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CASE REPORT

Mosaic embryo transfer after pre-implantation genetic testing for structural rearrangement: a case study

Shweta Mahalingam[1*], Rashmi Rasalkar[1], Meenakshi Lallar[1,2], Avinash Pradhan[1], Sneha Khairnar[1], Satish Kariyaiah[1], Angela Devanboo[1], Eswarachari Venkataswamy[1], Vedam L. Ramprasad[1], Priya Kadam[1]

ABSTRACT

Background: Identifying the cause of recurrent pregnancy loss (RPL) helps in the direct management of future pregnancies. In 3%-4% of cases, a chromosomal structural rearrangement is identified in the couple. Pre-implantation genetic testing - structural rearrangements (PGT-SR) is an accepted practice for such couples opting for pregnancy through *in-vitro* fertilization. However, the result interpretation and clinical outcome for mosaic embryo transfer have limited predictions.

Case Presentation: In this case study, we describe the genetic tests involved in establishing the cause of RPL followed by management through PGT and prenatal diagnosis. The couple was identified with a structural rearrangement and opted for PGT-SR (structural rearrangement) which revealed a mosaic embryo involving multiple chromosomes. The case study describes the implication of the transfer of mosaic embryos, followed up with prenatal tests with complex results, its interpretation, and genetic counseling.

Conclusion: The case reiterates the benefit of thorough genetic evaluation for pregnancy management by using multiple genetic tests for couples with RPL. It aids in decision-making for complex genetic results in prenatal scenarios.

Keywords: Recurrent pregnancy loss, PGT-SR, mosaic embryo, genetic counseling.

Introduction

Recurrent pregnancy loss (RPL) is observed in 1%-2% of the couples worldwide, whereas in the Indian population, RPL is noted in ~7% of the couples [1-3]. Identifying the cause of RPL is important for the management of future pregnancies, predicting pregnancy outcomes, and providing appropriate prenatal care. RPL due to structural chromosomal rearrangements in the parents can occur in 3%-4% of the cases [3]. One of the most common processes to follow in managing the pregnancy outcome for couples with RPL starts with genetic testing of the product of conception (POC). In about ~50% of sporadic miscarriage samples, a chromosomal abnormality is found [4]. Identifying an abnormality in the POC not only helps in the direct management of future pregnancies but also provides psychological closure for the couple. Karyotype and chromosomal microarray are the commonly prescribed tests for testing POC.

Genetic testing of the parents for underlying chromosomal or genetic changes is also an important step in the evaluation of RPL causes. Once a structural rearrangement is identified as the genetic cause for RPL, preimplantation genetic testing for structural rearrangements (PGT-SRs) can serve as a potential solution for selecting a euploid embryo followed by prenatal diagnosis to confirm.

Correspondence to: Shweta Mahalingam
MedGenome Labs Ltd., Bangalore, India.
Email: shwetamahalingam97@gmail.com
Full list of author information is available at the end of the article.
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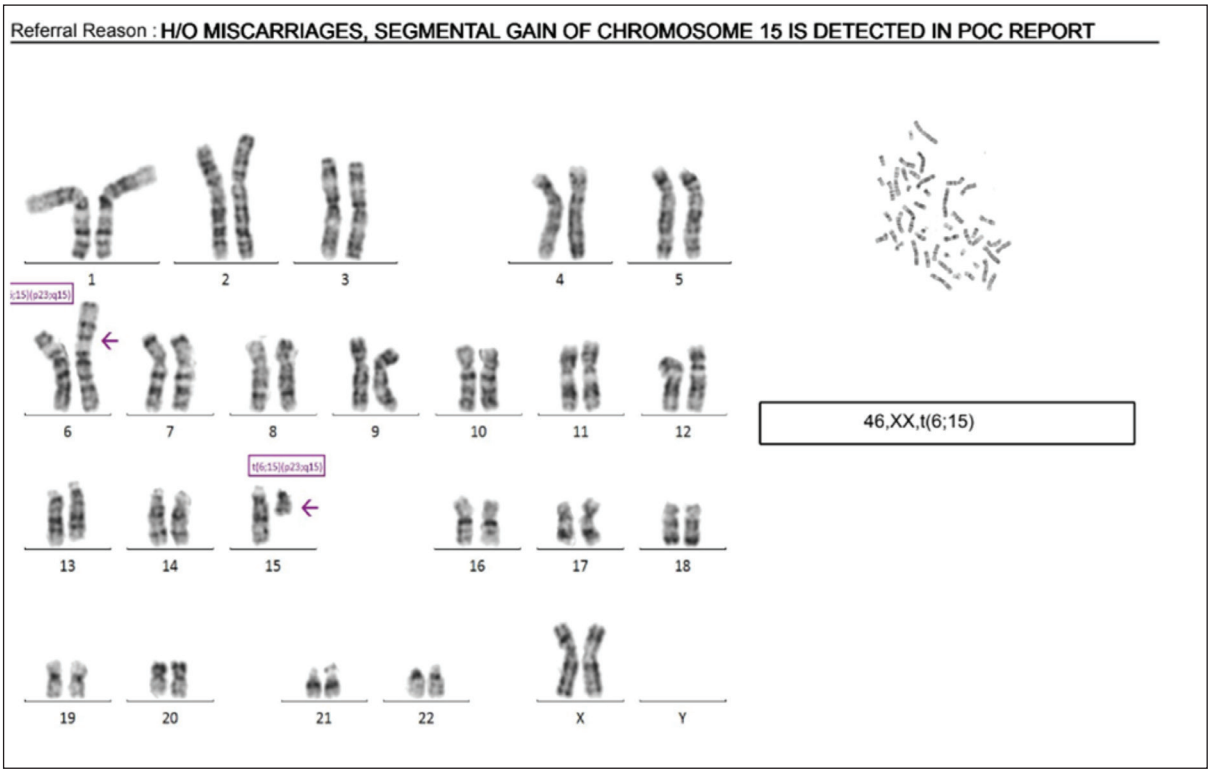


Figure 1. *Karyotype revealing balanced translocation between the short arm of chromosomes 6 and the long arm of chromosomes 15.*

Table 1. *Embryo biopsy information (Pre-analytical).*

Embryo ID	No. of cells biopsied	Biopsy day	Embryo grade	Nucleus seen
KN1	8-10	Day 5	High	Yes
KN2	6-8	Day 5	Low	Yes
KN3	6-8	Day 5	High	Yes
KN4	6-8	Day 5	Low	Yes

New technologies for PGT-SR such as next generation sequencing (NGS) and array comparative genomic hybridization can even detect mosaic aneuploidies (different genotypic cell lines in the same embryo) [5]. The latest statement by the Preimplantation Genetic Diagnosis International Society (PGDIS) provides evidence on transferring mosaic embryos and their implications. Recent studies also suggest there is no statistically significant difference in the implantation rate of mosaic versus. euploid embryos; however, the pregnancy outcome of such transfer still needs to be further documented [6].

Embryos are classified as low mosaic (20%-50% cells with mosaicism) and high mosaic (>50% cells with mosaicism) embryos. The live birth rate and miscarriage rate of both the groups were compared in Pin-Yao Lin et al. [7] and it revealed a comparable live birth rate (44.5% *vs.* 36%, $p = 0.45$ in low *vs.* high mosaic groups) and a higher miscarriage rate after transfer of high mosaic embryos (5.1% *vs.* 30.7%; $p = 0.012$). For some couples, the transfer of mosaic embryos might be the only choice. However, there is uncertainty with respect to clinical outcomes in such cases [8].

There are fewer case studies contributing to the evidence of transferring high mosaic embryos and good pregnancy outcomes. Yung-Liang Liu et al 2019 and Gauri Agarwal et al 2022 describe live births after the transfer of high mosaic embryos with abnormality in one chromosome. With more information, we will be able to find better evidence to draw conclusions about mosaic embryo transfer. Here, we describe a case study with the transfer of a high mosaic embryo (in multiple chromosomes) and its outcome in a 38-year-old pregnant woman with a history of RPL and who is a known carrier of a balanced chromosomal translocation. The case study describes the follow up tests undergone by the patients, their results, challenges in the interpretation of the result, genetic counseling, and the outcome of the pregnancy.

Case Report

A 38-year-old woman presented with a history of recurrent miscarriages was referred for genetic evaluation. Genetic

Table 2. *PGT-SR result.*

Embryo ID	Result	Transfer recommendation	Interpretation
KN1	Mosaicism detected	*Refer guidelines	Although no aneuploidy is detected structurally, it may either be normal or balanced translocation involving chromosomes 6 and 15 as in seen in mother. Additionally low-level mosaicism in chromosome 1, 2, 3, 4,12, 14, 17, 22, high level mosaicism in chromosome 6 and 11 and sex chromosomes.
KN2	Aneuploidy detected	Not recommended	Observed segmental gain in chromosome 15, unbalanced segregation of the balanced translocation seen in the mother was detected. Additionally segmental mosaic loss in chromosome 2 (30%), 12 (40%), mosaic gain in chromosome 3 (40%), 18 (25%) and segmental mosaic gain in chromosome 7 (38%).
KN3	Aneuploidy detected	Not recommended	Observed loss in chromosome 15, unbalanced segregation of the balanced translocation seen in the mother was detected. additionally segmental mosaic gain in chromosome 2 (30%) was observed.
KN4	Aneuploidy detected	Not recommended	Observed segmental gain in chromosome 15, unbalanced segregation of the balanced translocation seen in the mother was detected. Additionally gain, mosaic gain and mosaic loss are observed in multiple chromosomes.

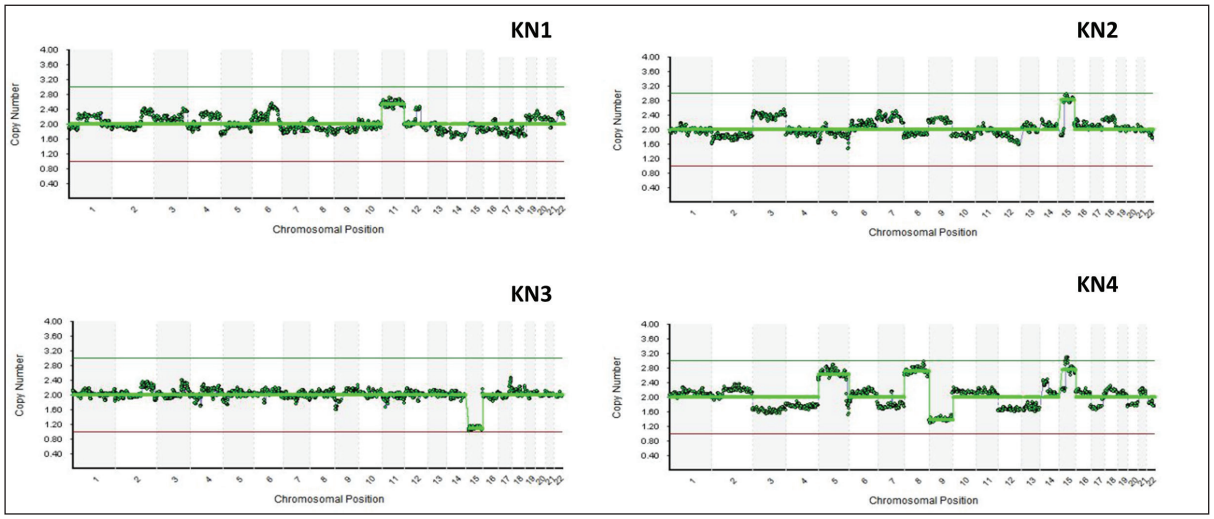
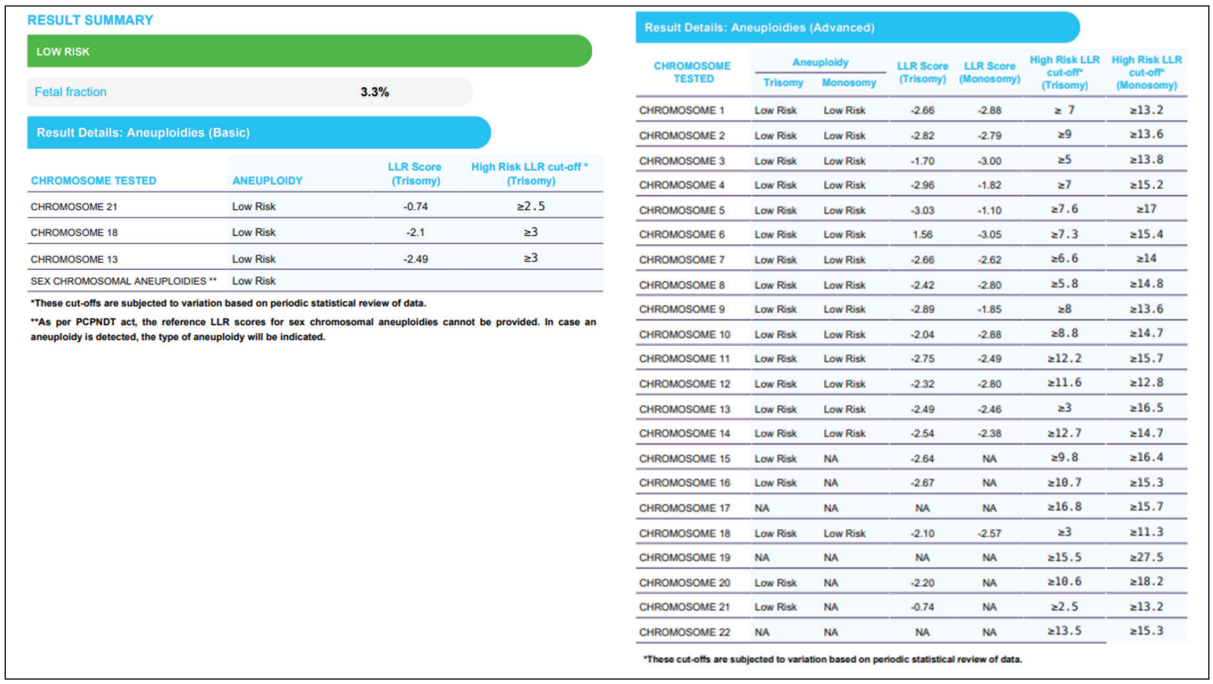


Figure 2. *PGT-SR analysis using BlueFuse Multi software (v4.4).*

testing of one of the products of the conceptus sample revealed segmental gain in chromosome 15. Parental karyotyping was suggested. The male karyotype was apparently normal, and the female karyotype revealed apparently balanced translocation between the short arm of one of chromosomes 6 and the long arm of one of the chromosomes 15 (Figure 1) - 46,XX,t(6;15)(p23;q15). The mother was a balanced translocation carrier. The couple was explained about PGT-SR, conception with donor ovum, and prenatal diagnosis options in genetic counseling. Hereafter, the couple opted for pregnancy through *in-vitro* fertilization followed by pre-implantation genetic screening to screen for inherited chromosomal structural abnormalities. The pre-implantation genetic screening was done using NGS technology. The biopsy material was subjected to a process called whole genome amplification (WGA). The WGA was then sequenced using a MiSeq sequencing platform along with control samples. Sequenced data was then analyzed using BlueFuse Multi software (v4.4) based on the read count produced to determine the copy number for all 23 pairs of chromosomes, thereby analyzing whole chromosome anomalies for the entire genome. It detects losses and gains of whole chromosomes with a specificity and sensitivity of >99%. Six to seven biopsied trophectoderm (TE) cells of four embryos were analyzed and aneuploidy was detected in three of them (Table 2, Figure 2).

The embryo KN1 revealed low-level mosaicism in chromosomes 1, 2, 3, 4, 12, 14, 17, and 22 and high-level mosaicism in chromosomes 6, 11, and sex chromosomes. The transfer recommendations were made as per the PGID guidelines. The couple opted to transfer the mosaic



RESULT SUMMARY

LOW RISK

Fetal fraction 3.3%

Result Details: Aneuploidies (Basic)

CHROMOSOME TESTED	ANEUPLOIDY	LLR Score (Trisomy)	High Risk LLR cut-off * (Trisomy)
CHROMOSOME 21	Low Risk	-0.74	≥2.5
CHROMOSOME 18	Low Risk	-2.1	≥3
CHROMOSOME 13	Low Risk	-2.49	≥3
SEX CHROMOSOMAL ANEUPLOIDIES **	Low Risk		

*These cut-offs are subjected to variation based on periodic statistical review of data.

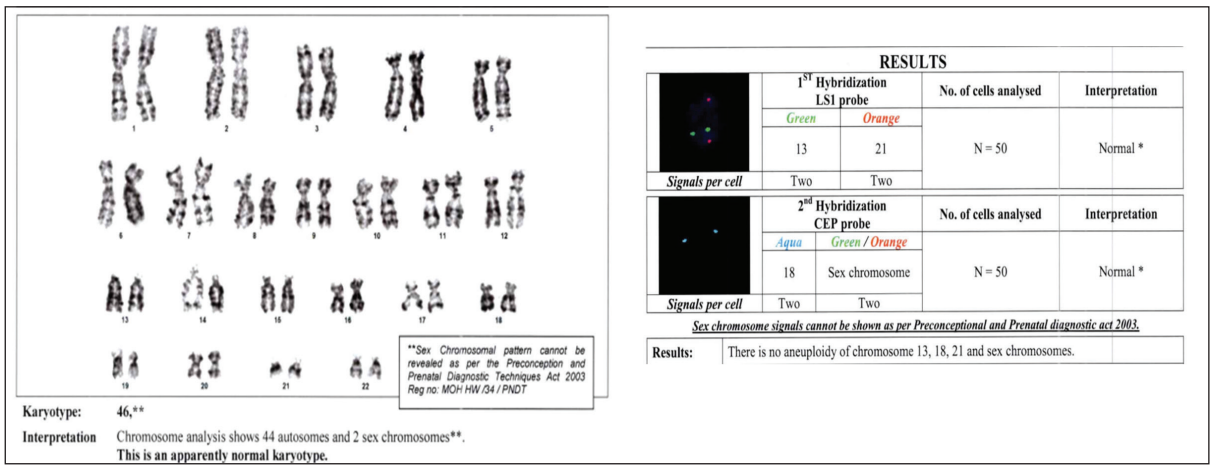
**As per PCPNDT act, the reference LLR scores for sex chromosomal aneuploidies cannot be provided. In case an aneuploidy is detected, the type of aneuploidy will be indicated.

Result Details: Aneuploidies (Advanced)

CHROMOSOME TESTED	Aneuploidy: Trisomy	Aneuploidy: Monosomy	LLR Score (Trisomy)	LLR Score (Monosomy)	High Risk LLR cut-off* (Trisomy)	High Risk LLR cut-off* (Monosomy)
CHROMOSOME 1	Low Risk	Low Risk	-2.66	-2.88	≥ 7	≥13.2
CHROMOSOME 2	Low Risk	Low Risk	-2.82	-2.79	≥9	≥13.6
CHROMOSOME 3	Low Risk	Low Risk	-1.70	-3.00	≥5	≥13.8
CHROMOSOME 4	Low Risk	Low Risk	-2.96	-1.82	≥7	≥15.2
CHROMOSOME 5	Low Risk	Low Risk	-3.03	-1.10	≥7.6	≥17
CHROMOSOME 6	Low Risk	Low Risk	1.56	-3.05	≥7.3	≥15.4
CHROMOSOME 7	Low Risk	Low Risk	-2.66	-2.62	≥6.6	≥14
CHROMOSOME 8	Low Risk	Low Risk	-2.42	-2.80	≥5.8	≥14.8
CHROMOSOME 9	Low Risk	Low Risk	-2.89	-1.85	≥8	≥13.6
CHROMOSOME 10	Low Risk	Low Risk	-2.04	-2.88	≥8.8	≥14.7
CHROMOSOME 11	Low Risk	Low Risk	-2.75	-2.49	≥12.2	≥15.7
CHROMOSOME 12	Low Risk	Low Risk	-2.32	-2.80	≥11.6	≥12.8
CHROMOSOME 13	Low Risk	Low Risk	-2.49	-2.46	≥3	≥16.5
CHROMOSOME 14	Low Risk	Low Risk	-2.54	-2.38	≥12.7	≥14.7
CHROMOSOME 15	Low Risk	NA	-2.64	NA	≥9.8	≥16.4
CHROMOSOME 16	Low Risk	NA	-2.67	NA	≥10.7	≥15.3
CHROMOSOME 17	NA	NA	NA	NA	≥16.8	≥15.7
CHROMOSOME 18	Low Risk	Low Risk	-2.10	-2.57	≥3	≥11.3
CHROMOSOME 19	NA	NA	NA	NA	≥15.5	≥27.5
CHROMOSOME 20	Low Risk	NA	-2.20	NA	≥10.6	≥18.2
CHROMOSOME 21	Low Risk	NA	-0.74	NA	≥2.5	≥13.2
CHROMOSOME 22	NA	NA	NA	NA	≥13.5	≥15.3

*These cut-offs are subjected to variation based on periodic statistical review of data.

Figure 3. *The NIPT result revealing 3.3% fetal fraction and low risk for aneuploidies in Chromosome complement.*



**Sex Chromosomal pattern cannot be revealed as per the Preconception and Prenatal Diagnostic Techniques Act 2003 Reg no: MOH/HW/34/PNDT

Karyotype: 46,**

Interpretation: Chromosome analysis shows 44 autosomes and 2 sex chromosomes**.
This is an apparently normal karyotype.

RESULTS

	1ST Hybridization LSI probe		No. of cells analysed	Interpretation
	Green	Orange		
	13	21	N = 50	Normal *
Signals per cell	Two	Two		

	2nd Hybridization CEP probe		No. of cells analysed	Interpretation
	Aqua	Green / Orange		
	18	Sex chromosome	N = 50	Normal *
Signals per cell	Two	Two		

Sex chromosome signals cannot be shown as per Preconceptional and Prenatal diagnostic act 2003.

Results: There is no aneuploidy of chromosome 13, 18, 21 and sex chromosomes.

Figure 4. *FISH result revealing no aneuploidy of chromosome 13,18, 21 and sex chromosomes.*

embryo (embryo KN1) after understanding the risks and benefits of the option. They were recommended for prenatal testing as per the guidelines. Post implantation, the ultrasound at 7 weeks of gestation showed a single intrauterine fetus with normal cardiac activity. The nuchal translucency scan at 13 weeks showed no gross fetal abnormalities. The couple opted for whole genome non-invasive prenatal testing (NIPT) at 13 weeks and 2 days of gestation. The NIPT result revealed a 3.3% fetal fraction and low risk for aneuploidies in chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 18, and sex chromosomal aneuploidies. Further, it was low risk for Trisomy 15,16, 20, and 21 (Figure 3). However, a risk status could not be assessed for Monosomy 15,16, 19,20, 21, and 22 and Trisomy 17, 19, and 22 due to the limit of detection for these conditions. They opted for further genetic counseling to discuss the NIPT result. As discussed in pre-test counseling, the couple opted for fluorescent *in-situ* hybridization (FISH), Karyotype, and chromosomal microarray. It was done on uncultured amniotic fluid cells collected at 16 weeks of gestation. Maternal cell contamination was ruled out for the collected sample using AmpFlSTR Identifiler® PCR Amplification Kit (Qiagen, Valencia, CA). There are 16 loci used to determine the MCC status (*CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, vWA, D2S1338, D19S433, and Amelogenin marker*) and the STR data were analyzed using Chimer marker V 3.1.0.

The FISH result revealed no aneuploidy of chromosome 13,18, 21, and sex chromosomes. Karyotype analysis

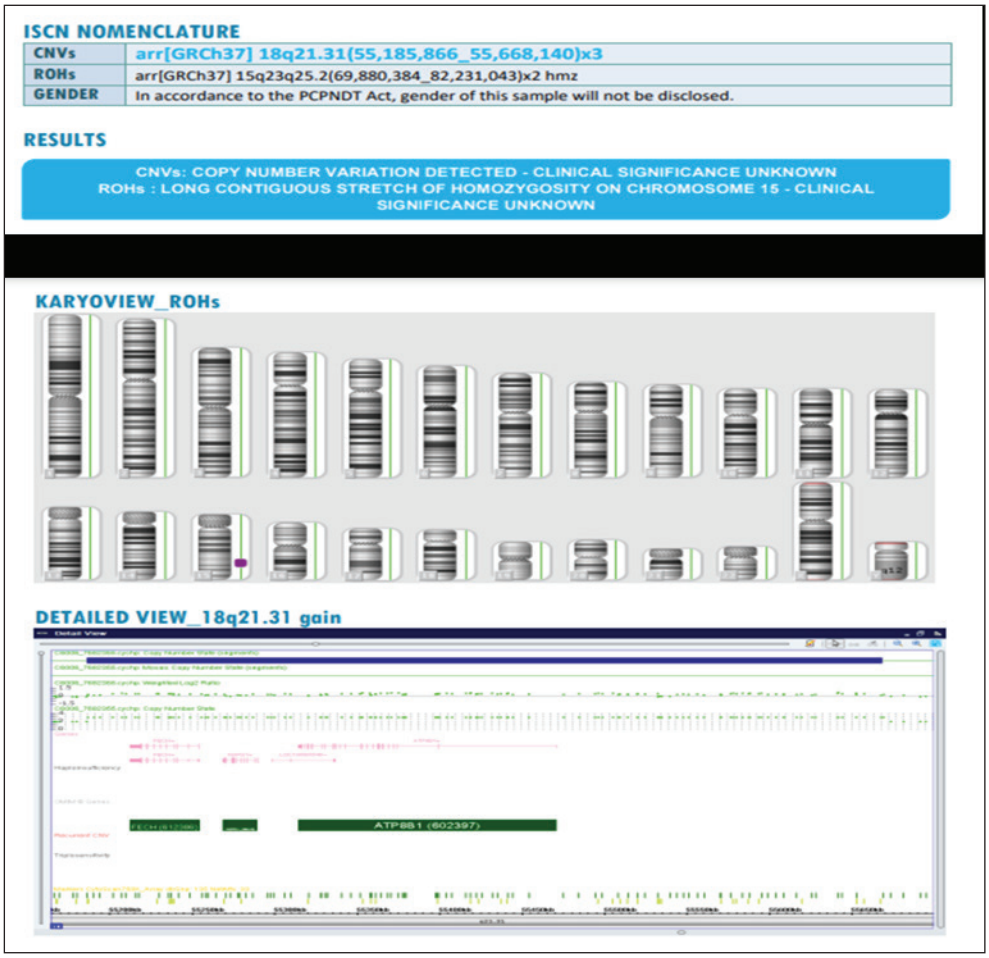


Figure 5. *Chromosomal microarray analysis revealing a 0.5 MB gain involving chromosome 18 at cytoregion 18q21.31 indicating trisomy for this region.*

revealed normal karyotype (Figure 4). Furthermore, a chromosomal microarray analysis was done to check for copy number variations and breakpoints. We have used a CytoScan 750K chipset fabricated by ThermoFisher Scientific. It is a hybrid dual design including clinically relevant copy number probes combined with the power of high-density single nucleotide polymorphism (SNP) markers for confident breakpoint determination. This chipset had a total number of 750,436 copy number markers (Number of non-polymorphic markers: 550,000; number of SNP markers: 200,436). Chromosomal microarray analysis revealed a 0.5 MB gain involving chromosome 18 at cytoregion 18q21.31 indicating trisomy for this region - variant of uncertain significance (VUS) (Figure 5) - arr[GRCh37] 18q21.31(55,185,866_55,668,140) x3 - This breakpoint contained a total of 127 copy number markers (95 non-polymorphic markers and 32 SNP markers). The segment consisted of 3 OMIM genes - *FECH* (ferrochelatase gene), *ATP8B1* (ATPase phospholipid transporting 8B1 gene), and *NARS1* (asparaginyl-tRNA synthetase 1 gene). Microarray analysis furthermore revealed a region of heterozygosity in the cytoregion arr [GRCh37] 15q23q25.2(69,880,384_82,231,043). Parental segregation by chromosomal microarray suggested for the copy number variation observed was not opted by the couple. No ultrasound abnormalities were noted throughout the pregnancy and a follow-up on the pregnancy outcome revealed a healthy and alive baby with normal developmental milestones at the time of study.

Discussion

Evaluation, genetic testing, and genetic counseling for RPL remain a challenge when the cause for such occurrence is not diagnosed. However, with evolving genetic technologies, the percentage of unknown etiology may be reduced and aid in good pregnancy outcomes for such couples. Prenatal care does not stop at finding the cause for such occurrences, but also in managing the future steps. It needs to be in the best interest of the patient. In this case, the segmental gain of chromosome 15 in the POC paved the way for parental investigation and subsequent findings in parental karyotype revealed the cause of RPL. European society of human reproduction and embryology and American society for reproductive medicine guidelines favor the use of PGT-SR for translocation carriers as studies report RPL in ~30% of families with translocation. In the case of reciprocal translocations, during meiosis I, the normal and the translocated chromosomes form a quadrivalent structure and undergo segregation that leads to unbalanced gamete

segregation. Depending on the mode of segregation, the gametogenesis will produce both balanced or unbalanced gametes for the translocated chromosome [8]. In this case, we observed for embryos KN2, KN3, and KN4, a gain or segmental gain in chromosome 15 for which the reasons can be traced to the translocation observed in the mother leading to unbalanced gametogenesis. The clinical outcome of imbalances determined by the CNVs are dependent on the genes involved and how well the genes have been studied to predict the phenotypic consequences.

Current literature also adds evidence to how with PGT-SR treatments, translocation carriers with bad obstetric history may have better pregnancy outcomes, and lower risk for miscarriage and birth defects [9]. In the current era, genetic tests are still evolving and might reveal a plethora of information that needs to be interpreted more carefully and in respect to the patient's advantage. The transfer of high-grade mosaic embryos remains debatable due to limited evidence on the outcome of the transfer. However, in this case study, we have reported a healthy live birth after the transfer of a high-grade mosaic embryo.

PGDIS recommends prenatal diagnostic tests for pregnancy achieved through the transfer of euploid or mosaic embryos [6]. In situations where mosaic embryo is the only option, genetic counselling must include thorough education about the implications of transferring mosaic embryos, the follow up tests, and possible outcomes. There is limited published evidence for discordance between PGT results and pregnancy outcomes, most of them only comparing the birth rate or implantation rates. Sachdev, et al compare the concordance rate with re-biopsied samples including the same cell line or inner cell mass and describes the concordance percentage for mosaic embryos to be 35.3% [6]. Similarly, Pin Yao Lin et al describes the concordance percentage for mosaic embryos between initial biopsy and inner cell mass to be 37% [6]. The reason for discordance can be due to various underlying biological reasons and technical reasons. The most common biological explanation states the self-correcting nature of the embryos, where an aneuploidy cell line is eliminated naturally due to its growth disadvantage by apoptosis during development [10]. Discordance between TE and fetus could also arise due to the region biopsied [11]. The biopsy technique, sample handling, sub-optimal DNA amplification or library construction, choice of algorithm used, and technique used for normalizing the mapping bins are some of the technical reasons for mosaic results [6]. There is a further need to investigate all plausible explanations for discordant results in PGT to improve the clinical outcome. There is limited evidence of pregnancy outcome with respect to the type of mosaic chromosome and number of chromosomes involved after mosaic embryo transfer. Furthermore, an unpredicted variation in microarray made the genetic counseling of this case very challenging. Around 2%-6% of prenatal chromosomal microarray testing might reveal a VUS [12]. The patient must be aware about the possibility of VUS as one of the test outcomes before opting for the test. The long-term implications on the developmental milestones of the child are also yet to be studied.

Conclusion

This case adds evidence to pregnancy management in translocation carriers, multi-chromosome high mosaic embryo transfer after PGT-A, follow up prenatal diagnostic tests, and its possible outcomes. It highlights the benefit of synchronous use of multiple genetic tests now available leading to good outcomes in couples with RPL.

Conflict of interest

Authors Shweta Mahalingam, Rashmi Rasalkar, Meenakshi Lallar, Avinash Pradhan, Sneha Khairnar, Satish Kariyaiah, Angela Devanboo, Eswarachari Venkataswamy, V. L. Ramprasad, and Priya Kadam are/were employed with MedGenome Laboratories during the course of the project.

Consent for publication

Informed consent was obtained, and all reasonable efforts were made to maintain patient confidentiality.

Ethical approval

Ethical approval is not required at our institution to publish an anonymous case report.

Author details

Shweta Mahalingam[1], Rashmi Rasalkar[1], Meenakshi Lallar[1,2], Avinash Pradhan[1], Sneha Khairnar[1], Satish Kariyaiah[1], Angela Devanboo[1], Eswarachari Venkataswamy[1], Vedam L. Ramprasad[1], Priya Kadam[1]

1. MedGenome Labs Ltd., Bangalore, India
2. Prime Diagnostics and Prenatal Imaging, Chandigarh, India

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