Complex chromosomal rearrangement—a lesson learned from PGS

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Abstract

Purpose The aim of the study is to report a case of non-diagnosed complex chromosomal rearrangement (CCR) identified by preimplantation genetic screening (PGS) followed by preimplantation genetic diagnosis (PGD) which resulted in a pregnancy and delivery of healthy offspring.

Methods A 29-year-old woman and her spouse, both diagnosed previously with normal karyotypes, approached our IVF-PGD center following eight early spontaneous miscarriages. PGS using chromosomal microarray analysis (CMA) was performed on biopsied trophectoderm. Fluorescence in situ hybridization (FISH), as well as re-karyotype, were performed on metaphase derived from peripheral blood of the couple. Subsequently, in the following PGD cycle, a total of seven blastocysts underwent CMA.

Results A gain or loss at three chromosomes (3, 7, 9) was identified in six out of seven embryos in the first PGS-CMA cycle. FISH analysis of parental peripheral blood samples demonstrated that the male is a carrier of a CCR involving those chromosomes; this was in spite of a former diagnosis of normal karyotypes for both parents. Re-karyotype verified the complex translocation of 46,XY,t (3;7;9)(q23;q22;q22).

Subsequently, in the following cycle, a total of seven blastocysts underwent PGD-CMA for the identified complex translocation. Two embryos were diagnosed with balanced chromosomal constitution. A single balanced embryo was transferred and pregnancy was achieved, resulting in the birth of a healthy female baby.

Conclusions PGS employing CMA is an efficient method to detect unrevealed chromosomal abnormalities, including complicated cases of CCR. The combined application of array CGH and FISH technologies enables the identification of an increased number of CCR carriers for which PGD is particularly beneficial.

Keywords Complex chromosomal rearrangement (CCR) · Chromosomal microarray analysis (CMA) · Preimplantation genetic screening (PGS) · Preimplantation genetic diagnosis (PGD)

Case report

Preimplantation genetic screening (PGS) is a procedure for aneuploidy screening aimed at selecting euploid embryos and improving implantation and pregnancy outcome following in vitro fertilization (IVF) when the biological parents are presumed to be chromosomally normal. Indications for PGS include infertile couples with previous failed IVF cycles, recurrent pregnancy loss, advanced maternal age, and men with severe male factor. These patients are considered to be at relatively high risk for aneuploid embryos, and they may choose PGS as a treatment option in order to select only the euploid embryos for transfer. PGS can also be offered when single embryo transfer is recommended. In some clinics, PGS is

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offered in combination with preimplantation genetic diagnosis (PGD) when there is a genetic indication [1].

Currently, chromosomal microarray analysis (CMA) is the most popular technology for PGS, a molecular cytogenetic-based method that allows the analysis of all 24 chromosomes demonstrating copy number changes (gain/loss of chromosomal segments). PGS is performed on five to ten trophoctodal cells biopsied from day 5 blastocysts. Blastocysts have a significant rate of mosaicism but a relatively lower rate compared to cleavage-stage embryos [2, 3]. The biopsied cells undergo whole genome amplification (WGA) and array comparative genomic hybridization (array CGH) [4–7].

Embryonic chromosomal aberration is the main cause (~67%) for spontaneous miscarriages [8]. In our center, PGS-CMA analysis in the setting of IVF is offered to couples with greater than four miscarriages and couples with good quality embryos that have experienced greater than five repeated implantation failures, and PGD-CMA is offered to couples with a genetic indication that are carriers of known chromosomal rearrangements, including translocations, insertions, and deletions. Approximately 5% of couples that experience recurrent spontaneous abortions reportedly carry balanced translocations between two non-homologous chromosomes [9]. These individuals are known to have high rates of unbalanced gametes following meiotic segregation, resulting in embryos with abnormal chromosomal constitution. PGD for translocations carriers minimize the risk for implantation of a chromosomally abnormal embryo, thus significantly lowering the risk for pregnancy loss [10–13].

In this report, we describe a unique case of a couple with a history of eight spontaneous miscarriages in which a complex chromosomal rearrangement (CCR) was not detected by classical cytogenetic techniques and which was diagnosed by PGS-CMA, leading to the birth of a healthy child following PGD for a complex translocation carrier.

The report refers to a 29-year-old female and her spouse who presented to our center following a history of eight spontaneous miscarriages. All their previous pregnancies were lost early in the first trimester, and none of the fetuses had been analyzed chromosomally. Both partners had a normal G-banding karyotype. Under the assumption that most of the repeated miscarriages were a result of aneuploid embryos, the couple was offered PGS by CMA.

An IVF protocol using GnRH analogue (Decapeptyl; Ferring Kiel, Germany) and human menopausal gonadotropin (Gonal F; Serono, Aubonne, Switzerland) for controlled ovarian stimulation yielded a total of 22 oocytes. Twenty mature oocytes (MII) were fertilized by intracytoplasmic sperm injection (ICSI), resulting in 18 embryos (82% fertilization rate). The embryos were grown in a continuous single culture complete medium with human serum albumin (CSCM-C, Irvine

Scientific, Santa Ana, CA, USA) in 5% O₂/5%CO₂ conditions in an embryoscope time-lapse microscope (Vitrolife, Göteborg, Sweden) until day 5. Seven embryos developed to top-quality full blastocysts on day 5, and five to ten trophoctodermal cells were biopsied from each of them by means of laser micromanipulation (Zilos; Hamilton Thorne Biosciences, Beverly, MA). The biopsied cells were lysed, and their DNA was subjected to WGA using the SurPlex kit (BlueGnome, Cambridge. Cat. 415101-00). Amplified DNA samples from each embryo and male reference DNA were fluorescence labeled (green for the samples and red for the control male) and co-hybridized to 24sure+ array (BlueGnome, Cambridge. cat. 408602-PK). DNA chip arrays were washed, scanned, and analyzed by BlueFuse Multi software (BlueGnome, Cambridge) according to the manufacturer's protocol.

All seven embryos were found to be chromosomally abnormal. A gain or loss at chromosomes 3, 7, and 9 was demonstrated in different combinations in six of them (Table 1, Fig. 1). These findings let us to hypothesize that one of the partners is a carrier of a balanced CCR involving these three chromosomes, in spite of the fact that both partners initially presented with a normal karyotype. It is well accepted that although CMA can detect unbalanced chromosomal changes, it is unable to detect structural chromosome aberrations, such as balanced translocation. For that reason and in order to confirm the presence of CCR, we performed fluorescence in situ hybridization (FISH) analysis on peripheral blood of both partners. We used specific probes for chromosomes 3, 7, and 9, which were designed to detect segments on both sides of the predicted chromosomal break in each tested chromosome [Cep 9 SpectrumOrange (Vysis Cep9), Cep 7 SpectrumAque (D7Z1), Cep 3 SpectrumOrange (D3Z1); Abbott, USA Medical Laboratories and Subtelomere 9q SpectrumGreen (RP11-112N13), Subtelomere 3q SpectrumGreen (RP11-196F4), Subtelomere 7q SpectrumRed (RP11-2000A5); Cytocell, Cambridge]. The probes were hybridized with metaphase plates derived from their lymphocytes, as described by us elsewhere [14]. Briefly, extracted lymphocytes were stimulated to proliferate using phytohemagglutinin-M (PHA-M, Biological Industries, Beit Haemek, Israel, 0.2/5 ml peripheral blood karyotyping medium) for 72 h, and metaphase spreads were prepared using 0.2 ml of colcemide solution (10 µg/ml, Biological Industries, Beit Haemek, Israel) following standard cytogenetic techniques. FISH analysis was performed using probes as described above [14].

FISH results on peripheral blood demonstrated that the male partner carried an apparently balanced CCR with a triple chromosomal translocation, 46,XY, ish t(3;7;9)(q23;q22;q22)(RP11-196F4-, RP11-112N13+, RP11-2000A5-, RP11-196F4+; RP11-112N13-, RP11-2000A5+) [15], involving chromosomal fragments with relatively similar sizes and banding (Fig. 2). This may clarify why this chromosomal aberration was overlooked

Table 1 Results of all embryos analyzed in the first CMA cycle

Embryo ID	Result
1	Unbalanced (−3s, +9s)
2	Unbalanced (−7s, −16, +9s)
3	Unbalanced (−9s, +7s, +22)
8	Abnormal (−8)
12	Unbalanced (−9s, +3s)
14	Unbalanced (−7s, +3s, +22)
16	Unbalanced (−3 s, +7s)

s structural aberration

in the prior karyotype analysis. The male partner underwent repeated karyotype analysis, which identified a three-way translocation: 46,XY,t(3;7;9)(q23;q22;q22) (Fig. 3), confirming the CCR that had been diagnosed in their embryos but failed to be detected in his previous karyotype. This subsequent analysis was done from standard 72-h lymphocyte culture and G banding was achieved by the trypsin-Giemsa method as describe previously [16]. The resulted karyotype resolution was estimated as a 550-band level and metaphases were analyzed for less condensed chromosomal karyotype. The male partner underwent CMA to ensure that there was no loss of genetic material and that the 3-way translocation was indeed balanced.

Following the acquisition of this new information, the couple now had a genetic indication for treatment and was thus offered an IVF cycle in which PGD for chromosomal translocation (rather than PGS) would be performed by CMA in order to select euploid embryos for transfer.

Twenty oocytes were retrieved in the ensuing IVF-PGD cycle. ICSI was performed and resulted in 15 embryos (a 75% fertilization rate). Trophectoderm biopsy was performed on seven good quality blastocysts on day 5, and the biopsied embryos were subsequently frozen. DNA from trophectodermal samples was subjected to WGA and CMA as described above (Fig. 4). Two embryos were found to be chromosomally balanced and suitable for transfer. Five additional embryos were determined to be unbalanced due to aberrant chromosomal segregation in the paternal gamete caused by the CCR (Table 2).

The two euploid embryos were transferred in two subsequent frozen IVF cycles. Pregnancy was achieved in the second cycle. Following the PGD cycle, the couple was referred once more for genetic consultation. The rare possibilities of UPD of chromosome 7, as well as misdiagnosis, were discussed with the couple, and confirmation of the PGD results by amniocentesis was recommended according to our lab standards. Having suffered many miscarriages, they refused any further invasive procedure during the pregnancy. A 3195 grams female baby was born at week 39 of pregnancy. The child is reportedly healthy and normal.

Discussion

PGS-CMA is an invasive procedure and it does not necessarily improve the pregnancy rates during IVF retrieval compared to cumulative transfer of all embryos through multiple frozen embryo transfers [1]. Proponents of PGS claim that this procedure will shorten the time to conception and that it will decrease the miscarriage rate per pregnancy. Opponents of PGS claim that there is not sufficient evidence to justify broad application of this technique [1–3, 17, 18].

Fig. 1 Representative figure of CMA results of abnormal embryos that were diagnosed by PGS. The *green lines* demonstrate a gain of chromosomal regions and *red lines* demonstrate a loss of chromosomal regions

Fig. 2 The FISH results on proliferating leukocytes isolated from peripheral blood of the male partner demonstrate a balanced CCR involving a triple chromosomal translocation [46,XY, ish t(3;7;9)(q23;q22;q22)(RP11-196F4-, RP11-112N13+, RP11-2000A5-, RP11-196F4+, RP11-112N13-, RP11-2000A5+)]. Fluorescence probes for three chromosomal regions were used

in two rounds of hybridizations: CEP 9 (*orange*; Vysis Cep9), CEP 7 (*aqua*; D7Z1), and TEL 9q (*green*; RP11-112N13); CEP 3 (*orange*; D3Z1), TEL 3q (*green*; RP11-196F4), and TEL 7q (*red*; RP11-2000A5). *Chr* chromosome (Chr 3; Chr 7; Chr 9), *Der* derivative, which is a product of the chromosomal break and translocated (Der 3; Der 7; Der9)

One of the indications for CMA is couple carriers of known chromosomal rearrangements, mostly translocations, as part of a PGD program. Chromosomal translocations are caused by exchange of genetic material between two different chromosomes due to aberrant chromosomal breaks and repairs. CCRs are structural abnormalities involving several chromosome breakpoints with reciprocal exchanges of three or more segments. CCRs can include inversions, insertions, or deletions, but the great majority of the cases involve compound translocations, mostly three-way translocations. CCRs are rare events, which can be inherited or acquired *de novo* during gametogenesis or early embryonic development. To date, approximately 255 cases of CCRs involving three or more

chromosomes have been reported, including only a few cases of successful pregnancy outcome [19].

Carriers of apparently balanced CCRs are considered to be at extremely high risk for spontaneous miscarriage or chromosomally abnormal offspring as a result of unbalanced chromosomal segregation of hexavalent or even more complex configuration at meiosis I. Nonaka et al. [9] estimated that the frequency of balanced reciprocal translocation involving three chromosomes is about 0.1% (two cases out of 1415 couples that underwent chromosome analysis) among a population with a history of recurrent spontaneous miscarriages. Only six cases of PGD that were analyzed by FISH technique for carriers of CCRs were reported in the literature [20, 21]. Each

Fig. 3 **a** Re-karyotyping for the male partner demonstrates CCR 46,XY,t(3;7;9)(q23;q22;q22). The *red arrows* point at derivatives 3, 7, and 9. **b** A schematic presentation of the balanced CCR of the male partner. *Chr* chromosome, *der* derivative

Fig. 4 **a** A representative of PGD-CMA results (24sure⁺ microarray). **b** A schematic presentation of an unbalanced embryo carrying a loss of 3q23 → 3qter in *red* and a gain of 7q22 → 7qter in *green*. (Gain of X in *green* and loss of Y in *red* demonstrate a female constitution compared to a male reference)

of these couples had a history of repeated pregnancy loss. Among them, only three cases included three-way translocation CCR. In those three cases, a total of 43 embryos were biopsied through seven PGD-FISH cycles, and 40 embryos

Table 2 Results of all embryos analyzed in the second CMA cycle

Embryo ID	Result
1	Unbalanced (−9s, +7s)
3	Unbalanced (+3s, +7s)
4	Unbalanced (−7s, +3s)
6	Unbalanced (+3s, +7s)
9	Balanced
16	Unbalanced (−3s, +7s)
17	Balanced

s structural aberration

underwent diagnosis, resulting in the detection of three balanced embryos. Combining this information with our results (two balanced embryos out of a total 14 diagnosed during two PGS/PGD cycles), we estimate that the chance for balanced embryos for CCR carriers is approximately 9.25% (5/54), compared to a 20–30% chance of balanced embryos in cases of reciprocal translocation between two chromosomes [21]. The couples should be made aware of this extremely low rate of chromosomally balanced embryos in CCRs, and it should be taken into consideration when planning a controlled ovarian stimulation cycle.

In the reported case, CCR carrier status was detected after PGS-CMA, in spite of an apparently normal karyotype. The comparable sizes of all three exchanged fragments (~50 MB) probably contributed to the difficulty in arriving at the correct diagnosis. A second karyotype with a resolution of the 550-

band level done in our center focused on the chromosomes that according to the CMA results were suspected to be involved, and it corroborated the three chromosomal translocations even in G-banding technique. In conclusion, our case demonstrated the additive value of PGS-CMA in detecting unbalanced chromosomal abnormalities which may be below the resolution of the classical chromosomal karyotype analysis technique. Given that high-resolution CMA will detect only unbalanced chromosomal changes, FISH and conventional karyotyping analyses nevertheless remain essential for delineating balanced CCR carriers. The combined application of array CGH technologies and FISH enables the identification of an increased number of CCRs, and PGD is particularly beneficial for these carriers.

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