Diagnosis of parental balanced reciprocal translocations by trophectoderm biopsy and comprehensive chromosomal screening

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Abstract

Purpose This study investigates a case series of eight couples who underwent trophectoderm (TE) biopsy and comprehensive chromosomal screening (CCS) for routine aneuploidy screening and were found to have CCS results concerning for previously undetected parental balanced reciprocal translocations.

Methods In each case, controlled ovarian hyperstimulation and in vitro fertilization (IVF) yielded multiple blastocysts that each underwent CCS with high-density oligonucleotide microarray comparative genomic hybridization (aCGH).

Results Parental translocations were suspected based on the finding of identical break point mutations in multiple embryos from each couple. Confirmation of these suspected translocations within blastocysts was performed with next-generation sequencing (NGS). Subsequent parental karyotypic evaluation resulted in a diagnosis of parental balanced reciprocal translocation in each case.

Conclusions We demonstrated that high-resolution aCGH and NGS on TE biopsies can accurately detect parental reciprocal translocations when previously unrecognized.

Keywords Reciprocal translocation · Comprehensive chromosomal screening (CCS) · Preimplantation genetic screening (PGS) · Blastocyst trophectoderm biopsy · Recurrent pregnancy loss (RPL)

Introduction

Preimplantation genetic screening (PGS) has been utilized for over 20 years with the goal of improving outcomes in assisted reproductive technologies. Comprehensive chromosomal screening (CCS) evaluates all 23 pairs of autosomes and sex chromosomes to determine if an embryo is chromosomally balanced, with the ultimate goal of identifying embryos with the highest implantation potential. CCS may be of particular benefit with embryo selection for transfer in patients with a poor prognosis due to advanced maternal age, previous implantation failures, or recurrent pregnancy loss (RPL) [1–6].

Individuals with infertility or RPL are at higher risk for having a balanced translocation compared to the general population. This is independent of age with 0.5–5% of couples with reproductive issues carrying a balanced translocation [7–10]. Carriers of balanced translocations are most often identified by parental karyotyping or by genetic analysis of products of conception after embryonic demise. Affected couples may elect for IVF with CCS to expedite the timeline to a successful pregnancy by selecting normal/balanced embryos for transfer.

Array comparative genomic hybridization (aCGH) was the first technology to become widely available for CCS [1]. Next-generation sequencing (NGS) is a newer technology that is increasingly being used for CCS [11]. Both aCGH and NGS are able to detect chromosomal abnormalities such as deletions, duplications, and sex chromosomal aberrations. Despite these advances, the technologies to date have been

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limited in their ability to distinguish an embryo that is a carrier of a balanced translocation from one that is truly normal in embryos derived from translocation carrier parents because these technologies describe the relative amount of chromosomal material present, but not the arrangement [12, 13].

In an attempt to detect balanced translocations, translocation breakpoint-specific and closely flanking fluorescence in situ hybridization (FISH) probes have been used to detect both structural and numerical aberrations in either interphase cells or in polar bodies in the past [14, 15]. The design and optimization of these patient-specific FISH probes is time-consuming and additionally requires the translocation breakpoint to be known prior to testing. More recently, Treff et al. reported that single-nucleotide polymorphism (SNP) array-based CCS can distinguish normal from balanced translocation carrier embryos [13]. However, parental DNA and at least one unbalanced IVF embryo were necessary to make this diagnosis. Breakpoints were not identified with the technology, so the genetic risk of the carrier is unable to be evaluated.

In sum, there are no effective methods for all carrier couples to screen and identify breakpoints or markers for both carrier embryo diagnosis and genetic risk evaluation. We, however, describe a case series of eight infertile couples attempting IVF, where the presence of a parental balanced reciprocal translocation was suspected due to abnormalities detected after trophectoderm (TE) biopsy and CCS with both aCGH and NGS. All couples had a previously undiagnosed or undetected balanced reciprocal translocation that was later confirmed by parental karyotypic evaluation.

Materials and methods

Study subjects

After obtaining approval from the Institutional Review Board of the University of California, Los Angeles, patients' data were reviewed and analyzed from August 2013 to January 2017. All patients undergoing IVF with TE biopsy for CCS were eligible for the study.

Ovarian stimulation, insemination, and embryo biopsy

Controlled ovarian hyperstimulation was performed using recombinant follicle-stimulating hormone (Gonal F®—Serono, Geneva, Switzerland, or Follistim®—Merck, Kenilworth, New Jersey, USA) and human menopausal gonadotropin (Menopur®—Ferring Pharmaceuticals, New Jersey, USA). A gonadotropin-releasing hormone (GnRH) agonist suppression protocol (short or long) or GnRH antagonist flexible protocol according to ovarian reserve and anti-müllerian hormone values were utilized. Once the lead follicle reached a minimum mean diameter of 18 mm, final oocyte maturation was

achieved with human chorionic gonadotropin (hCG) subcutaneously (3300–10,000 IU) or a combination of hCG 1000 IU and leuprolide acetate 1 mg subcutaneously. Oocyte retrieval was performed 35–36 h after hCG injection, and all metaphase II oocytes underwent intracytoplasmic sperm injection.

Embryos were grown in Quinn's Advantage Plus Cleavage media until they reached the blastocyst stage. Assisted hatching was performed on day 5 or 6 prior to TE biopsy with a non-contact 1.48-μ diode laser to create a circular 6- to 9-μ-diameter opening in the zona pellucida. Blastocyst grading was performed based on the Gardner and Schoolcraft criteria [16]. On the day of biopsy, 5–10 TE cells were aspirated with a biopsy pipette (inner and outer diameters 35 and 49 μ, respectively; COOK Ireland Ltd.; Limerick, Ireland) and the specimen cleaved from the embryo via laser. The TE cells were washed in sterile phosphate-buffered saline solution (PBS), placed into microcentrifuge tubes containing 2 μl PBS and sent for CCS (PacGenomics Inc., Aguora Hills, California, USA).

Comprehensive chromosomal screening

For whole-genome amplification, TE cells were first lysed and genomic DNA was randomly fragmented and amplified using Repli-G single cell kit (Qiagen; Hilden, Germany). This method was selected to ensure the highest possible genome coverage, ranging from 84 to 99% of the genome [17, 18]. The DNA was then quantified by the broad-range DNA assay kit Qubit (London, UK). After amplification, DNA was labeled, purified, and hybridized for aCGH performed overnight. For each sample, two negative controls (one male control, one female control) underwent the same treatment.

Amplified DNA was assessed for chromosome number using a previously validated high-density oligonucleotide aCGH (Agilent Technologies; Santa Clara, California, USA) [19]. Briefly, whole-genome amplification products were fluorescently labeled with Cy3 using SureTag DNA-labeling kit (Agilent Technologies). Labeled samples were then mixed with Cy5 control-labeled samples. The labeled samples and controls were purified with SureTag DNA-labeling purification column (Agilent Technologies), dried and dissolved in hybridization buffer containing Cot-1 DNA, 10× aCGH blocking agent, and 2× HI-RPM Hybridization buffer (Agilent Technologies). Samples were then loaded onto SurePrint G3 human CGH 8 × 60K Oligo Microarray (Agilent Technologies). After overnight hybridization at 65 °C, microarrays were washed and then scanned with SureScan Microarray Scanner (Agilent Technologies) at 3 μM. Images obtained were analyzed by modified Cytogenomics software (Agilent Technologies). Bioinformatics were performed with the same software, and the normalized ratio of each sample versus the control was retrieved following Agilent CGH data analysis protocol.

NGS was performed to determine if the newer NGS technology detected similar results as aCGH, using the Illumina NGS platform (San Diego, California, USA) with 150 cycles DNA sequencing. Modified Nexus Copy Number software (Illumina) was used for NGS processing with call thresholds set at 50 kilobases.

Breakpoint detection

In our laboratory, aCGH testing features are nearly identical to the testing features of NGS with 1 million reads per sample. In terms of resolution, segmental abnormalities larger than 3.5 megabases were reported in both aCGH and NGS platforms. Aberrations below this size were below the limits of detection in validation studies and thus were unable to be clinically reported. All reported cases were identified based on judgment calls. An inherited translocation was suspected when (1) at least two embryos of partial aneuploidy shared the exact same breakpoint(s); (2) the breakpoint call log₂ values were close to + 0.59 (gain)/− 1.0 (loss) to show no or very low mosaicism and signifying that the likely inherited translocation was most likely non-mosaic; and (3) multiple embryos shared the same whole chromosome loss/gain. Once the laboratory identified a suspected translocation, the referring provider was notified for further parent evaluation.

Parental karyotype

Parental karyotypes were performed on couples with a history of RPL as part of a routine workup. In cases where blastocyst CCS results were suggestive of balanced translocation and no previous karyotype was performed due to no suggestion of a karyotypic abnormality such as RPL noted in the history, parental karyotyping was performed. In one case where a prior karyotype was performed and noted as normal, a repeat karyotype with attention to the specific breakpoint mutation was obtained. Karyotyping was performed using peripheral blood from the parent couples. Metaphase chromosomes were inspected by G banding with at least 20 metaphases analyzed for each patient.

Results

Eight patient couples from the study cohort were identified as having a recurring identical breakpoint mutation in their embryos via CCS, raising suspicion for a parental balanced translocation. A total of 1847 CCS cases were performed during the same time period, excluding previously known translocation cases, indicating an incidence of just over 0.4% of cases undergoing PGS for routine aneuploidy screening. The patients ranged in age from 28 to 38 years and male partners from 35 to 39 years. Patients elected for CCS and had a history

of infertility or RPL (Table 1). A translocation was suspected when a segmental abnormality was detected with the identical chromosomal breakpoint mutation in more than one embryo (Fig. 1). Of these cases, a total of 87 embryos were evaluated from 14 IVF cycles. Twenty distinct breakpoint mutations were identified (Table 2). When a recurring breakpoint mutation was identified suggestive of a translocation, NGS testing was performed to replicate and validate the aCGH findings. In all cases, the high-density oligonucleotide aCGH and NGS results were identical.

The presence of a parental balanced reciprocal translocation was confirmed in one parent in all cases. In one case (patient 2), a conventional karyotype had been performed prior to IVF and was reported as normal in both the male and female. After the CCS results for this couple were reviewed, the karyotypes were repeated with special attention to the breakpoint noted in the embryos. The resultant karyotype, 46 XX t(5, 17)(q32q23), confirmed the female patient as a translocation carrier.

Discussion

To our knowledge, this is the largest study describing identification of previously undetected translocations via TE CCS. Two CCS methods were utilized (high-density oligonucleotide aCGH and NGS) with identical findings, demonstrating that both technologies have the potential to identify these aberrations. Because translocations occur as a result of a double break in two different chromosomes and exchange of fragments between the chromosomes, identification of recurrent breakpoint mutations in multiple embryos appears to be a suitable method for detection of a parental translocation. In each couple presented, multiple blastocysts with the same chromosomal breakpoint mutation were identified leading to suspicion of a translocation in one of the parents. Subsequent parental karyotyping corroborated a balanced translocation carrier.

Table 1 Patient characteristics

Couple	Female age	Male age	Diagnosis
1	35	Unavailable	RPL
2	32	39	RPL
3	32	35	Secondary infertility
4	38	38	Primary infertility—PCOS
5	28	36	Primary infertility—PCOS
6	34	35	Unavailable
7	34	35	Primary infertility—male factor
8	34	35	Unavailable

RPL, recurrent pregnancy loss; PCOS, polycystic ovary syndrome

Fig. 1 An example of a patient's PGS results with the recurrent breakpoint of 6q23.2q27

#	Embryo Grade	NGS Result	PGS Result	Interpretation	Notes
1	5AA		46, XY	Euploidy	
2	5AA		46, XX	Euploidy	Dup of 6q23.2q27
3	5AA		45, XY, -13	Aneuploidy: monosomy 13	
4	5AA		46, XY	Euploidy	
5	5AB		46, XX	Euploidy	Del of 6q23.2q27
6	3BB		46, XX, -3, +4, +8, -16	Multiple chromosomal abnormalities.	Dup of 6q23.2q27
7	5AB		Female	Multiple chromosomal abnormalities.	
8	3BB		49, XY, +15, +17, +21	Aneuploidy: trisomy 15, 17, 21	Del of 6q23.2q27
9	3BB		46, XX	Euploidy	

Treff et al. [20] initially described the ability of CCS to identify a balanced translocation using SNP microarray or qPCR-based CCS technology in three patient couples. Due to the increased sensitivity of high-density oligonucleotide

Table 2 Summary of breakpoints detected and translocation carrier karyotypes

Couple	Number of blastocysts analyzed	Breakpoints	Carrier karyotype
1	29	6q14.1q27 13q14.11q34 6q12q14.1	46 XX t(6, 13) (q13;q14.1)
2	6	5qter 17qter	46 XX t(5, 17)(q32q23)
3	9	10q26.13q26.3 13q31.3q34 10q11.21q26.13 13q12.11q31.1	46XX t(10, 13)(q26.1;q31.2)
4	8	3p26.3p24.3 4p16.3p15.31 3p24.3p11.1 4p15.1p11	46 XY t(3, 4)(p25; p16.1)
5	11	4pter	46 XX t(4, 9)(q13; q32)
6	10	4q11q35.2	Translocation confirmed base on breakpoint ^a
7	5	4p13p16.3	Translocation confirmed base on breakpoint ^a
8	9	6p23.2q27	Translocation confirmed base on breakpoint ^a

ter, terminus

^a Translocation confirmed via verbal report, exact karyotype unavailable

aCGH and NGS, the ability to detect balanced translocation carriers may be even greater, as demonstrated with the patient couple that had a previously false-normal karyotype; these newer technologies may provide added sensitivity to detect translocations beyond routine chromosomal evaluation.

A recent analysis estimates the ability of CCS to detect any segmental imbalance in both cleavage state and blastocyst biopsies in the range of 4 to 58% [21]. The limitation of these technologies lies in the level of resolution of detection, where the greater the resolution of the technology used, the more likely the detection of an abnormality that is present within the cells tested. Even if a high-resolution method was utilized, balanced translocation carriers can produce embryos with unbalanced chromosomes resulting in sub-chromosomal copy number changes, or de novo segmental imbalances that alter the size of the chromosomal imbalance potentially below the limit of detection. A more recently recognized phenomenon, embryonic mosaicism, adds another layer of complexity, as mosaic embryos resulting from mitotic cell division errors and can lead to a mix of chromosomally normal cells and abnormal cells with a loss or gain of chromosomal material that could influence the CCS result.

The long-proposed interchromosomal effect, where translocated chromosomes influence synapses and disjunction of other chromosomes leading to increased incidence of aneuploidy in chromosomes unrelated to the translocation, should also be noted [22]. The data presented here are consistent with more recent CCS studies that fail to validate this association [9]. Multiple studies have shown that live birth rates are increased, and miscarriage rates are decreased using CCS with

subsequent transfer of euploid/balanced embryos. Clinical pregnancy rates range from 60 to 70% after transfer of a euploid/balanced embryo, which is equivalent to euploid embryos produced from non-translocation carriers [23, 24].

In conclusion, previously undetected parental translocations can be identified when recurrent breakpoint mutations are detected in multiple embryos from the same cohort using either high-density oligonucleotide aCGH or NGS. Use of CCS with these latest technologies can detect reciprocal translocations even with previously errantly normal parental karyotypes. There does not appear to be an increase in aneuploidy above the age baseline, and with properly selected embryos, clinical pregnancy rates approach that of non-translocation carriers.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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