

# Healthy Baby Born to a Robertsonian Translocation Carrier following Next-Generation Sequencing-Based Preimplantation Genetic Diagnosis: A Case Report

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## Abstract

Preimplantation genetic diagnosis (PGD) is well established method for treatment of genetic problems associated with infertility. Moreover, PGD with next-generation sequencing (NGS) provide new possibilities for diagnosis and new parameters for evaluation in, for example, aneuploidy screening. The aim of the study was to report the successful pregnancy outcome following PGD with NGS as the method for 24 chromosome aneuploidy screening in the case of Robertsonian translocation. Day 3 embryos screening for chromosomal aneuploidy was performed in two consecutive in vitro fertilization (IVF) cycles, first with fluorescent in situ hybridization (FISH), and then with NGS-based protocol. In each IVF attempt, three embryos were biopsied. Short duration of procedures enabled fresh embryo transfer without the need for vitrification. First IVF cycle with the embryo selected using PGD analysis with the FISH method ended with pregnancy loss in week 8. The second attempt with NGS-based aneuploidy screening led to exclusion of the following two embryos: one embryo with 22 monosomy and one with multiple aneuploidies. The transfer of the only euploid blastocyst resulted in the successful pregnancy outcome. The identification of the euploid embryo based on the NGS application was the first successful clinical application of NGS-based PGD in the case of the Robertsonian translocation carrier couple.

## Keywords

- ▶ aneuploidy screening
- ▶ next-generation sequencing
- ▶ preimplantation genetic diagnosis
- ▶ Robertsonian translocation

Robertsonian translocations are classified as the most common balanced structural chromosomal rearrangements in the human population occurring with the frequency of approximately 1 in 1,000 newborns.<sup>1</sup> The majority of Robertsonian translocation cases involve two different acrocentric

chromosomes. Carriers of heterologous Robertsonian translocations are diagnosed more frequently among the infertile couples (especially in oligospermic males) than in healthy population.<sup>2</sup> Because of the well-known risk of unbalanced conceptions in carriers of Robertsonian translocations, the

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male carriers are the candidates for intracytoplasmic sperm injection (ICSI)-IVF along with preimplantation genetic diagnosis (PGD).<sup>3,4</sup>

Previously published cases of PGD concerning Robertsonian translocation carriers usually involved the diagnosis of ploidy status using the multicolor FISH method. However, the reports of imbalances of other embryo chromosomes in such cases<sup>5</sup> indicated the need for broadening the analysis to include additional chromosome copy number abnormalities. The next-generation sequencing (NGS) has been used routinely in various PGD protocols at INVICTA Fertility Clinic since August 2013. This report presents NGS-based PGD application in the case of a Robertsonian translocation carrier.

## Patients and Methods

In 2011, a couple with 5 years of primary infertility (females 31 and males 46) was seeking treatment at the INVICTA Fertility Clinic. In 2009, male infertility factor with severe oligospermia was diagnosed and determined as the reason of infertility.

The couple underwent standard clinical investigation and all the hormonal results were normal including AMH (anti-Mullerian hormone) at 2.4 ng/mL (AnshLabs I generation assay) and unchanged female karyotype. Male karyotype was abnormal with Robertsonian translocation 45,XY,der(14;15)(q10;q10). The couple's decision to undergo IVF was followed by genetic counseling regarding PGD options and presentation of success rates, risk of misdiagnosis, and possible genetic, clinical, and social outcomes. Because of the low risk of father-to-child transmission in case of father being Robertsonian heterozygote, the couple decided not to perform PGD directed to 14 and 15 uniparental disomy.<sup>1</sup>

Translocation carrier state is associated with high risk of aneuploidy in offspring, therefore, we proposed FISH analysis for fused chromosomes 14q and 15q. A written consent form, in which a possible risk of the in vitro procedure and PGD misdiagnosis was stated, was obtained. In addition, confirmatory prenatal diagnosis was recommended for each pregnancy achieved after PGD.

The IVF program, according to the standard long protocol used at INVICTA Fertility Clinic started in January 2012.<sup>6</sup> The female was stimulated with 225 IU of Menopur (Ferring Pharmaceuticals, Saint Prex, Switzerland) daily for 9 days. We received 12 cumulus oocyte complexes with 6 metaphase II cells. Blastomeres biopsies from three embryos (6C each) were performed on day 3. Laser technology (Anritsu 1488 nm in Saturn 3 Research Instruments, Falmouth, UK) was used to create an opening in each zona pellucida and one blastomere was gently aspirated from each embryo.

After the biopsy, each embryo was washed, transferred to G2 medium (Vitrolife, Sweden), and cultured for 2 more days. The used FISH probes included LSI (Abbott Molecular, Chicago, IL) and TelVysion probes (unique DNA sequence probes, LSI) specific for investigated chromosomes, which were purchased from Vysis Inc. (Abbott Molecular, Chicago, IL) and Kreatech (Kreatech Diagnostics, Netherlands). The specificity and sensitivity of the probes had previously

been tested using patient lymphocyte cultures. The probes had specificity of 100% and efficiency of 84 to 95%. Slides of the biopsied blastomeres were prepared as described by Coonen et al.,<sup>7</sup> and probe mixtures were prepared according to the manufacturer's protocol. FISH signals were independently scored and interpreted by two technologists. The euploid blastocyst with morphology of 2 BC was transferred on day 5 of the culture. Pregnancy was confirmed 11 days later—hCG 94 mIU/mL. The pregnancy ended with miscarriage in week 8. Patients did not take advantage of cytogenetic investigation of the material from the miscarriage.

During the second IVF cycle, patients decided to perform diagnosis of translocation and aneuploidy of all 24 chromosomes simultaneously with the use of new NGS-based PGD method. In September 2013, the ICSI procedure and the embryo culture with preparation for PGD were performed as described earlier. Three embryos obtained in second IVF cycle were biopsied on day 3. Cells were transferred into thin-walled 0.2 mL polymerase chain reaction (PCR) tubes containing sodium dodecyl sulphate (SDS) lysis buffer with proteinase K and frozen at  $-20^{\circ}\text{C}$ . After that, cells were lysed by incubation for 20 minutes at  $37^{\circ}\text{C}$  and 15 minutes at  $65^{\circ}\text{C}$ .

PGD NGS method was designed at the INVICTA Fertility Clinic Molecular Laboratory Department. Whole genome amplification (WGA) was performed according to the manufacturer's protocol (PicoPlex WGA kit, New England Biolabs, Ipswich, MA). Concentration of DNA after WGA was quantified with Qubit 2.0 Fluorometer and Qubit dsDNA HS Assay (Life technologies, CA). For DNA fragmentation, Ion Xpress Plus Fragment Library Kit, Life Technologies, according to manufacturer's protocol for 10 to 100 ng of gDNA input was used. For barcoding, Ion Xpress Barcode Adapters 1–96 Kit (Life Technologies) were used. Ion Xpress Equalizer Kit (Life Technologies) was used for library input normalization, according to manufacturer's protocol. Barcoded libraries were clonally amplified with The Ion PGM Template OT2 200 Kit (Life Technologies) using Ion One Touch 2 System. After chip loading, sequencing was performed using Ion PGM Sequencing 200 Kit v2 (Life Technologies) on Ion 314 and 316 chips. Preliminary analysis, for example, base calling and reads mapping against human genome reference sequence (Hg19) were performed with Ion Torrent Suite Software (Life Technologies, Carlsbad, CA). The INVICTA Bioinformatics Team Script was used for further computational calculations. Read coverage for each chromosome was corrected for guanosine-cytosine (GC)-bias, and aneuploidy detection was performed using sample results comparison to baseline values obtained from 72 males and 52 females euploid samples processed beforehand with established protocol described earlier. The INVICTA algorithm was introduced to eliminate the influence of sample-to-sample reads coverage variance on false-positive calls. Male control samples were processed together with probes from blastomeres and underwent the same computational analysis to exclude any performance malfunctions. Negative control sample was processed to exclude contaminations. The protocol was validated using cell lines and was accurate regarding detection of whole chromosome aneuploidies.

On day 5, the transfer of the single healthy, euploid blastocyst was performed. Pregnancy was confirmed and prenatal diagnosis was performed at week 15.

## Results

Three blastomeres biopsied from three embryos on day 3 of the culture were analyzed in both cycles. The embryological and PGD results for this couple are shown in **Table 1**.

## Discussion

Originally, the main reason to introduce PGD was to avoid pregnancy termination in case of single gene diseases with severe outcome.<sup>8</sup> Soon thereafter, the indications for the PGD were expanded to add late-onset diseases with high predisposition for malignancy,<sup>9</sup> human leukocyte antigen typing<sup>10</sup> and chromosomal aberrations.<sup>11</sup> Owing to improvements in embryos selection methods, screening for aneuploidies related to age of parents became one of the main reasons for PGD along with desire to avoid transmission of inherited genetic abnormalities.<sup>12–15</sup> Similar approach of screening embryos not only for the derivatives of abnormal segregation of translocation but also for the risk of aneuploidies of other chromosomes was proposed by some authors.<sup>16</sup> The interchromosomal effect in Robertsonian carriers reproductive cells was supported by some authors,<sup>5,17</sup> and regarded as doubtful with the need for further studies by others.<sup>18</sup> Nevertheless, in the case of the male carriers with oligospermia, it seems to be crucial to

analyze both aspects of the observed higher aneuploidy rates.<sup>19,20</sup>

The first molecular, cytogenetic technique—FISH—was introduced as an easy and simple PGD procedure.<sup>21</sup> Even though it has some limitations, for example, defective nucleus fixation, abnormal patterns of hybridization, and the intensity of fluorescence from simultaneously hybridized probes, it is, currently, the most frequently used screening methods. However, those examples suggest that FISH is not a completely reliable method. That is the reason why the interpretation of the results is often doubtful and requires rehybridization. Also, it may cause delay of the transfer decision both with overestimation of aneuploid blastomeres.<sup>15</sup>

To perform genome-wide scans, the researchers developed comparative genomic hybridization methods. However, the technological advances directed the laboratory efforts toward the golden standard of the molecular genetics sequencing.<sup>22</sup> NGS is a rapidly developing technology which produces enormous amount of data with the wide range of applications. The development of the different NGS platforms and diminishing costs enabled their introduction into the preimplantation diagnostics. The semiconductor sequencers are another step forward allowing analysis of embryos in fresh IVF cycles achieved by the faster turnaround time.<sup>23</sup> In cases of screening for the abnormal segregation of the translocation, it was used to count multiple amplicons from each chromosome and compared with the relative ratio of fragments obtained from the healthy individuals. NGS-based PGD allows the precise analysis of number of all human chromosome copies in a probe by achieving high coverage of the

**Table 1** The embryological and PGD results for investigated couple

	PGD FISH program	PGD NGS program
Start date	January 2012	September 2013
Antral follicles count	17	10
Stimulation duration (d)	9	10
hMG total dosage (IU)	2,025	2,250
Number cumulus oocyte complexes	12	15
MII	6	11
2PN	4	5
Number of embryos biopsied on day 3	3	3
Embryos PGD results	1: (14q D14S1419 × 2) (D15Z4 × 2)	1: normal, 46, XX
	2: (14q D14S1419 × 0) (D15Z4 × 1)	2: unbalanced, 45 (–22)
	3: (14q D14S1419 × 3) (D15Z4 × 2)	3: unbalanced, 44, (–7, –11, –17, +21)
Total reads	1 embryo: 4	1 embryo: 51,810
	2 embryo: 1	2 embryo: 73,847
	3 embryo: 5	3 embryo: 70,246
Embryos transferred	1	1
4 wk 2 d hCG level (mIU/mL)	94	322,3
Ongoing pregnancy	No (miscarriage in wk 10)	Healthy baby born

Abbreviations: FISH, fluorescent in situ hybridization; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin; MII, metaphase II; NGS, next-generation sequencing; PGD, preimplantation genetic diagnosis.

human genome sequence. Concluding, we confirm the positive impact of NGS-based PGD in the case of the Robertsonian translocation carrier. We believe that this first report of the healthy baby birth achieved after the application of new NGS technology is just a first step leading to further studies reaffirming reliability of NGS-based PGD in screening for the embryonic ploidy status.

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