Discrepancy of Karyotype and CMA /NGS in PGD Patient with Cryptical Complex Chromosomal Rearrangement

TING WANG (kevinwtwt@163.com)
Guangdong Women and Children Hospital https://orcid.org/0000-0002-9261-9016
Hanbiao Chen
Guangdong Women and Children Hospital
Jian Lu
Guangdong Women and Children Hospital
Weiwei Huang
Guangdong Women and Children Hospital
Huamei Huang
Guangdong Women and Children Hospital
Chuangqi Chen
Guangdong Women and Children Hospital
Li Guo
Guangdong Women and Children Hospital

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Abstract

Background: Complex chromosome rearrangement (CCR) is a structural rearrangement involving more than two breakpoints. CCR carriers are at high risk for phenotypic abnormalities or reproductive failure, such as chromosomal abnormalities in fetuses and infertility.

Methods: We presented a carriers with chromosome (3,18) apparent balanced translocation diagnosed in eleswhere, whose fetus had duplications in chromosome 3 and deletions in chromosome 10 demonstrated by chromosome microarray analysis(CMA).

Results: Through the high resolution of GTG-banding, a cryptical translocation in chromosome 10 was found and the karyotype of the carrier was revised as 46,XY,t(3;10;18) (p26.3;q26.1;q21.1).In the cycle of preimplantation genetic diagnosis (PGD),21 oocytes were retrieved, and 15 were fertilized. At last 7 embryos were biospied and sent to diagnosis by next generation sequencing(NGS).Unfortunately, none of the NGS results from the 7 biopsy embryos were normal. Combining previous literature and our results, we assessed the odds of a balanced embryo in a CCR carrier to be about 9.3%(28/302).The transferable embryo rate was approximately 71.4%(20/28) and healthy live born delivery rate was 55%(11/20).

Conclusions: NGS and CMA featured high automation, relatively low cost, high throughput, and high repeatability, which made them commonly used during prenatal diagnosis and PGD. The multiple technology combination can provide more accurate diagnosis and better fertility services for CCR patients.

1 Introductions

Complex chromosome rearrangement (CCR) is a structural rearrangement involving more than two breakpoints.The most common CCR involves three chromosomes and three breakpoints.Constitutional CCR are rare and approximately 250 cases had been reported so far[1]. The CCR occurrence in newborns was about 0.5%[2].Balanced CCR carriers are prone to recurrent miscarriage, and unbalanced CCR is often associated with mental retardation and congenital malformations.In addition.CCR are not only associated with rare structural abnormalities, but occasionally occurs with gene deletion and duplication.These are mainly through the damage of dose-sensitive genes, cis-regulatory elements, so as to affect the expression of candidate disease genes through long-term positioning effect[3].CCR carriers had a less than 6% chance of retrieving a normal/balanced blastocyst.The chance of getting pregnant is less than 4 %[4].During meiosis, CCR can produce a large number of gametes and unbalanced chromosomes [5].The application of preimplantation genetic diagnosis (PGD) to CCR carriers can reduce the risk of spontaneous abortion, thus producing fetuses with balanced chromosomes and avoiding the generation of embryos with unbalanced chromosomes[6].

The resolution of traditional karyotype analysis is limited, and it can only detect the abnormality of > 10MB fragments in general. Moreover, due to personal subjective judgment, it is easy to miss small structural changes.In recent years, with the development of molecular and cell biology technology, chromosome microarray analysis (CMA) and next generation sequencing(NGS) had been increasingly applied in prenatal diagnosis.CMA can cover the whole genome DNA, with high throughput, high resolution and fast detection speed[7].Schluth-Bolard C et al had proven that high-resolution NGS was effective in detailed studies of CCR[8].Constantia A et al found a cryptic breakpoint by whole-genome mate-pair sequencing in a CCR carrier,which was misdiagnosed by karyotype[9]. Frumkin T et al revealed a gain or loss at three chromosomes (3, 7, 9) by PGS-CMA in six embryos,whose parents had a former diagnosis of normal karyotype[10].

In this study, we presented a carriers with chromosome (3,18) balanced translocation,whose fetus had duplications in chromosome 3 and deletions in chromosome 10 demonstrated by CMA.By revealing the cryptical translocation, we aimed to provide CCR carriers with more accurate risk assessment of abnormal pregnancy and better assisted reproduction with CMA and NGS.

2 Materials And Methods

2.1 Subjects

A 35-year-old woman who had a live born healthy child referred to our genetic medical center due to the high risk of noninvasive prenatal test(NIPT) performed in other hospital which demonstrated 8M deletions in chromosome(chr) 10.Low resolution (400-band level) initial chromosome results elsewhere suggested that the woman's husband had a significant balanced translocation on chr3 and chr8 [46,XY,t(3;18)(p26;q21.1)],while a normal karyotype[46,XX] detected in the woman.In view of the inconsistency between the karyotype analysis and the NIPT results, we recommended that the couple have their chromosomes reexamined.Informed consents were obtained from the couple before all examination and this case report was approved by Ethics Committee

2.2 Karyotype and chromosome microarray analysis

The couple accepted our suggestion and their peripheral blood was drawn in a vacuum heparin tube for phytohemagglutinin (PHA)-stimulated lymphocytes cultures.To obtain chromosomes with a resolution higher than 550 bands, we used thymine(Sigma,USA) and deoxycytidine(Sigma,USA) to synchronize the lymphocytes and inhibited chromosome contraction.According to the standard protocol,20 metaphases were acquired for karyotype using Ikaros system version 5.8.2(Zeiss,Germany). The distal translocation of Chr10 was found to be involved in the husband's karyotype.Hence, the karyotype of the husband was revised as 46,XY,t(3;10;18) (p26.3;q26.1;q21.1).(Fig. 1),and his wife's reexamination of karyotype was normal.

An amniocentesis was performed at 23 weeks gestation due to the high risk of NIPT.

10 ml of amniotic fluid were used for DNA extraction by QIAamp DNA Blood Mini Kit(QIAGEN,Germany), NANODROP 2000(Thermo USA) was applied to determine DNA concentration. CytoScan 750K chip (Affymatrix,USA) were used for CMA detection, including enzyme digestion, ligation, PCR amplification, purification, fragmentation, labeling, hybridization, washing, scanning. All the procedures were operated according to the manufacturer's protocol. The results were analyzed with ChAS3.1.0.15 (Affymatrix USA) software.The copy number variants(CNV) showed the fetus had 4.7Mb duplications in chr3 and 9.7Mb deletions in chr10(Fig. 2).The couple decided to terminate the pregnancy because chromosomal structural abnormalities of the fetus involved many genes, which could lead to fetal malformation.PGD was advised to them by our genetic conselor on account of CCR.

3 Results

An IVF protocol was performed as described by Shen et al[11]. Controlled ovarian hyperstimulation was applied with gonadotropin-releasing hormone agonist long protocol.The cycle was followed by intramuscular injection of gonadotropin (Gn) after descending regulation, and the Gn dose was adjusted according to the follicle diameter monitored by ultrasonography.When the diameter of follicle was greater than 16mm, human chorionic gonadotropin (Serono,Switzerland) was injected for 6000 ~ 10000u, and 21 oocytes were retrieved after 34 ~ 36h.The collectde oocytes were cultured in medium for 3 hours, those in metaphase II were fertilized by intracytoplasmic sperm injection (ICSI).The 15 fertilized oocytes were separately cultured in a complete medium (Chang,USA) at incubator (Thermo,USA) with 37℃ 5%CO_2 .After five days 7 high-quality embryos of 13 blastocysts were picked for blastocyst biopsy.Four to ten trophectodermal cells were biopsied from each of them using a 35mm inner diameter biopsy micropipette (Humagen,USA). The biopsied cells were lysed and the whole genome amplification were performed with the SurPlex kit(Illumina,USA), including fragmentation, library preparation and amplification.All the 7 biopsied blastocysts were successfully amplified.A whole genome sequencing was performed by Ion Torrent PGM (ThermoFisher,USA) platform according to the manufactor's protocol[12].Unfortunately, none of the NGS results from the 7 biopsy embryos were normal as Table.1 showed.NGS result of embryo no. 3 confirmed our previous CMA result and karyotype about chr10's breakpoint(Fig. 3).The couple decided to abandon any embryo transfer and planned on next cycle.

4 Discussions

Reciprocal balanced translocations typically involved two chromosomal breakpoints, whereas CCR had three or more breakpoints.Theoretically, a balanced translocation carrier had only 1/9 chance of delivering offspring with a normal phenotype.Due to the nature of CCR and the number of chromosomes involved, it was particularly difficult to analyze the meiotic behavior of CCR and its consequences. Therefore, there were relatively few data on meiotic separation of human CCR[13].Previous studies had suggested that in the population with recurrent spontaneous abortion the frequency of CCR was 0.1%[14].The risk of

offspring with an unbalanced karyotype was different in carriers of CCR.Natural pregnancy was discouraged in CCR carriers. Prenatal diagnosis was recommended even if a couple with CCR became pregnant naturally[15].

Preimplantation genetic diagnosis (PGD) ,provided to carriers of CCR, might reduce the risk of spontaneous abortion and the opportunity for offspring to carry chromosomal imbalances, and also could increased the chance of pregnancy by selecting against cleavage-stage embryos carrying unbalanced CCR[16].The identification of CCR and the accurate description of breakpoints depended on the quality of chromosome analysis. Preliminary identification of CCR was performed using traditional cytogenetic methods based on GTG-banding and high-resolution karyotype.By introducing molecular cytogenetics technology (fluorescence in situ hybridization,FISH), the properties of CCR had been greatly improved.Various FISH methods for studying CCR had been tested, and a growing number of reports suggested that CCR may be more complex and common than initially thought[9, 10, 16–19]. However, FISH is limited to detecting specific chromosomes and requires a large number of probes to obtain reliable results. With the development of molecular technology,the application of PGD based on NGS provided an accurate method for detecting the unbalanced segmental rearrangement of embryos[4, 10, 20, 21]. Unlike the traditional low-throughput PCR technique, the next generation sequencing technology can comprehensively detect 23 pairs of chromosomes at high resolution. In addition, NGS can detect de novo copy number variants. High automation, high throughput, and high repeatability make NGS the most widely used technology in PGD.In this case we also employed CMA as control for comparison.

To our knowledge,this is the first study to summarize clinical outcome of PGD patients with CCR reported by previous literatures(Table 2).Combining previous literature and our results (0 balanced embryo out of a total 7 diagnosed in one PGD cycle), we assessed the odds of a balanced embryo in a CCR carrier to be about 9.3%(28/302), while the chance was 20–30% in the case of two chromosomal translocations[19].The transferable embryo rate was approximately 71.4%(20/28) and healthy live born delivery rate was 55%(11/20) according to the data demonstrated in Table 2.The couple was informed that the rate of balanced embryo acquired via PGD in CCR carrier is extremely low,and this should be taken into consideration in planning the next controlled ovarian hyperstimulation cycle.

In this case,the couple was re-karyotyped by reason of discrepancy in cytogenetic result and CMA.The cryptic translocation was finally found in chr10 with high resolution G-banding technique(550-bands level).This indicated that a high level of banding is necessary in cytogenetic examination.It could avoid misdiagnoses in initial consultation and reduce patient's dissatisfaction and unnecessary medical expenses.We did not conduct FISH verification on the breakpoint of chr10 for the reason as follow.First,customed FISH probes were expensive, which could add extra cost.Second, 550-bands level karyotype was sufficient to identify translocation on chr10 as demonstrated by Fig. 1.Third,we found the deletion of chr10 in the CMA results of the fetus,meanwhile there were chr10 gain or loss in 5 of the 7 biospied embryos according the NGS results(Table 1).

In conclusion,NGS featured high automation, relatively low cost, high throughput, and high repeatability, which made them commonly used during prenatal diagnosis and PGD.The combination of molecular and cytogenetic technology can provide more accurate diagnosis and better fertility services for CCR patients.

Abbreviations

CMA; chromosome microarray anlysis;NGS:next generation sequencing;PGD:preimplantation genetic diagnosis;CCR:complex chromosome rearrangement;Chr:chromosome;NIPT:noninvasive prenatal testing;GTG-banding:Giemsa trypsin banding;FISH:fluorescence in situ hybridization

Declarations

Ethics approval and consent to participate

This study was performed with the approval of Medical Ethics Committee of

Guangdong Women and Children Hospital.

Consent for publication

Written consent for publication of medical data and genetic testing results was obtained from the affected couple.

Availability of data and materials

The datasets used and/or analyzed during the current study are available

from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors'contributions

All authors have materially participated in the study and manuscript preparation.

TW analyzed the clinic data, drafted the manuscript; LG and HC carried out

the clinic data analysis, and participated in the design of the work; JL and WH

participated in NGS and CMA analysis and conceiving the work. LG and HC participated in the karyotyping analysis. CC participated in the PGD.HH participated in the follow-up. All authors have approved the final article.

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Tables

Table.1 NGS results of seven embryos

Embryo ID		
	NGS results	outcome
1	47,XN,+18	no transfer
2	46,XN,-(3)(p26.3-p26.1)(4.8Mb),+(10)(q26.13-q26.3)(9.61Mb)	no transfer
3	46,XN,+(3)(p26.3-p26.1)(4.8Mb),-(10)(q26.13-q26.3)(9.61Mb)	no transfer
4	47,XN,+3,-(10)(p15.3-q26.13)(127.06Mb),-(18)(q21.1-q23)(31.90Mb)	no transfer
5	47,XN,-(3)(p26.3-p26.1)(4.8Mb),+10,-(18)(p11.32-q21.1)(46.10Mb)	no transfer
6	46,XN,-(10)(q26.2-q26.3)(6.76Mb),+(18)(q21.1-q23)(31.90Mb)	no transfer
7	46,XN,+(18)(p11.32-q21.1)(46.10Mb)	no transfer
NGS:next generation sequencing		

Table.2 clinical outcome of PGD patients with CCR reported by our study and previous literature

literature	cycle	oocytes	fertilized oocytes	blastocyst	biospied embryos	analyzed embryos	balance/normal embryos	transfer	healthy live born delivery
Hu L et al.(2018)	8	145	110	51	47	46	3	2	2
Brunet BCFK,et al(2018)	7	84	66	25	25	25	6	4	4
Frumkin T et al. (2017)	2	42	33	14	14	14	2	2	1
Vanneste E et al. (2011)	2	26	18	16	16	16	4	2	0
Lim CK et al. (2008)	4	80	61	56	56	54	4	4	1
Escudero T et al. (2008)	13	NM	NM	NM	151	140	9	6	3
our study	1	21	15	13	7	7	0	0	0
total	37				316	302	28(9.3%[a])	20(71.4%[b])	11(55%[c])

Figures

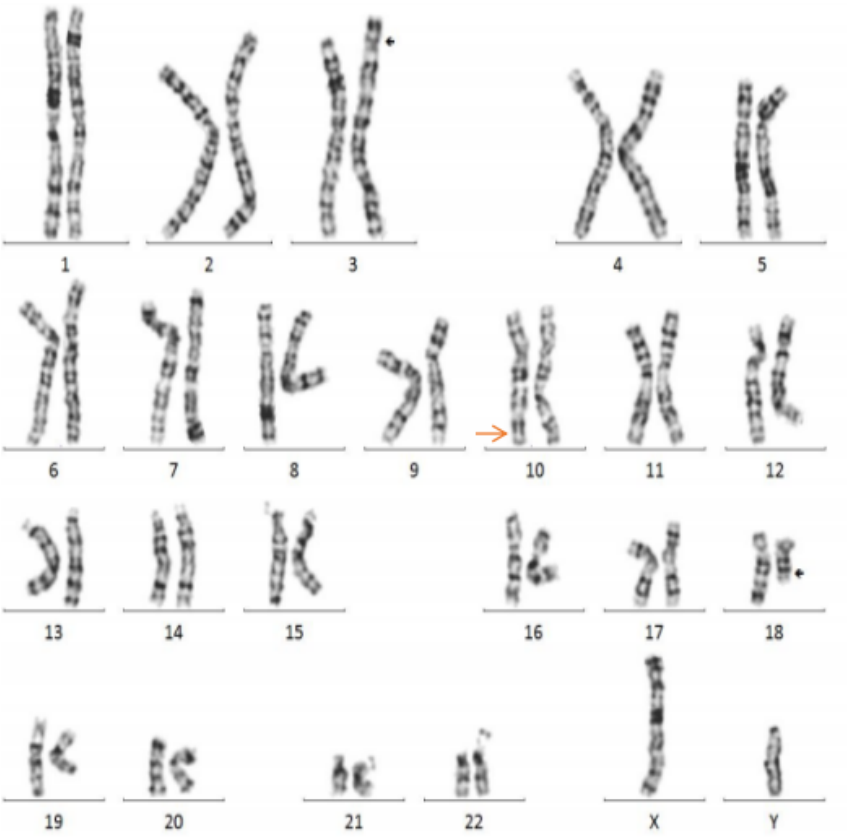

Figure 1

karyotpye of the husband in 550-bands level black arrow:apparent translocation;red arrow:cryptical translocation

Figure 2

the CMA result of the fetus:arr[hg19]:3p26.3p26.1(61,891-4,742,752)×3,10q26.13q26.3(125,728,807-135,426,386)×1

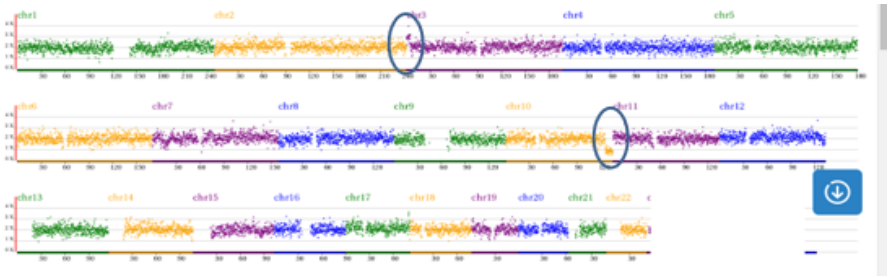

Figure 3

NGS result of embryo no. 3:+(3)(p26.3-p26.1)(4.8Mb),-(10)(q26.13-q26.3)(9.61Mb)