Whole-genome mate-pair sequencing of apparently balanced chromosome rearrangements reveals complex structural variations: two case studies

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Case Report

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Abstract

BACKGROUND: Apparently balanced chromosome rearrangements (ABCRs) in non-affected individuals are well-known to have high reproductive risks such as infertility, abnormal offspring, and pregnancy loss. However, caution should be exercised in genetic counseling and reproductive intervention because cryptic unbalanced defects and genome structural variations beyond the resolution of routine cytogenetics may get omitted.

CASE PRESENTATION: Two cases of ABCRs were recruited in this study. In family 1, the couple suffered two terminated pregnancies and underwent labor induction. Single nucleotide polymorphism (SNP) array analysis of the aborted sample from the second pregnancy showed a 10.8 Mb heterozygous deletion at 10q26.13q26.3 and a 5.5 Mb duplication at 19q13.41-q13.43. The non-affected father was diagnosed as a carrier of three-way complex chromosomal rearrangement [t(6;10;19)(p22;q26;q13)] by karyotyping. Whole-genome mate-pair sequencing revealed a cryptic breakpoint on derivative (der) chromosome 19 indicating that the karyotype was a more complex structural rearrangement including four breakpoints. Three genes, FAM24B, CACNG8, and KIAA0556, were disrupted without causing any abnormal phenotype in the carrier. In family 2, the couple suffered from a spontaneous miscarriage. They had an affected child with multiple congenital malformations and an unbalanced karyotype, 46,XY,der(11)t(6;11)(q13;p11.2). The female partner was considered a balanced translocation carrier with the karyotype 46,XX,t(6;11)(q13;p11.2)dn. Further SNP array and fluorescent in situ hybridization (FISH) indicated a cryptic insertion between chromosome 6 and chromosome 11. Finally, whole-genome mate-pair sequencing revealed an extremely complex genomic structural variation, including a cryptic deletion and 12 breakpoints on derivative chromosome 11[der(11)], and 1 breakpoint on derivative chromosome 6 [der(6)].

CONCLUSIONS: Our study investigated two rare cases of ABCRs and demonstrated that whole-genome mate-pair sequencing is a powerful approach to analyze the genome complex structural variation. In case of ABCRs detected by conventional cytogenetic techniques, whole genome sequencing (WGS) based approaches should be considered for accurate diagnosis, effective genetic counseling, and correct reproductive intervention to avoid recurrence risks.

Background

Apparently balanced chromosome rearrangements (ABCRs), including translocation, inversion, and insertion, involve exchange of genomic regions between non-homologous chromosomes without the gain or loss of genetic material. Simple ABCR is a two-breakage rearrangement, whereas complex chromosomal rearrangement (CCR) indicates chromosome abnormalities with three or more breakpoints. CCRs are rare in the population and only around 380 cases have been reported till date [1, 2]. Although most ABCRs are associated with a normal phenotype, they show high reproductive risks including infertility, recurrent spontaneous miscarriage, offspring with developmental defects and so on [3–5].

Reproductive risk increases with an increase in the number of breakpoints, and the particular characteristics of the rearrangement are associated with a peculiar mis-segregation mode in meiosis. For example, in reciprocal translocation, small translocated segments are prone to adjacent-1 segregation, small centric segments are apt for adjacent-2 segregation, and a small quadrivalent is usually associated with 3:1 disjunction. Indeed, some translocation carriers have more than 50% risk of bearing an abnormal child, whereas others have less than 1% risk. Therefore, it is crucial to accurately diagnose the karyotype for ABCR carriers with normal phenotype to determine the reproductive risk and choose an appropriate mode of bearing to avoid birth defects; these methods would include spontaneous pregnancy combined with prenatal diagnosis, preimplantation genetic testing, and use of donor sperm or egg.

Accurate cytogenetic diagnosis depends on the resolution of the testing techniques used. Previously, identification of ABCRs and further breakpoint confirmation were mainly based on high-quality G-banding of the metaphase chromosomes. However, due to the limited resolution of conventional cytogenetic technologies, only large structural rearrangements (> 5 Mb) could be identified. As a result, submicroscopic structural rearrangements could still be missed, even if high-resolution banding technology were used. Molecular testing techniques including FISH, CMA, and WGS, have greatly improved the ability to identify ABCRs [6–10]. Recent studies have shown that WGS can detect extremely complex balance rearrangements, including genome structural variations at the molecular level, which cannot be detected by conventional G banding techniques [11].

In this study, we analyzed the structural features of two cases of CCR using low pass whole-genome mate-pair sequencing in order to perform risk evaluation of ABCR carriers, and to examine complex genome structure variations in ABCRs detected by conventional cytogenetic technology.

Case presentation

Family 1: A couple (I-1 and I-2 in Fig. 1A), with the husband aged 32 years and the wife aged 27 years, visited our hospital for genetic counseling and birth guidance. They had conceived two natural pregnancies, which were terminated due to an abnormal fetus image during routine prenatal check-up. In the first pregnancy, B-ultrasonography at week 13 of pregnancy showed fetal growth retardation, equivalent to the size of a 12 W fetus, NT thickening, and a small amount of regurgitation in the reverse tricuspid valve of the venous catheter. In the second pregnancy, four-dimensional B-ultrasonography revealed a thickened fetal neck skin fold, lymphatic hydrocystoma of the neck, wide eye distance, short nasal bone, and hydramnios. Chromosome microarray (CMA) testing using SNP 750K for amniotic fluid cells (AFC) showed that the fetus had an unbalanced chromosome rearrangement between chromosomes 10 and 19, i.e. $\text{arr}(\text{hg}19)10\text{q}26.13\text{q}26.3(124,625,736 - 135,426,386) \times 1$ (10.8 Mb deletion) and $\text{arr}(\text{hg}19)19\text{q}13.41\text{-q}13.43(53,487,026 - 58,956,816) \times 3$ (5.5 Mb duplication), respectively. The pregnancy was subsequently terminated. Karyotype analysis showed a normal karyotype in the wife, whereas the husband was a complex translocation carrier with the karyotype $46,\text{XY},\text{t}(6;10;19)(\text{p}22;\text{q}26;\text{q}13)$ (Fig. 1B). The husband had normal semen parameters.

Family 2: This couple (II-3 and II-4 in Fig. 2A), with both the husband and wife aged 36 years, visited our hospital to conceive a healthy baby. They had four natural pregnancies after marriage. The first and the third pregnancies were naturally miscarried in the first trimester due to unknown reasons. At the second pregnancy, they delivered a healthy daughter with a normal chromosome karyotype. At the fourth pregnancy, they gave birth to a boy with anal atresia, congenital heart disease, and penile scrotal transposition; his karyotype analysis at birth showed an unbalanced translocation 46,XY, der(11)t(6;11)(q13;p11.2)mat. He only survived for more than 100 days after birth. The male partner (II-3) in this couple had a normal karyotype, whereas the female partner carried a de novo translocation between chromosome 6 and chromosome 11; her karyotype was interpreted as 46,XX,t(6;11)(q13;p11.2)dn. The parents (I-1 and I-2) of the female partner both had a normal karyotype.

Materials And Methods

Karyotyping analysis was performed for eight individuals including I-1 and I-2 in family 1, and I-1, I-2, II-3, II-4, III-3, and III-5 in family 2. SNP array analysis was performed for II-2 in family 1. II-4 in family 2 underwent SNP array analysis and FISH testing. WGS was performed for both I-1 in family 1 and II-4 in family 2.

Karyotyping analysis, FISH, and SNP array were performed as per our previous study [12]. Briefly, metaphase G-banding of approximately at the 400–550 band level was performed using peripheral blood lymphocytes. Metaphase FISH was performed using whole chromosome painting probe 6 (WCP6) and centromere-enumeration probe 11 (CEP 11). Single nucleotide polymorphism (SNP) microarray analysis was performed using Cytoscan 750K chip (Affymetrix, Santa Clara, CA, USA) separately as per our previous report [13]. The data were analyzed using chromosome analysis software ChAS (Affymetrix, USA).

DNA was isolated from peripheral blood lymphocytes using a QIAamp® DNA blood midi kit (QIAGEN, Hilden, Germany). Low-coverage whole genome massively parallel sequencing was performed in two carriers for excluding the cryptic chromosome structural rearrangements. Briefly, a non-size selected mate-pair library was prepared using ~ 3 µg of genomic DNA and was then subjected to 50-bp-end multiplex sequencing on the Illumina HiSeq™ X10 platform. After automatically removing adaptor sequences and low-quality reads, high quality paired-end reads were aligned to the NCBI human reference genome (GRCh37/hg19) using SOAP2. Uniquely mapped reads were selected for subsequent analysis as previously described in detail [12]. After bioinformatics analysis, we obtained the candidate breakpoint regions. The precise breakpoints were further confirmed by PCR and Sanger sequencing, and the genomic locations of the breakpoints were analyzed according to the February 2009 (GRCh37/hg19) assembly in the UCSC Genome Browser (<http://genome.ucsc.edu>). To validate the imbalanced karyotype, FISH was performed on the metaphase chromosomes from peripheral blood lymphocytes using whole chromosome probes (WCPs) for chromosomes 6 (CytoTrend, HK, China) and the centromere probe for chromosome 11 (Abbott-Vysis, USA) following the manufacturer's instructions.

Results

Family 1. SNP array analysis of the aborted sample in the second pregnancy showed a 10.8 Mb heterozygous deletion at 10q26.13q26.3 and a 5.5 Mb duplication at 19q13.41-q13.43. G-banding karyotype analysis showed that the female partner had a normal karyotype, whereas the male partner had a complex translocation between chromosome 6, 10, and 19 (Fig. 1B). To precisely determine the karyotype, further WGS analysis was performed and two rearrangement breakpoints on chromosome 19 were indicated, 19q13.41 and 19q13.42, respectively (Fig. 1C). The intermediate segment 19q13.41-19q13.42 of the translocated chromosome 19 was inserted into the derived chromosome 10. Finally, the four breakpoints of chromosome 6, 10 and 19 were confirmed to be involved in the complex balanced rearrangement of karyotypes, and a total 3 genes, FAM24B, CACNG8, and KIAA0556, were disrupted.

Family 2. G-banding karyotype analysis showed that the karyotype of the male partner (II-3) was normal, whereas the karyotype of the female partner showed a reciprocal translocation between chromosome 6 and chromosome 11 (Fig. 2B). Fluorescence in situ hybridization (FISH) analysis with a chromosome 6 painting probe revealed that an intermediate segment 6q21-q25.1 was inserted into 11p14.1, the middle region of the short arm of chromosome 11 (Fig. 2D). In order to precisely map the insertion rearrangement, WGS was performed and revealed 12 breakpoints in the 106.448-150.463 Mb region of the long arm of chromosome 6 (6q21-q25.1), associated with a heterozygous deletion in the 145.943-147.066 Mb region of the long arm of chromosome 6 (Fig. 2E). Chromosome microarray (CMA) using SNP (750K, Affymetrix) for the female partner showed a cryptic heterozygous microdeletion in the 146–147 Mb region of chromosome 6q (Fig. 2C), which confirmed the reliability of WGS analysis. The karyotypes of the parents of the female partner and her healthy daughter were normal, whereas her affected son inherited her derived chromosome 11, which could interpret the normal phenotype for her daughter and the abnormal phenotype for her son. Surprisingly, 13 breakpoints in chromosome 6 and 11 were identified in the karyotype of the female partner, indicating that WGS can detect complex genome structural variations. Due to insufficient DNA, precise breakpoint analysis by PCR was not carried out.

Discussion

Detection of ABCR carriers is very important in ABCRs with normal phenotype owing to a high risk of recurrent spontaneous miscarriage and birth defects [11, 12, 14]. In this study, two couples from different families had a history of recurrent adverse pregnancies, suggesting the necessity for cytogenetic testing. Karyotype analysis and further WGS revealed that the patients harbored complex chromosome rearrangements. Our study shows the advantages of WGS in karyotype analysis.

The conventional techniques can easily miss submicroscopic chromosome abnormalities or cryptic rearrangements with similar chromosome band modes due to the limitations of testing sensitivity [11]. Our study showed that low-coverage WGS technique has all the advantages of the previously used testing techniques; it can detect the chromosome rearrangement of large segments as in traditional

karyotype analysis, and can detect submicroscopic copy number variation similar to a chromosome microarray. In particular, it can detect cryptic genome structure variation.

Insertion is a three-break rearrangement with an incidence of only 1/80000 in previous studies [15]. However, a submicroscopic insertion was found in each family included in this study. In family 1, a fragment of chromosome 19 was inserted into chromosome 10, and in family 2, a large fragment of chromosome 6 was inserted into chromosome 11. Type IV CCRs configurations will be formed during the pachytene stage of meiosis for the carrier in family 1 (Fig. 1D) [1, 16]. The CCR with an inserted middle segment can produce new rearrangements and result in higher reproductive risk, increased unbalanced gamete production, and consequently, affected offspring. Our study indicates that insertion might be found to be much more common after the testing resolution is increased. As insertion itself can lead to risk of spontaneous abortion and birth defects, the reproductive risk is found to be higher compared with that obtained in the previous testing results showing a three-way translocation in family 1 and a simple translocation in family 2.

Findings of more submicroscopic chromosome abnormalities might illustrate some unexplained spontaneous miscarriages and birth defects, and could provide a basis for accurate genetic counseling. However, improvement of testing sensitivity may also cause new problems for the interpretation of results and clinical genetic counseling, such as how to express the complexity of chromosome structure rearrangement due to a lack of nomenclature based on WGS, how to judge the pathogenicity of small fragments of CNV, how the cryptic genome structural rearrangements alter the pattern of meiotic recombination, and so on. Thus, additional data on genome structural variations using WGS technique are needed to answer these questions.

Conclusions

In conclusion, we present two rare cases of ABCRs with cryptic insertion and minute imbalance structural abnormalities identified by whole-genome mate-pair sequencing. Our study showed that WGS breakpoint studies can facilitate improved understanding of complex genomic structural variations. In the future, the incidence of cryptic CCR should be investigated in a large cohort carrier with ABCR diagnosed by traditional G banding karyotype.

Abbreviations

WGS: whole-genome sequencing; SNP: single-nucleotide polymorphism; FISH: fluorescence in situ hybridization; CNV: copy number variation; PCR: polymerase chain reaction; WCP: whole chromosome painting.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of the Reproductive and Genetic Hospital of Citic-Xiangya, and written informed consent was obtained from all the participants prior to the genetic study.

Consent for publication

Written informed consent forms were obtained from the two couples.

Availability of data and materials

The datasets generated and 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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Authors' contributions

TY performed the karyotyping analysis experiments, interpreted the results, and, drafted the initial manuscript. CD collected patient data, performed the FISH experiments, and interpreted the results. TYQ conceived the study, participated in its design, helped draft the initial manuscript, and wrote the manuscript. All authors have read and approved the final manuscript.

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Figures

Figure 1

Molecular cytogenetic analysis for Family 1. (A) The pedigree of family 1 with the proband (II-2) indicated by an arrow. Black spot, induced labor; open symbols, unaffected individuals; square with black spots, carriers; circles, females. (B) The husband in family 1 was a carrier for a complex translocation between chromosome 6, 10, and 19 by G-banding analysis. (C) Whole genome DNA sequencing of peripheral blood from the carrier revealed four breakpoint rearrangement karyotypes. The genetic material from chromosomes 6, 10, and 19 is illustrated as purple, yellow, and blue lines, respectively. BP stands for breakpoint. (D) Type IV CCR Hexavalent Configurations. type IV CCR, as refined by whole-genome mate-pair sequencing in the current study. The additional breakpoint as well as possible recombination at the “middle segment” in type IV CCR increases the percentage of unbalanced gametes, and subsequent reproductive risk. Genetic material from chromosomes 6, 10, and 19 is illustrated in purple, yellow, and blue lines, respectively.

Figure 2

Molecular cytogenetic analysis for Family 2. (A) The pedigree of family 2. (B) The wife in family 2 showed recombination chromosome 6 and 11 by G-banding analysis. (C) The results of the CytoScan750K_Array. The figure shows the signal of loss (Red arrow) in chromosome 6. (D) The results of WCP 6 (Red signal)/CEP 11 (White signal) FISH show insertion translocation between chromosome 6 and 11. (E) Whole genome DNA sequencing of peripheral blood from the carrier revealed thirteen breakpoints and rearrangement in the karyotypes with a cryptic deletion on chromosome 6. Genetic material from chromosomes 6 and 11 are illustrated as purple and blue lines, respectively. BP stands for breakpoint.