A novel male 2;4;14 complex chromosomal translocation with normal semen parameters but 100% embryonic aneuploidy

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Abstract

We report a case of a couple with a history of six spontaneous miscarriages in which a novel complex chromosomal rearrangement was detected in the male partner who had a totally normal semen analysis. Preimplantation genetic testing of their embryos demonstrated 100% aneuploidy.

Keywords Male complex translocation · Recurrent miscarriages

Introduction

Complex chromosomal translocations (CCTs) are translocations which involve more than two chromosomes at more than two breakpoints. These CCTs can be balanced or unbalanced, de novo, or familial. Literature suggests CCTs are associated with neoplasm as well as infertility.

Most CCTs associated with infertility report multiple first trimester losses. One report of a female patient with infertility was found to have CCT involving chromosomes 1, 8, and 11 in a manner identical to her mother and biological brother [1]. Another case report presents a known parental balanced complex chromosome translocation which resulted in a de novo, simple balanced translocation in the fetus [2]. This suggests crossover can impact the complexity of the translocation and therefore impact fecundity.

A karyotype analysis of 1063 Chinese couples with reproductive abnormalities yielded 339 pregnancies in patients with known CCT. Of these pregnancies, nearly 78% were spontaneous losses while nearly 10% resulted in phenotypically abnormal live born pregnancies [3]. Complex chromosomal relocations involving chromosome 1 were associated with

abnormal sperm in males while chromosome 8 was associated with phenotypically abnormal pregnancies.

The main cause for spontaneous miscarriages (SABs) is embryonic chromosomal aberration, estimated to be the case in 67% of SABs [4]. Approximately 5% of couples with recurrent pregnancy loss reportedly carry balanced translocations between two non-homologous chromosomes [5]. These individuals are known to have high rates of unbalanced gametes following meiotic segregation, resulting in embryos with abnormal chromosomal constitution. Further investigation is warranted to uncover which kinds of, and to what extent chromosomal involvement impacts phenotype [6].

Structural chromosomal aberrations are up to ten times more common in infertile men than fertile controls (3% in oligozoospermic men and 19% in men with non-obstructive azoospermia) [7–9], making karyotypes an important test in the work-up of infertile men. The incidence of chromosomal abnormalities increases with worsening semen parameters. Less than 1% of men with normal sperm concentration are identified to have chromosomal anomalies, while 5% of men with severe oligospermia have chromosomal abnormalities [10, 11]. Therefore, chromosomal analysis is not routinely performed, except for men with severe oligospermia or azoospermia, couples with recurrent pregnancy loss, or unexplained repeat failures of assisted reproductive techniques. Dong et al. [12] analyzed the relationship between male infertility and chromosomal translocations. A retrospective cohort of infertile men was recruited for chromosomal analysis using standard Giemsa stain banding. Among the 1056 infertile men, 2.1% had chromosomal translocations, including 0.7% with Robertsonian translocations, 1.0% with autosome-

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autosome reciprocal translocations, and 0.4% with gonosome-autosome reciprocal translocations.

This case report presents a novel complex translocation involving chromosomes 2, 4, and 14 in a phenotypically normal male with normal semen parameters and recurrent pregnancy loss at different gestational ages. Few publications have reported on the pregnancy outcomes of male carriers of individual translocations involving chromosomes 2 and 14. Zhang et al. [13] studied the clinical features of carriers of reciprocal chromosomal translocations involving chromosome 2 and the relationship of translocation breakpoints with infertility and recurrent pregnancy loss. Of the 82 translocation carriers, nine (11%) were carriers of a chromosome 2 translocation. Four cases had oligozoospermia or infertility, while five had normal semen. A literature review of 55 patients who were carriers of chromosome 2 translocations show that the most common break points observed in six patients were at 2p13 and 2q31. Breakpoints at 2p23, 2p13, 2p11.2, 2q31, and 2q37 were associated to both pregestational and gestational infertility, while other breakpoints were associated with gestational infertility. All breakpoints at chromosome 2 were correlated with gestational infertility.

Zang et al. [14] found that of 82 patients with balanced reciprocal translocations, 14 were carriers of the chromosome 4 translocations: four presented with pregestational infertility, clinical manifestations such as oligozoospermia, severe oligozoospermia, or azoospermia. Ten presented with gestational infertility, that is, able to conceive but with a tendency to miscarry. The breakpoint at 4q12 was associated with pregestational infertility, whereas the breakpoints at 4q13, 4q21, 4q25, and 4q32 were associated with gestational infertility. However, the breakpoint at 4q35 was associated with both pregestational and gestational infertility.

Sperm meiotic segregation was studied in four males heterozygous for the rare Robertsonian translocations t(13;15) and t(14;15) [15]. The frequencies of normal and balanced sperm resulting from the alternate mode of segregation ranged from 77.6 to 92.8%, confirming the prevalence of alternate segregation over other segregation modes in all Robertsonian translocations. The incidences of unbalanced complements ranged from 6.7 to 20.4%, with a significant excess of disomy rates over the complementary frequencies of nullisomy.

Three cases of rare Robertsonian translocation t(14;22) were investigated for meiotic segregation in sperm samples from male carriers using the fluorescent in situ hybridization (FISH) procedure [16]. The three carriers included two men with an abnormal semen analysis and one with normal semen parameters. In the three carriers, similar frequencies, ranging from 78.53 to 81.76%, were found for normal and balanced spermatozoa resulting from alternate segregation. The total proportion of unbalanced spermatozoa resulting from adjacent modes of segregation ranged from 17.59 to 20.94%.

Grasshoff et al. [17] reported a de novo CCT with five breakpoints involving chromosomes 4, 10, and 14 in a male carrier who was ascertained through a son presenting with mental retardation/congenital malformations due to an unbalanced karyotype with partial trisomy 14 and partial monosomy 4.

The variability on meiotic segregation might reflect differences in the location of breakpoints in translocated chromosomes, leading to the variable production of unbalanced gametes and the variable alterations of semen parameters.

Case presentation

We present a couple self-referred to our infertility clinic with a history of six recurrent first trimester SABs. The female partner is a 30-year-old G6 P0060 with no medical problems or contributory family medical history who had had six conceptions with the same partner, four of them resulting in biochemical pregnancies, one in an embryonic gestation, and the last one in a spontaneous miscarriage at approximately 13-week gestation with previous detection of fetal cardiac activity at 8-week gestation. None of these miscarriages had genetic testing of the products of conception.

Evaluation of the female partner resulted in normal hysterosalpingogram, normal hysteroscopy with benign proliferative phase endometrium; normal thrombophilia profile, normal prenatal laboratory results, normal baseline endocrine profile (AMH 3.99 ng/ml, FSH 5 mIU/ml, LH 2 mIU/ml, estradiol 36 pg/ml, TSH 1.02 uIU/ml, prolactin 4 ng/ml, blood type O+, carrier screening negative for 213 diseases), and normal female 46 XX karyotype performed using cultures peripheral blood lymphocytes and GTG banding (500 band resolution).

The male partner, a healthy phenotypically normal 33-year-old, had a normal semen analysis (32 million/ml, 84% motility, 7% normal forms) but an abnormal karyotype. Culture of peripheral blood lymphocytes and GTG-banding (500 band resolution) demonstrated a complex balanced translocation involving the long arm of chromosome 2, the short arm of chromosome 4 and the long arm of chromosome 14: 46, XY, t(2;4;14)(q21.1;p15.2;q22) (Fig. 1).

He has three brothers, two who had not attempted conception yet, and one with a history of a still birth at approximately 17-week gestation with no fetal karyotype obtained. Professional genetic counseling was obtained and no relevant family history was elicited. This couple did not wish to disclose this finding to his family, so further karyotyping of other members of the family could not be pursued.

The couple underwent 2 cycles of in vitro fertilization with intracytoplasmic sperm injection (ICSI) in two different institutions (one private and one academic) and preimplantation genetic screening (PGS) performed by two different commercial laboratories. The first cycle rendered seven metaphases II

Fig. 1 Male karyotype by G-banding technique (G band level 500–550) showing complex chromosome translocation involving chromosomes 2, 4, and 14 (arrows indicate break points)

(mII) oocytes, four with normal diploid fertilization (2PN) from which three developed to Gardner 3BB blastocysts on day 5. The second IVF cycle rendered 15 mIIs and 2 mIs (metaphase one) oocytes with 17 2PN after ICSI from which seven blastocysts Gardner grade 5BB were biopsied. PGS was performed with chromosomal microarray analysis (CCS) by dual-indexed sequencing of pooled libraries. Testing involved whole genome amplification followed by tagmentation of the DNA, and subsequent PCR reaction of adapter sequences. All embryos were vitrified immediately after biopsy while awaiting PGS results. Nine of the ten blastocysts biopsied were found to be abnormal and one blastocyst had no diagnosis due to lack of DNA amplification. A gain or loss at chromosomes 2, 4, and 14 was demonstrated in different combinations (Table 1). This couple did not have any embryos transferred.

Discussion

CCTs are rare events: approximately 70% of complex chromosomal rearrangements are detected in people with a normal

phenotype; 20–25% in patients with congenital abnormalities and/or mental retardation and 5–10% are detected during prenatal diagnosis [18]. Among phenotypically normal CCT carriers, most suffer reproductive failures and male infertility [6, 19]. Madan [20] reviewed 18 men referred for infertility and compared them to 17 fertile males. The number of extra complex CCTs in 10/18 infertile males did not significantly differ from 9/17 in fertile males, showing that an extra complex CCT is not necessarily associated with male infertility. To date, approximately 255 cases of CCTs involving three or more chromosomes have been reported, including only a few cases of successful pregnancy outcomes [18]. Frumkin et al. [21] estimates that the chance for balanced embryos for CCT carriers is approximately 9.25%, compared to a 20–30% chance of balanced embryos in cases of reciprocal translocation between two chromosomes [22]. Chen et al. [23] analyzed the characteristics of CCT in Chinese male carriers and its influence on male fertility. They found 49 CCT carriers among the 1625 males studied: 34.7% had three-way exchanges, 34.7% had two-way exchanges, and 30.6% had exceptional combinations. Azoospermia- or oligospermia-induced infertility was found in 38.8% of the CCT carriers.

Table 1 PGS results of day 5 embryos obtained after ICSI for the second IVF treatment

	Embryo						
	2	5	6	8	12	15	16
Chromosome							
1	2		2	2	2	2	2
2pter-q21.1	3		3	2	2	1	2
2q21.1-qter	3		3	1	1	1	3
3	2		2	2	2	2	2
4pter-p15.2	2		2	2	3	2	2
4p15.2-qter	2		2	1	2	2	2
5	2		2	2	2	2	2
6	2		2	P1	2	2	2
7	2		2	2	2	2	2
8	2		2	2	2	2	2
9	2		2	2	2	2	2
10	2		2	2	2	2	2
11	2		2	2	2	2	2
12	2		2	2	2	2	2
13	13		2	2	2	2	2
14pter-q22	1		1	3	2	2	2
14q22-qter	1		1	2	1	2	1
15	2		2	2	2	2	2
16	2		2	2	2	2	2
17	2		2	2	2	2	2
18	2		2	2	2	2	2
19	2		2	2	2	2	2
20	2		2	2	2	2	2
21	M1		2	2	2	M3	2
22	2		2	2	2	2	2
Sex	XY		XX	XY	XX	XY	XY
Interpretation	*	No DNA amplification	*	*	*	*	*

M mosaic, *P* partial

*Unbalanced translocation

A total of 87 pregnancies were achieved, among which 75.9% had spontaneous abortions, 9.2% had a dead malformed fetus, and 14.9% had a phenotypically normal offspring. Recurrent abortion was associated frequently with breakpoints on CCT-involved chromosomes 6, 7, 8, 11, and 16, while dyszoospermia mostly with breakpoints on CCT-involved chromosomes 10 and 14. A more recent study of 1063 Chinese couples with reproductive abnormalities showed that 124 of them were CCT carriers with a 77.6% risk for spontaneous abortions and 9.7% risk for an abnormal child [3]. Abnormal pregnancy was frequently associated with CCTs on chromosome 8, while dyszoospermia was frequently associated with CCTs on chromosome 1 among the males. So, male carriers of CCT can present with normal or abnormal semen parameters as well as with favorable or unfavorable reproductive outcomes.

This case report is unique since it identifies a fertile male CCT carrier with normal semen parameters but probably high degree of aneuploid spermatozoa based on recurrent first trimester miscarriages and lack of euploid embryos after PGS. It would be important to assess if the male partner carries any normal or balanced sperm prior to proceeding to IVF, but, unfortunately, all laboratories performing sperm fluorescent in situ hybridization contacted in the USA responded that there were no probes available in enough different colors to cover all the fragments involved in a single hybridization. Scriven et al. [24] reported meiotic outcomes of carriers of three-way translocations ascertained in cleavage-stage embryos. Fluorescent in situ hybridization probes were directly or indirectly labeled and sourced from different European manufacturers. A total of five probes were used for each translocation in order to detect all the theoretical unbalanced translocation products. Of the 29

embryos from the male heterozygotes, 6 (20.7%) were diagnosed to be normal or balanced, and 23 (79.3%) to be unbalanced. For male heterozygotes, using a PGD test with at least five probes reduced the risk of an unbalanced conception to < 1% with at least a 20% chance of a transferable result per embryo tested and a > 75% chance of transfer-testing six embryos (the median number in their experience for couples with a translocation, [25]). Using only three probes for the translocated segments, the risk of an unbalanced translocation product after PGD was 22.37%. Based on their models, these authors calculated the risk of a natural pregnancy live birth with an unbalanced translocation between 1 and 5.8% for a male carrier and 17.9% for a female carrier.

This case also reports a novel CCT involving chromosomes 2, 4, and 14 associated with recurrent pregnancy loss at various gestational ages ranging from preclinical to the second trimester. Stephenson and Sierra [26] reported that 36% of miscarriages associated with a parental carrier of a structural chromosomal rearrangement were found to have an unbalanced structural chromosome rearrangement, but, due to lack of pregnancy tissue in non-visualized pregnancy losses, chromosomal composition of these early losses is unknown. Kolte et al. [27] reported that non-visualized pregnancy losses contribute negatively to the chance for live birth: each non-visualized pregnancy loss confers a relative risk (RR) for live birth of 0.90 (95% CK 0.83; 0.97), equivalent to the RR conferred by each additional clinical miscarriage, raising the question of potential usefulness for performing parental karyotype screening on couples with multiple biochemical losses.

Patients should be made aware of this extremely low rate of chromosomally balanced embryos in CCTs, and it should be taken into consideration when planning a controlled ovarian stimulation cycle.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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