



Balanced reciprocal translocation at amniocentesis: cytogenetic detection and implications for genetic counseling

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Genet. Mol. Res. 15 (3): gmr.15038556
Received February 15, 2016
Accepted April 8, 2016
Published August 18, 2016
DOI <http://dx.doi.org/10.4238/gmr.15038556>

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ABSTRACT. Balanced translocation is a common structural chromosomal rearrangement in humans. Carriers can be phenotypically normal but have an increased risk of pregnancy loss, fetal death, and the transmission of chromosomal abnormalities to their offspring. Existing prenatal screening technologies and diagnostic procedures fail to detect balanced translocation, so genetic counseling for carriers remains a challenge. Here, we report the characteristics of chromosomal reciprocal translocation in 3807 amniocentesis cases. Of the 16 detected cases of fetal reciprocal translocation, 8 cases (50%) showed positive biochemical marker screening; 3 cases (18.75%) were the parental carriers of a chromosomal abnormality; 2 (12.5%) were of advanced maternal age, 2 (12.5%) had a previous history of children with genetic disorders, and 1 case (6.25%) was associated with positive soft markers in obstetric ultrasound. Chromosomes 5 and 19 were the most commonly involved chromosomes in balanced translocations. Of the 13

cases with fetal balanced translocations, 8 (61.5%) were inherited from a paternal chromosome, 3 (23.1%) from a maternal chromosome, and 2 (15.4%) cases were *de novo*. The incidence of balanced translocation at amniocentesis was 0.42%. Male carriers of reciprocal chromosome translocation appear to have a higher chance of becoming a parent of a child born by normal childbirth than female carriers.

Key words: Balanced translocation; Amniocentesis; Genetic counseling; Giemsa banding (GTG banding)

INTRODUCTION

Balanced translocations, whereby two different chromosomes exchange segments with no gain or loss of genetic material, are common structural chromosomal rearrangements in humans. Frequency rates have been reported to range from 0.08 to 0.3% in the general population (Zhang et al., 2015a). Carriers of balanced chromosomal translocations may be phenotypically normal, but are associated with an increased risk of pregnancy loss, fetal death, and the transmission of chromosomal abnormalities to their offspring through the production of a higher number of unbalanced spermatozoa (Godo et al., 2013). Because the carriers of balanced translocations have an increased risk of pregnancy loss, fetal death, and the transmission of chromosomal abnormalities to their offspring, or have a phenotypically normal child, genetic counseling for carriers of balanced translocations remains a challenge. In most couples with recurrent pregnancy loss, the number of female carriers with balanced chromosomal aberrations significantly exceeds that of male individuals (Kochhar and Ghosh, 2013). The success rate of natural pregnancy in couples where men carry translocations ranges from 30 to 70% (Ozawa et al., 2008). Paoloni-Giacobino et al. (2000) have also reported that carrier males suffer from primary infertility with severe oligoasthenoteratospermia or azoospermia, while at least two of three carrier women are fertile. Patients carrying balanced reciprocal translocations are subject to meiosis nondisjunction risk. Indeed, the mispairing of translocated chromosomes during the first meiotic division can give rise to different forms of segregation, which can result in balanced, unbalanced, and normal gametes of the translocated chromosomes (Pourjafari et al., 2012). The exact formation is dependent on the breakpoints and the chromosomes involved (De et al., 2015).

Several different technologies are used for prenatal screening tests and diagnostic procedures, including ultrasonography, the double-marker test [pregnancy-associated plasma protein A and free β -human chorionic gonadotropin (β -HCG)], the triple marker test (α -fetoprotein, free β -HCG, and unconjugated estriol), and array-based comparative genomic hybridization of fetal DNA in the maternal circulation (Sheth et al., 2015). These technologies are, however, limited to detecting the most common aneuploidies such as trisomies 13, 18, and 21, and do not detect balanced translocations (Wapner et al., 2012; Novelli et al., 2013; Konialis and Pangalos, 2015).

Prenatal testing and, alternatively, preimplantation genetic diagnosis (PGD), are recommended aspects of genetic counseling for balanced translocation carriers because these individuals are at an increased risk of implantation failure, miscarriage, or the delivery of affected offspring (Vozdova et al., 2013). Although PGD is currently widely available for and utilized by recurrent miscarriage couples carrying a structural chromosomal abnormality,

clinical characteristics including spontaneous abortion do not differ between those couples that accept and those that decline PGD (De Krom et al., 2015).

Hence, reproductive performance and genetic counseling for carriers with balanced translocation merit further research. The present study aimed to assess balanced reciprocal translocation at amniocentesis in Jilin Province, China. Moreover, the relationship between chromosomal breakpoints and reproductive performance is discussed.

MATERIAL AND METHODS

Women presenting a 16-22-week singleton gestation at the Center for Prenatal Diagnosis, First Hospital, Jilin University, China, for amniocentesis between February 2011 and December 2014 were offered participation in our study. Amniotic fluid cells were obtained by amniocentesis at 16-22 weeks' gestation. The amniocenteses were for indications including advanced maternal age, a positive aneuploidy screening result, abnormal ultrasound, and paternal/maternal carriers. Amniotic fluid cells for centrifugation were cultured in CHANG Amnio® Medium (Irvine Scientific, Santa Ana, CA, USA), followed by treatment with colcemid. A total of 3807 amniocentesis samples (excluding 19 culture failure samples) were processed. They also underwent Giemsa banding (GTG banding) to determine the karyotype using our previously reported methods (Zhang et al., 2015b). The results were reported according to the recommendations of the International System for Chromosome Nomenclature 2009 (Shaffer et al., 2009).

For cases of fetal chromosomal balanced translocation, we performed cytogenetic analysis of the couples, and suggested genetic counseling if appropriate. Finally, we performed a follow-up to determine the outcome of pregnancy. The process is shown in Figure 1. The study protocol was approved by the Ethics Committee of The First Hospital, Jilin University, Changchun, China, and all participants provided their written informed consent.

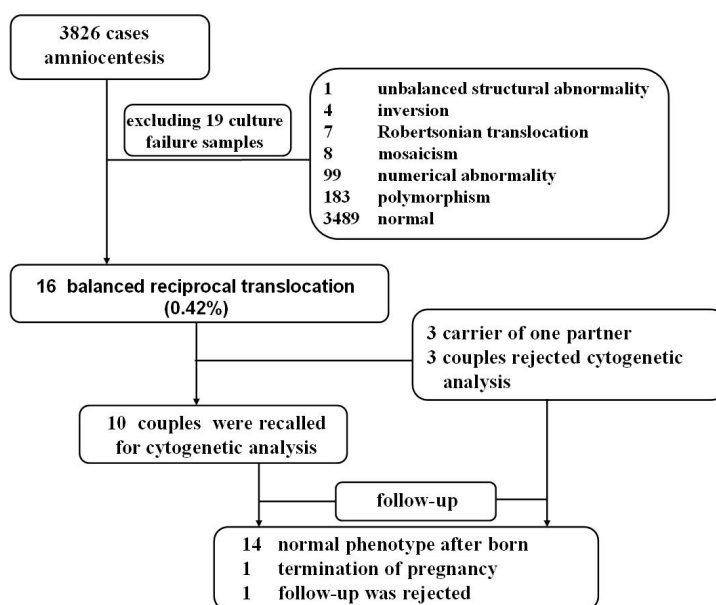


Figure 1. Flow chart of genetic counseling and reproductive choice after amniocentesis.

RESULTS

Of the 3807 amniotic fluid cell chromosomes analyzed, the frequency of balanced translocation was 0.42% (16/3807). Of these, eight (50%) cases had positive biochemical marker screening, three (18.75%) were the parental carriers of a chromosomal abnormality, two (12.5%) were studied because of advanced maternal age, two (12.5%) had a previous history of children with genetic disorders (children with aneuploidy), and one case (6.25%) was associated with positive soft markers in obstetric ultrasound (Table 1).

Table 1. Analysis of fetal karyotype and reproductive outcomes.

Case	Indication for amniocentesis	Maternal age (years)	Fetal karyotype	Familial/ <i>de novo</i>	Outcomes after born
1	Previous aneuploid child	27	46,XX,t(5;20)(q13;q12)	Paternal	Normal phenotype
2	Advanced maternal age	36	46,XX,t(17;19)(p11;q13)	Paternal	Normal phenotype
3	Previous aneuploid child	34	46,XX,t(5;11)(p14;p15)	Paternal	Normal phenotype
4	Paternal carrier	31	46,XX,t(10;19)(q24;p13.1)	Paternal	Normal phenotype
5	BMS positive	34	46,N,t(X;1)(p11;q11)	?	-
6	Abnormal ultrasound	34	46,XX,t(4;14)(q13;q11)	Maternal	Normal phenotype
7	BMS positive	32	46,XX,t(1;22)(p22;q11)	Paternal	Normal phenotype
8	BMS positive	34	46,XX,t(4;20)(q21;q13.3)	<i>De novo</i>	Normal phenotype
9	BMS positive	27	46,XX,t(2;10)(q11;q11)	?	Normal phenotype
10	Maternal carrier	30	46,XX,t(17;22)(q21;q11)	Maternal	Normal phenotype
11	Advanced maternal age	41	46,XX,t(3;19)(q12;q13)	<i>De novo</i>	Normal phenotype
12	BMS positive	32	46,XX,t(5;11)(p15;q13)	?	Normal phenotype
13	BMS positive	31	46,XX,t(3;9)(q21;q22)	Paternal	Normal phenotype
14	BMS positive	30	46,XX,t(5;13)(q13;q12)	Paternal	Termination of pregnancy
15	Maternal carrier	24	46,XX,t(1;13)(p22;q32)	Maternal	Normal phenotype
16	BMS positive	34	46,XX,t(3;19)(q12;q13)	Paternal	Normal phenotype

BMS = biochemical marker screening.

Fetal karyotypes and abnormal chromosomes are shown in Table 1 and Figure 2, respectively. Chromosomes 5 (N = 4) and 19 (N = 4) were the most commonly involved chromosomes in the balanced reciprocal translocations (Table 1).

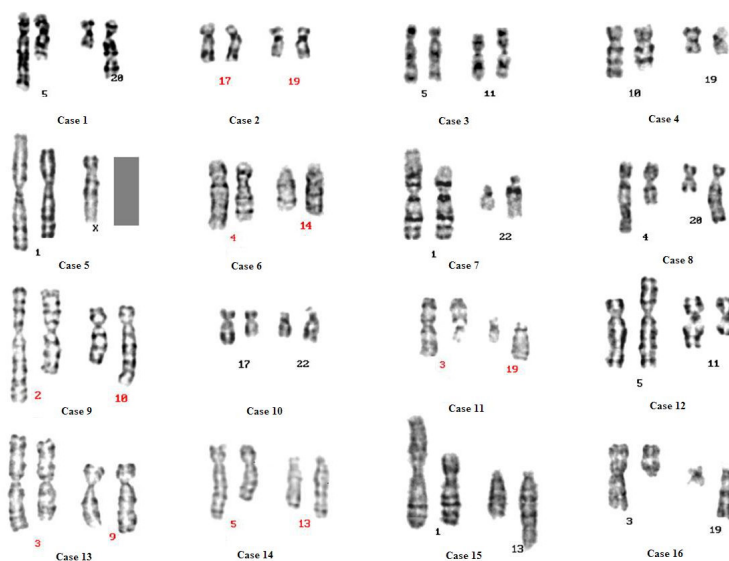


Figure 2. Abnormal fetal karyotype.

Although we tried to recall all 16 couples for cytogenetic analysis, three couples refused. Of the other 13, 8 (61.5%) cases of fetal balanced reciprocal translocations were shown to be inherited from the paternal chromosome, 3 (23.1%) were inherited from the maternal chromosome, and two cases (15.4%) were *de novo* (Table 1). Through follow-up, we found that 14 children were born with a normal phenotype. Pregnancy was terminated in one case, and follow-up was refused in another (Table 1).

DISCUSSION

Microscopy can only detect gross chromosome abnormalities; therefore, chromosomal microarray analysis is used to identify additional, clinically significant cytogenetic information. It is at least as good at identifying aneuploidies and unbalanced rearrangements as karyotyping. However, it is not able to identify balanced translocations or triploidies (Wapner et al., 2012). Instead, it can detect small microdeletions/microduplications (copy number variations) with unknown functional roles and difficult genotype/phenotype correlations. Copy number variation syndromes are often associated with variable phenotypic outcomes, ranging from normal to severely affected individuals. Because of a supposed lack of experience by laboratory staff, clinical geneticists, and counselors at managing the outcomes of microarray-based analysis (Novelli et al., 2013), conventional cytogenetic analysis remains an important method in prenatal diagnosis.

Invasive prenatal diagnostic techniques are feasible tools for confirming fetal chromosomal abnormalities (Zhang et al., 2010), and amniocentesis is a reliable and low-risk method of achieving suitable genetic material (Grether-González et al., 2010). Routine chromosome analysis has been the gold standard for prenatal cytogenetic diagnosis (Wilson et al., 2009); it identifies reciprocal chromosomal translocations as the most common type of structural chromosomal aberrations in the human population (Vasilevska et al., 2013).

The frequency of balanced reciprocal translocation in the general population is estimated from prenatal and newborn studies to be approximately 1 in 625 (0.16%) (Nonaka et al., 2015). Sheth et al. (2015) detected abnormal karyotypes in 125/1728 cases (7.2%), with structural abnormalities such as reciprocal and Robertsonian translocation detected in 20 cases (1.2%). Wu et al. (2013) reported an abnormal fetal karyotype frequency of 4.6%. Structural aberrations of chromosomes were identified in 43 cases, including 21 cases (0.5%) of balanced reciprocal translocation. Both Peng et al. (2006) and Chang et al. (2013) reported similar frequencies of reciprocal translocations in amniocentesis cases of 0.44% (74/16,749) and 0.53% (66/12,468), respectively. Specifically, 0.256% had a reciprocal translocation. Balkan et al. (2011) observed 52 cases (4.9%) with chromosomal aberrations, of which two were reciprocal translocations, while 41 cases (0.84%) were balanced translocations in a study of prenatal cytogenetic examinations by Park et al. (2001). The findings from the present study were in line with these, detecting abnormal karyotypes in 318/3807 cases (8.35%), of which 16 (0.42%) were balanced reciprocal translocations.

Indications for amniocentesis include advanced maternal age (AMA), maternal serum screening results, ultrasonographic findings for congenital malformations or markers of aneuploidy, and chromosomal abnormalities in a previous pregnancy (Wilson et al., 2009; Danisman et al., 2013; Suzumori et al., 2015). Positive prenatal screening and AMA are the main clinical indications (Mademont-Soler et al., 2011). Prenatal diagnosis is offered to carriers of a balanced chromosomal rearrangement because they may be predisposed to offspring with an unbalanced karyotype (Bache et al., 2007; Chang et al., 2013). Chang et

al. (2013) reported that the highest proportion of reciprocal translocations was found in cases with indications of AMA (52.7%, 39/74), abnormal biochemical markers in the maternal serum (12.2%, 9/74), and a parent with an abnormal karyotype (17.6%, 13/74). Balkan et al. (2011) reported that the highest detection rate of chromosome aberrations was in cases undergoing amniocentesis for abnormal maternal serum screening combined with abnormal ultrasound findings (8.0%). Meng et al. (2015) reported that over a period of 13 years, the indication of abnormal maternal serum screening and abnormal ultrasound findings increased, while AMA decreased for prenatal diagnosis of cytogenetic abnormalities, and a continuous trend of reduced invasive procedures was observed. The present study showed that of 16 cases of balanced translocation, eight had a positive biochemical marker screening, three were the parental carriers of a chromosomal abnormality, two were studied because of advanced maternal age, two had a previous history of children with genetic disorders, and one case was associated with positive soft markers in obstetric ultrasound.

Infertility and spontaneous abortion are closely associated with reciprocal chromosome translocations. Because of various mechanisms of abnormal meiotic segregation, the size of unbalanced chromosome segments in offspring determines the degree of risk for reproductive abnormalities. To evaluate this risk in different pedigrees, it is important to assess the type of chromosomal translocation and the location of the breakpoints (Midro, 1992). In the present study, chromosomes 5 and 19 were the most commonly affected in the 16 fetal karyotypes with a balanced reciprocal translocation.

Male carriers of reciprocal chromosome translocations have a higher chance of becoming a parent of a child who undergoes normal childbirth than female carriers. Vasilevska et al. (2013) reported six cases of reciprocal translocations of different autosomes, of which five were of paternal origin and one was inherited from the mother. Only the cases of 46,XY,t(6;10)(p21;q26) pat. and double translocation 46,XX,t(1;21)t(7;16) mat. were associated with reproductive problems. Couples with a fetal chromosome 46,XN,t(5;20)(q13;q12) pat. and 46,XN,t(5;11)(p14;p15) pat. had a previous child with aneuploidy. Peng et al. (2006) reported an incidence of *de novo* reciprocal translocations of 0.08%, which is much lower than that observed in the present study (15.4%). This difference might reflect the low sample number in the present study. In our study, 14 children of the 16 cases with reciprocal chromosome translocations were born with a normal phenotype, so had no reproductive problems.

In conclusion, the incidence of balanced reciprocal translocation at amniocentesis was 0.42% in Jilin Province, China. The highest proportion of reciprocal translocations was observed in cases with indications of positive biochemical marker screening. Chromosomes 5 and 19 were most commonly involved in fetal karyotypes with a balanced reciprocal translocation. Male carriers of reciprocal chromosome translocations have a higher chance of becoming a parent of a child born by normal childbirth than female carriers.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We would like to thank all patients for their cooperation. Research supported by the National Natural Science Fund (#81471515).

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