43,640,359-46,894,358) × 1. The partial monosomy could be reflected by a shift in the LOH probability of the SNPs toward 1 on the graph and the partial trisomy could be reflected by a greater frequency and distribution of all probes between 0 and 1 in the LOH probability graph.

Conflict of interest

No potential conflicts of interest were disclosed by the authors.

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