Familial complex chromosome rearrangement (CCR) involving 5 breakpoints on chromosomes 1, 3 and 13 in a severe oligozoospermic patient

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Introduction

Complex chromosome rearrangement (CCR) is a structural chromosomal aberration that involves three or more breakpoints located on two or more chromosomes. CCRs may be grouped into three major categories depending on different characteristics. According to chromosome numbers involved and the breakpoints per chromosome, CCR can be classified into double two-way exchanges, three-way exchanges, and more complicated or the exceptional CCRs [17, 26]. Based on the origin or means of transmission, CCR can be divided into familial and de novo [11, 18]. Depending on unchanging or loss/gain of genetic materials, CCR can be classified into balanced or unbalanced. It is well-known that carriers of unbalanced CCR may lead to significant clinical consequences such as dysmorphic features, multiple congenital anomalies and mental retardation or subnormality in their offspring [2, 3] while carriers of the balanced CCR usually have normal phenotypes and remain undetected in family members through multiple generations. Recently, studies have revealed

that carriers of the balanced CCR are prone to infertility and recurrent abortions, and predispose to have offspring with unbalanced rearrangements due to either malsegregation of the derivative chromosomes or formation of a recombinant chromosome [17]. In general, most familial cases have a normal phenotype with apparently balanced rearrangements [20]. In contrast, half of de novo CCRs are unbalanced, and remaining half are apparently balanced but associated with multiple structural anomalies as well as mental retardation [7].

In the present study, we describe a severe oligozoospermic patient who inherited from his mother a balanced CCR that involves five breakpoints on three chromosomes 1, 3, and 13. To the best of our knowledge, this is a new case of CCRs identified in the human population.

Case report

The patient was a 26-year old man being an IT worker who got married for 3 years with normal libido but his wife never got pregnant. His wife carries a normal karyotype with regular menstruation. His mother was 51 years old already in menopause and described a history of irregular menstruation, but didn't experience any miscarriages or other kinds of abnormal pregnancy. The patient is the only child who was born during the third year after parental marriage. The karyotype of his father is normal.

Semen analysis was performed according to the World Health Organization guidelines for semen analysis [36]. The result demonstrated oligozoospermia with 2.0 mL in volume with sperm concentration $<1 \times 10^6$ mL, forward motility $<1.0\%$ at 1 h, and no motility after 4 h, and actually only one sperm with motility was identified in 20 microscopic fields with $40\times$ magnification. Total ejaculate contained $<2 \times 10^6$ sperms in 2 mL on the average, but the sperm morphology looks normal.

Capsule An infertile male with severe oligozoospermia was inherited a complex chromosomal rearrangement involving 5 breakpoints and 3 chromosomes (1, 3 and 13) from his mother and detected no Y-chromosome microdeletions.

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The hormone test was done. The values for testosterone (T), prolactin (PRL), follicle-stimulating hormone (FSH), progesterone (P), luteinizing hormone (LH), and estradiol (eE2) were all within the normal range.

The ethics consideration

This study was performed at Linyi People's hospital in Shandong province. The hospital institutional ethical review committees (Ethics Committee of Linyi People's Hospital, Shandong, China) approved the protocol for this study, and the proband and his relatives provided written informed consent.

Cytogenetic analysis

Lymphocytes from peripheral blood samples were cultured for 72 h in RPMI-1640 medium supplemented with 10 % fetal bovine serum as described [24]. G-banding was performed with Olympus BX53 microscope (Olympus, Japan) equipped with Beion karyotype analysis software (Beion Medical Technology, Shanghai, China) following the published procedure [38]. At least 50 metaphases were analyzed, and 5 metaphases were photographed to determine the patients' karyotype. The karyotype was named according to ISCN [10].

Fluorescence in situ hybridization

Fluorescence In Situ Hybridization (FISH) was performed with bacterial artificial chromosome (BAC) clones (Acquired in cooperation with State Key Lab of Medical Genetics, Central South University, Changsha, China) including the region-specific probes for human chromosome 1: (RP11-421C4 for 1p36.33, RP11-634B7 for 1q44); the region-specific probes for human chromosome 3: (RP11-91P19 for 3p21.31, RP11-114L5 for 3q26.1.); and the region-specific probes for human chromosome 13: (RP11-893E5 for 13q14.2.). The FISH experiments were performed with Leica CW4000 cytogenetic workstation and Leica Image Processing software (CW4000Karyo, Leica, Germany). The counterstaining was done with DAPI.

Genetic testing of Y-chromosomal microdeletions

Genomic DNA was extracted from the peripheral blood of patients and controls using standard procedure. Six STSs, including sY84, sY86 for AZFa, sY127, sY134 for AZFb, and sY254, sY255 for AZFc, were chosen to detect microdeletions

according to EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions [31]. SRY gene (sY14) was used as internal control, and the DNA samples from fertile male and female served as positive and negative controls respectively. PCR amplifications were carried out in a final volume of 20 μ L solution containing 40 ng of genomic DNA, 2.5 μ M dNTPs, 1 μ M of each primer, 2.5 μ M $MgCl_2$, and 2.5 U Taq polymerase. The reaction profile was 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s with a final extension at 72 °C for 5 min. The PCR products were electrophoresed for 40 min at 130 V in a 2 % Tris-acetate-ethylenediaminetetraacetic acid (TAE) agarose gel containing ethidium bromide at a concentration of 1 mg/mL at room temperature. To confirm the accuracy, all the PCRs were repeated for three times and it was accounted if each of the results stay the same.

Results

The oligozoospermic male has an aberrant karyotype of complex chromosome rearrangement

To understand what the possible cause of the male's sterility is, a G-banding analysis was first performed on his peripheral white blood cells. The result revealed a complex chromosome rearrangement involving chromosomes 1, 3 and 13 (Fig. 1a). Carefully analysis finally established an unique karyotype as 46, XY, der(1) (3qter→3q12::1p32→1q42::3p13→3pter), der(3) (1pter→1p32::3p13→3q12::13q14.1→13qter), der(13) (13pter→13q14.1::1q42→1qter).

Verification of the derivative chromosomes using the region specific probes

To further verify the chromosomal breakpoints and translocation in der(1), der(3), and der(13), we used the region specific probes derived from the human chromosomes 1, 3 and 13 (for details, see materials and method). The probes were labeled with 3 colors, green, pink, and red. The result confirmed all the translocated segments at the right breakpoints as established (Fig. 2).

The aberrant karyotype is inherited from his mother

To explore if the aberrant karyotype is in a familial pattern, i.e., if the patient's CCR is inherited from his parents, we performed the G-banding analysis on the peripheral lymphocytes collected from the parents. The result demonstrated that his father's karyotype was normal, but his mother's was aberrant. Carefully characterization of the mother's karyotype with the same techniques described above revealed a maternal

Fig. 1 Chromosomes G-banding shows the rearrangement involving chromosomes 1, 3 and 13. **a** shows the G-banding result for the patient, and **b** shows the G-banding result for the patient's mother. The arrows in the figures mark the derivative chromosomes

complex chromosome rearrangement (CCR) with the same chromosomal breaking points involving chromosomes 1, 3 and 13 (Fig. 1b), indicating the patient's CCR was inherited from his maternal side.

Tracing the CCR in his mother's family

To trace more about the history of the CCR inheritance, we conducted cytogenetic analysis on his grandparents in maternal side and on all his mother's siblings. The result shows that except the patient's mother, all the others are normally both in karyotype and phenotype (Fig. 3).

Detection of Y-chromosomal microdeletions

To explore if the CCR described above is merely an accidental coincidence but not the real chromosomal anomaly causing the infertility, we performed a genetic testing with PCR to detect the Y-chromosomal microdeletions. Six STSs were chosen from AZFa, AZFb, and AZFc regions according to the EAA/EMQN best practice guidelines [31]. The result shows no Y-chromosomal microdeletions (Fig. 4).

Discussion

In the present report, we describe a severe oligozoospermia in an adult male who inherits a balanced CCR involving 3 chromosomes and 5 breakpoints from his mother but presents an apparently otherwise normal phenotype. The three derivative chromosomes are described as der(1) (3qter→3q12::1p32→1q42::3p13→3pter), der(3) (1pter→1p32::3p13→3q12::13q14.1→13qter), der(13) (13pter→13q14.1::1q42→1qter). The five breakpoints locate at 1p32, 1q42, 3p13, 3q12, and 13q14.1. According to chromosome and breakpoint numbers involved, this CCR should be classified as the complicated or type II CCR [26]. Until now less than 255 CCRs have been reported [9, 27], but only a few carriers of familial and balanced CCR were reported to be associated with the oligozoospermia.

Currently the mechanism of CCRs is not fully understood yet, however, it is generally believed that the transmission of the familial CCR occurs mainly via the maternal line, and the origin of de novo CCR can be paternal through spermatogenesis arrest [2, 5, 20]. Our data confirmed the patient's CCR is inherited from his mother, but we can not present any data to verify the original de novo CCR to be paternal. We believe the CCR may randomly come from either of grandparents by gametogenesis.

Very little can be interpreted for a male's prone at risk of sterility even the CCR is inherited from a phenotypically normal and fertile mother. Our PCR detection result of Y-chromosomal microdeletions proved this furthermore. Some data suggest that an acrocentric chromosome involved in a CCR may predispose to male sterility [30], which seems to be consistent to our case since chromosome 13 is typically acrocentric. De Gregory et al. suggested that some cells escape the mechanism for proper crossing-over during spermatogenesis in which chaotic breaks allow reunion of several chromosomes and lead to CCRs [8]. On the other side, it has been suggested that testis may play a central role in the process of gene birth and evolution based on genomic studies [15, 35, 37]. One of the evidences is that retrogenes are extremely active in testis which may partly explain why the chaotic breaks of chromosomes can happen and the reunion of the chromosomal segments can occur for the de

Fig. 2 Fluorescence in situ hybridization to verify the corresponding rearrangements in der(1), der(3), and der(13) using the region-specific probes from human chromosome 1, 3, and 13. F2-1 shows the FISH result for the patient, and F2-2 shows FISH result for the mother. In each set of the pictures, A: FISH showing signals on the normal chromosome 3 and the derivative chromosome 1 (der-1). The green color represents the probe RP11-114L5 (3q26.1), and the pink color represents probe RP11-91P19 (3p21.31) respectively; B: FISH showing signals of the normal chromosomes 1, 13 and the derivative chromosomes 3, 13. The red color represents the probe RP11-421C4 (1p36.33), the pink color represents the probe RP11-893E5 (13q14.2), and the green color represents the probe RP11-634B7 (1q44) respectively

novo CCRs. It is very clear that CCR is a very rare event and also a disaster for individual patients, but it is also one kind of the genetic materials to drive the evolution in the biology view.

Batista et al. stated that familial CCRs tend to involve fewer chromosomes and breakpoints than de novo CCRs [3]. In our case, the CCR involves 3 chromosomes and 5 breakpoints which may be an exception for Batista's generalization. Ergul et al. reported an oligozoospermic male with a familial CCR involving 3 chromosomes and 4 breakpoints [9]. Kim et al. analyzed 10 male cases of familial CCRs with fertility problems, 4 of which belong to complicated CCRs. They conclude that severe degree of the sterility is proportional to complexity (more than one breakpoint per chromosome) rather than number of chromosomes and location of breakpoints involved. They also concluded that the breakpoints on

chromosomes 3, 4q, 11q, 12q, and 13q are related to spermatogenic failure, which are closely consistent with our data and support our findings [17]. Bache et al. studied 464 infertile male with balanced rearrangements and found that the breakpoint at chromosome 1 is most predominant. These data are consistent with our case [1].

In the present case of oligozoospermia, the CCR involves balanced rearrangements of autosomes. It is well-established that spermatogenesis is determined by genes located on the sex chromosome such as sex region on Y chromosome (SRY) and azoospermic factor region (AZF). So it raises the question about how the autosomes can be responsible for oligozoospermia. It was reported that among the men with chromosomal abnormalities, 13.7 % were detected as azoospermia, and 4.6 % were detected as oligozoospermia [34]. Lissitsina et al. reported that sex chromosome abnormalities

Fig. 3 The family pedigree describes the CCR transmission from the mother to the patient

Fig. 4 PCR result of Y-chromosomal microdeletions detection. Six STSs were used to detect Y-chromosomal deletions in AZFa (sY84, sY87), AZFb (sY127, sY134) and AZFc (sY254, sY255) regions according to the EAA/EMQN best practice guidelines. **S** stands for DNA sample from the patient, **P** stands for the positive control DNA from fertile men, and **N** stands for the negative control DNA from female

are predominant (12.6 %) among azoospermic men, whereas autosomal abnormalities are a little more frequent (3 %) among oligozoospermic male [21], suggesting that most of the oligozoospermia are not related to sex chromosome or sex related genes located on autosomes such as SOX9 [12]. Several lines of the evidences have been reported to explain oligozoospermia caused by autosomal CCRs, but there are many contradicted data existing [22]. First of all, chromosomal rearrangements can change chromosomal structures and result in asynapsis during meiosis, which in turn cause impaired spermatogenesis [6]. Studies on mice show that the asynapsed regions can trigger the meiotic checkpoint machinery and in turn stops the gametogenesis, which may explain the association of chromosomal abnormalities with deficient sperm concentration in oligozoospermic patients [1, 25]. Secondly, the CCR associated derivative chromosomes can result in improper synapses and incorrect disjunction, which in turn cause abnormal segregation or meiotic failure leading to greatly reduced spermatogenesis. In the most common rearrangement form, for examples, the three-way CCRs, the segments from three chromosomes break off, translocate, and ligate. During meiosis, the three-way CCR heterozygote will come together and form a hexavalent configuration. Even the disjunction is symmetric, it can produce 20 possible gametic combinations, and 90 % of the combinations will produce unbalanced gametes by adjacent type segregation. If the disjunction is asymmetric, a large variety of extremely unbalanced gametes will occur, and most of them will die before the spermatogenesis finish, which could explain the underlying mechanism for oligozoospermia [32]. Kausch et al. calculated that at least 70 possible unbalanced gametic combinations occur for a carrier of the CCR involving 5 breakpoints in 4 chromosomes due to 4:4, 5:3, 6:2, and 7:1 segregations [16]. Accordingly, a greater complexity of rearrangement implies a greater range of abnormal gametes and much less normal sperms as final outcome. This is consistent with our case in which a complicated CCR leads to extreme low sperm concentration and almost no viable sperm can be seen in 20 microscopic fields with 40 $\times$ magnification. Kim et al.

reported that among 10 male cases of complex chromosome rearrangements (CCRs) with fertility problems, 6 carriers of double two-way or three-way exchanges showed recurrent abortions or oligozoospermia, and the remaining 4 carriers of extra complicated CCRs showed azoospermia [17]. In our case, the complicated CCR is associated with a severe oligozoospermia in stead of azoospermia, and it clearly demonstrated that a complicated CCR can be associated with oligozoospermia too. Madan reviewed the data for 18 infertile males and 17 fertile men with CCRs [22]. He found 10/18 infertile males and 9 /17 fertile men were carriers of the complicated CCRs, and mean breakpoint numbers for infertility and fertility groups are 4.3 and 4.1 respectively. His conclusion is that neither the complex nor the breakpoint numbers have the value to predict the infertility risk for a given CCR type. He also claims that no difference exists between de novo and familial CCRs. Thirdly, cryptic genomic imbalances may play a big role. Sismani et al. reported that cryptic genomic imbalances were detected in 50 % and 16.6 % of the patients with either de novo or familial balanced translocations and abnormal phenotypes respectively. Many of the smaller imbalances may have been missed due to the low resolution (1 Mb) array-CGH used by the author [32]. These findings further highlight the necessity of higher resolution CGH and advanced whole genome approaches in study of the apparently balanced rearrangements. Finally, a true balanced CCR may affect the gene functions too. Recent studies have demonstrated that the true balanced CCRs can disrupts genes, make sub-microscopic deletions, insertion or duplications around or at the breakpoints, which can lead to gene inactivation at the breakpoints [4, 5, 28]. On the other hand, CCRs can also affect regulatory elements in the rearranged chromosomes, which lead to severe meiotic disturbance or chromosomal imbalance and causes dramatic sperm reduction [19, 20].

It has been a long time that people classify all the CCRs into balanced and unbalanced according to loss/gain or unchanging of the genetic materials after translocation. In general, carriers of balanced CCR have normal phenotypes but at risk of infertility and recurrent abortions, or producing

offspring with unbalanced rearrangements. Therefore, it is always a challenging problem in prenatal counseling to estimate the infertility risk for a given carrier of balanced CCR. Madan et al. established an empiric prediction stating that a 50 % risk probability exists for a carrier of balanced CCR to have a spontaneous abortion; and a 20 % risk probability to have a liveborn but abnormal child [23]. Recently, Madan raised a new proposal based on data from 84 CCRs. The proposal splits all balanced CCRs into 4 types, each with a risk of a different type of imbalance [22]. Our case is an apparently balanced translocation (ABT) and belongs to the Type III CCR according to Madan's classification. Currently there are no ways to predict a CCR carrier associated with a given reproduction problems.

Until now, few researches focus on the molecular mechanism in base pair level underlying a given CCR corresponding to a certain phenotype. It is why many "apparently balanced" CCRs are associated with different phenotypes without any explanation, even the siblings inherited the same balanced CCR from the same parent show very different reproductive problems ranging from fertility to infertility [13]. The challenging question is not only why and how CCRs occur but also why and how CCRs affect a given phenotype, i.e., what are damaged in the genome by CCRs which affect the phenotypes. Obviously, with the technology improves, we will get more insights into CCRs. For examples, with the high resolution CGH, whole genome SNP genotyping, next generation sequencing tools, variety of PCRs, and chromosome sorting, now we understand that 40 % of patients observed as 'balanced translocations' were unbalanced and, remarkably, 20 % of the reciprocal translocations were, instead, complex rearrangements [8, 29, 33]. There are many cryptic genomic imbalances, such as micro-deletion, duplication and inversion, existing at or around the breakpoints in the so called "balanced" CCRs that make the subtle differences between them [32]. Recently Sobreira et al. combined the targeted capture and next-generation sequencing (TBCS) tools to efficiently and accurately sequenced 8 breakpoints from 3 balanced CCRs and found that the cytogenetically defined "balanced translocation" actually is unbalanced CCRs [33]. We believe that armed with the high-resolution technology, we will understand more about the cytogenetically "invisible" differences in a given familial CCR, and supply accurate prenatal analysis for the counseling patients, which is the most urgent demand as our case described.

Though the familial CCRs may cause infertility, it does not mean the patients will absolutely lose the opportunity to have their own children. With new technologies constantly emerging in assisted reproduction field, many helps can be offered to the affected individuals. Recently Joly-Helas et al. reported the first case of ICSI (intracytoplasmic sperm injection) performed in an CCR-caused infertile male which

resulted in a female baby who showed normal phenotype but carried the same CCR as her infertile father does [14]. To avoid the "ICSI transferred CCRs" as reported, it may be necessary for ICSI-fertilized embryos to go through pre-implantation genetic diagnosis (PGD) before pregnancy. It is interesting to see more cases like this in order us to understand more precisely about the benefits and feasibility of ICSI for the infertile parents carrying the chromosomal aberration. Will the CCR-carrying children through ICSI experience the same infertility problem as their parents do when they reach to adulthood? Can we avoid CICI-transferred CCRs by more efficient screening methods with more affordable cost? It is all we are concerning and should know.

In conclusion, CCR is a rare structural aberration, and most of reported cases are CCRs de novo. Familial CCR rarely conveys abnormal phenotypes, but progeny of the carriers may be at risk of fertility. The present case confirmed this situation. Our data demonstrate a balanced CCR with familial transmission can be as complicated as the unbalanced de novo CCR which contradicts with the general believe that familial and balanced CCRs normally contain low complexity of rearrangements. Why the reproductive function can be affected differently in the fertile parent and the infertile progeny even they carry the same type of CCR? We can not explain this currently with the routine analysis tools such as G-banding, FISH and Y-chromosomal micro-deletions. To serve the genetic counseling patients better, advanced technologies such as high resolution aCGH, whole genome SNP genotyping and the cost-efficient next generation sequencing are desperately needed.

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