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Translocation breakpoints of chromosome 4 in male carriers: clinical features and implications for genetic counseling

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ABSTRACT. Cytogenetic analysis remains a powerful and cost-effective technology, and has wide applicability in genetic counseling for infertile males. Chromosomal rearrangements are thought to be one of the major genetic factors that influence male infertility. Some carriers with balanced reciprocal translocation have been identified as having oligozoospermia or azoospermia, and there is an association between balanced translocation and recurrent abortion. Researchers have reported the involvement of chromosome 4 translocations in male factor infertility and recurrent miscarriages. A translocation breakpoint might interrupt the structure of an important gene, and it is associated with reproductive failure. However, the clinical characteristics of the breakpoints in chromosome 4 translocations have not been studied. Here, we report the breakpoints in chromosome 4 translocation and the clinical features presented in carriers to enable informed genetic counseling of these patients. Of 82 patients with balanced reciprocal

translocations, 14 were carriers of the chromosome 4 translocation: four presented with pregestational infertility (clinical manifestations: oligozoospermia, severe oligozoospermia, or azoospermia), whereas 10 presented with gestational infertility (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. Chromosome 4 translocation carriers with pregestational or gestational infertility should be counseled on chromosomal breakpoints and the different technologies available to assist reproduction.

Key words: Male infertility; Chromosome 4; Balanced translocation; Breakpoint; Genetic counseling

INTRODUCTION

Karyotype analysis is a powerful, cost-effective, and long-established technology that remains widely applicable in genetics (Pasquier et al., 2016). It is a diagnostic tool that provides valuable input in genetic counseling for infertile males (Poli et al., 2016). Chromosomal rearrangements are thought to be one of the major genetic factors that influence male infertility (Pastuszek et al., 2015). Although they may be phenotypically normal, carriers of reciprocal translocations can experience reduced fertility or spontaneous abortions (Harton and Tempest, 2012). These effects are dependent on the specific chromosomes involved in the translocation, the locations of the breaks, and the frequency of chiasmata (Harton and Tempest, 2012; Godo et al., 2013). In some male patients, one of the translocation breakpoints interrupts an important gene structure, leading to infertility (Pernice et al., 2002; Bianco et al., 2011).

Previous reports indicate the involvement of balanced reciprocal translocations on chromosome 4 in male infertility and recurrent pregnancy loss (Kochhar and Ghosh, 2013; Vozdova et al., 2013; Zhang et al., 2015c). The *Homo sapiens* sperm tail PG-rich repeat-containing 2 gene (*STPG2*) (formerly known as chromosome 4 open reading frame 37 (C4orf37), mapped at the chromosomal loci 4q22.3 and 4q23, may be associated with male factor infertility (Yakut et al., 2013). The NOP2/Sun RNA methyltransferase family member 7 gene (*NSUN7*) is located on chromosome 4 and comprises 12 exons. The transversion mutation in exon 7 of the *NSUN7* gene is associated with asthenospermia (Khosronezhad et al., 2015). Additionally, the translocation breakpoints of chromosome 4 in male carriers are associated with recurrent miscarriages in their partners (Kochhar and Ghosh, 2013; Zhang et al., 2015c; Tunç et al., 2016).

The aim of this study was to determine the correlation between the clinical characteristics of male infertility and the carriers of translocation breakpoints in chromosome 4. We have also highlighted the importance of genetic counseling for infertile patients.

MATERIAL AND METHODS

Patients

We recruited 5235 men who had been diagnosed with infertility or who were receiving

counseling for infertility owing to genetic causes from the outpatient clinic of the Centre for Reproductive Medicine at the First Hospital of Jilin University, Changchun, China, between July 2010 and December 2015. All patients underwent a thorough physical examination and semen analysis, and were required to complete a detailed questionnaire pertaining to their smoking habits, marital status, medical history, and working conditions. Azoospermia and oligozoospermia were defined as described previously (Zhang et al., 2015b). The study protocol was approved by the Ethics Committee of the First Hospital of Jilin University, and written informed consent was obtained from all participants.

Cytogenetic analysis

All patients were subjected to cytogenetic analysis. Peripheral blood (0.5 mL) was collected in sterile tubes containing 30 U/mL heparin. Lymphocytes were cultured in appropriate culture media (Yishengjun; Guangzhou Baidi Biotech, Guangzhou, China) for 72 h, and subsequently treated with 20 mg/mL colcemid for 1 h. G-banding of metaphase chromosomes and karyotype analysis were performed using our previously published methods (Zhang et al., 2015a).

RESULTS

A total of 5235 male patients were included in this study. Conventional cytogenetic analysis revealed a reciprocal translocation in 82 (1.57%) of the study participants. Of these, 14 patients (14/82; 17.07%) were carriers of a chromosome 4 translocation. Four (4/14) patients exhibited pregestational infertility (clinical manifestations: oligozoospermia, severe oligozoospermia, or azoospermia), whereas the remaining 10 patients exhibited gestational infertility (the patient's partners were able to conceive, but had a tendency to miscarry). The results of a karyotype analysis of the 14 patients expressing the chromosome 4 translocation are summarized in Table 1.

Table 1. Karyotypes of chromosome 4 translocation carriers and their clinical features.

Infertility causes	Clinical findings	Karyotype
Pregestational infertility	Oligozoospermia, severe oligozoospermia, or azoospermia	46,XY,t(Y;4)(p11;p14) 46,XY,t(1;4)(p36;q31) 46,XY,t(4;13)(q12;q12) 46,XY,t(4;14)(q35;q24)
Gestational infertility	Normal sperm density; a history of miscarriage	46,XY,t(1;4)(p34;q32) 46,XY,t(4;5)(q21;p15) 46,XY,t(4;9)(q35;p13) 46,XY,t(4;11)(q25;q21) 46,XY,t(4;12)(q13;p13) 46,XY,t(4;14)(q25;q24) 46,XY,t(4;16)(q35;q22) 46,XY,t(4;18)(q21;q21) 46,XY,t(4;21)(q21;q12) 46,XY,t(4;22)(q35;q11.2)

The breakpoints at 4q35 were the most common, and were observed in four patients. The breakpoints at 4p14, 4q12, and 4q31 were related to pregestational infertility, whereas the breakpoints at 4q13, 4q21, 4q25, and 4q32 were related to gestational infertility. However, the breakpoint at 4q35 was related to both pregestational and gestational infertility (Table 2).

Table 2. Incidence of breakpoints on chromosome 4.

Breakpoints	Number of patients with pregestational infertility	Number of patients with gestational infertility	Total (%)
p14	1		1 (7.14%)
q12	1		1 (7.14%)
q13		1	1 (7.14%)
q21		3	3 (21.42%)
q25		2	2 (14.29%)
q31	1		1 (7.14%)
q32		1	1 (7.14%)
q35	1	3	4 (28.57%)

DISCUSSION

Male infertility is associated with structural chromosomal abnormalities (Suganya et al., 2015). Reciprocal translocations are often detected in infertile men. The authors of previous studies have reported that the presence of translocations alters the process of spermatogenesis (Stouffs et al., 2014), and other authors have described the involvement of chromosome 4 translocation in male infertility and recurrent miscarriages (Kochhar and Ghosh, 2013; Vozdova et al., 2013; Zhang et al., 2015c). In this study, 14 patients were carriers of the chromosome 4 translocation. The major limitation of this study was the small number of carriers of the chromosome 4 translocation; moreover, the molecular effect of this translocation was not investigated. However, this study provides some information for male carriers of chromosome 4 translocation receiving genetic counseling.

Balanced translocations are associated with an increased risk of male infertility, fetal wastage, and death (Godo et al., 2013). The former is divided into two types of reproductive failure: pregestational and gestational infertility. In this study, the breakpoints on chromosome 4 were found to be associated with pregestational or gestational infertility. The breakpoints at 4p14, 4q12, and 4q31 were associated with pregestational infertility, whereas those at 4q13, 4q21, 4q25, and 4q32 were associated with gestational infertility. However, the breakpoint at 4q35 was associated with both pregestational and gestational infertility, and was identified in four patients (one case of pregestational and three cases of gestational infertility). The breakpoint at 14q24 was present in the pregestational patient. The heat shock protein family A (Hsp70) member 2 (heat-shock 70-kDa protein 2) gene (*HSPA2*), with the chromosomal locus 14q22-q24, is involved in major intrasperm protein transport during spermiogenesis (Huszar et al., 2000). The breakpoints at 4p14 and 4q31 were associated with Yp11 and 1p36 translocations, respectively. Some genes on Yp11 and 1p36 are associated with spermatogenesis (Schnieders et al., 1996; Kim et al., 2015). It has not been reported that related genes with chromosomal loci 4p14, 4q31, and 14q35 are involved in spermiogenesis or male infertility. This indicates that the breakpoints at 4p14, 4q31, and 14q35 do not affect spermatogenesis. However, serine peptidase inhibitor, Kazal type 2 (*SPINK2*), with the chromosomal locus 4q12, is required for maintaining normal spermatogenesis (Lee et al., 2011). The breakpoint at 4q12 can interrupt gene structure, leading to abnormal spermatogenesis.

The breakpoints at 4q13, 4q21, 4q25, 4q32, and 4q35 were associated with recurrent pregnancy loss. Similarly, previous reports have indicated a correlation between the breakpoints at 4q13, 4q21, 4q25, 4q32, and 4q35 and recurrent abortion (Kochhar and Ghosh, 2013; Vozdova et al., 2013; Dong et al., 2014; Ghazaey et al., 2015). An analysis of related literature published in recent years revealed a close association between breakpoints in chromosome

4 translocation carriers and male infertility and reproductive failure. The karyotypes of, and breakpoints in, chromosome 4, and their related clinical symptoms, are summarized in Table 3. The breakpoint at 4q21 is predominantly associated with pregestational infertility, whereas the breakpoints at 4q13, 4q21, 4q25, 4q32, and 4q35are associated with gestational infertility.

Table 3. Breakpoints in chromosome 4 translocation carriers and clinical features reported in previous literature.

Karyotype	Breakpoints	Clinical findings	Reference
t(1;4)	1q32;4q25	Spontaneous abortions	Campbell et al., 1995
t(1;4)	1q43;4q13	Infertility	Mierla et al., 2014
t(1;4)	1p36;4q31	Severe oligozoospermia	Zhang et al., 2015c
t(3;4)	3p25.2;4q25	Normospermic	Vozdova et al., 2013
t(3;4)	3q29;4q26	Recurrent pregnancy loss	Kochhar and Ghosh, 2013
t(4;5)	4q25;5p15.2	Abortions	Ghazaey et al., 2015
t(4;5)	4q35;5p15	Recurrent miscarriages	Dutta et al., 2011
t(4;6)	4q23;6q21	Abortions	Ghazaey et al., 2015
t(4;6)	4q31.3;6q21	Recurrent spontaneous pregnancy loss	Gada Saxena et al.,2012
t(4;6)	4q33;6q27	Asthenospermic	Vozdova et al., 2013
t(4;7)	4q31;7p22	Recurrent pregnancy loss	Kochhar and Ghosh, 2013
t(4;7)	4q2.7;7p14	Normospermic	Vozdova et al., 2013
t(4;7)	4q2.7;7p14	Normospermic	Vozdova et al., 2013
t(4;7)	4q31;7p22	Recurrent pregnancy loss	Kochhar and Ghosh, 2013
t(4;8)	4q35;8p11.2	Normospermic	Vozdova et al., 2013
t(4;9)	4p15.2; 9p13	Recurrent fetal wastage	Celep et al., 2006
t(4;9)	4q31.1;9p24	Recurrent fetal wastage	Celep et al., 2006
t(4;9)	4q23.2;9q22.3	Recurrent pregnancy loss	Kochhar and Ghosh, 2013
t(4;10)	4p16.2;10p11.1	Asthenospermic	Vozdova et al., 2013
t(4;10)	4q35;10q13	Infertility	Gada Saxena et al.,2012
t(4;11)	4q33;11p11.2	Normospermic	Vozdova et al., 2013
t(4;12)	4p15.1;12p12.2	Recurrent pregnancy loss	Kochhar and Ghosh, 2013
t(4;13)	4q21.3;13q22	Recurrent fetal wastage	Fryns and Van Buggenhout, 1998
t(4;14)	4q31.3;14q22	Recurrent fetal wastage	Fryns and Van Buggenhout, 1998
t(4;15)	4q25;15q26.3	Recurrent pregnancy loss	Kochhar and Ghosh, 2013
t(4;15)	4q25;15q?	Recurrent fetal wastage	Celep et al., 2006
t(4;16)	4p13;16q21	Normospermic	Vozdova et al., 2013
t(4;17)	4q21;17p13	Three miscarriages	Dong et al., 2014
t(4;20)	4q32;20p12	Abortions	Ghazaey et al., 2015

Carriers of chromosome 4 translocations must receive appropriate counseling to apprise them of suitable reproductive options. Patients with pregestational infertility related to oligozoospermia must be counseled regarding chromosomal breakpoints and *in vitro* fertilization/ intracytoplasmic sperm injection. However, patients with gestational infertility should be counseled regarding prenatal testing or preimplantation genetic diagnosis, because these patients are at an increased risk of implantation failure and miscarriage (Vozdova et al., 2013).

In conclusion, 14 patients were carriers of chromosome 4 translocations; four expressed pregestational infertility and 10 presented with gestational infertility. The breakpoint at 4q12 was associated with pregestational infertility, whereas the breakpoints at 4q13, 4q21, 4q25, 4q32, and 4q35 were correlated with gestational infertility. Carriers of chromosome 4 translocations with pregestational or gestational infertility should be counseled on chromosomal breakpoints and the various technologies available for assisted reproduction.

Conflicts of interest

The authors declare no conflict of interest.

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