

UNBALANCED KARYOTYPE IN A HUMAN FOETUS DUE TO A RECURRENT FAMILIAL TRANSLOCATION

Nicoleta ANDREESCU*, Dorina STOICĂNESCU*, Alina BELENGEANU**, Simona FARCAȘ*, Cristina POPA*, Monica STOIAN*, Valerica BELENGEANU*

* University of Medicine and Pharmacy "Victor Babeș" Timișoara, Department of Medical Genetics, Timișoara, România

** University of Medicine and Pharmacy "Victor Babeș" Timișoara, Department of Cell and Molecular Biology, Timișoara, Romania

Corresponding author: Nicoleta Andreescu, University of Medicine and Pharmacy "Victor Babeș" Timișoara, 2 Eftimie Murgu Square, 300041 Timișoara, România, tel. 000256224025, e-mail: nicollandreescu@yahoo.com

Abstract. Couples with multiple miscarriages are at risk for carrying a balanced translocation since they may produce unbalanced gametes. Chromosomal imbalances may lead to spontaneous abortions, or an offspring with multiple congenital anomalies. This report emphasizes the importance of the cytogenetic investigations in couples with recurrent spontaneous abortions. A couple was referred for cytogenetic prenatal testing because of a history of recurrent miscarriages and due to the fact that after an ultrasound examination the foetal heart rate was not perceived and the suspicion that the pregnancy stopped growing was raised. Chromosome studies of the chorionic villi from the foetus revealed a karyotype with deletion of the terminal region of chromosome 11. Analysis of the chromosomes of the couple revealed the presence of a chromosomal rearrangement, a balanced translocation (10;11) for the mother, while the father had a normal karyotype. Further investigation of the family from the mother side revealed the same balanced translocation for her mother and her brother. Fluorescence in situ hybridization (FISH) using telomere probes for the short arm of chromosome 10 and the long arm of chromosome 11 was performed to better characterize the balanced state in the mother. This helped delineate the 46,XX,der(11)(11pter→11q21::10p14→10pter) karyotype of the foetus. This paper confirms that a chromosomal abnormality carried by one of the parents is likely to be associated with a high rate of spontaneous abortion. The diagnosis of the chromosomal balanced translocation of the genitors has significant implications for management and options in this family. Chorionic villi sampling should be offered to women who present with first-trimester spontaneous abortions and it is the most reliable method of determining the karyotype of spontaneously aborted foetuses.

Keywords: balanced translocation, miscarriage, large deletion 11q, duplication 10p

INTRODUCTION

Chromosomal aberrations are disruptions in the normal number or structure of the chromosomes, representing a major cause of genetic disorders. Numerical aberrations involve the loss or gain of an entire chromosome, giving rise to a monosomy or a trisomy. Structural aberrations affect parts of the chromosomes, usually implying a break in the chromosome and give rise to different rearrangements. Translocations are the most frequent types of chromosomal rearrangements between two chromosomes, involving an exchange of genetic material. They may be either balanced or unbalanced, depending on whether the quantity of genetic material is modified or not. Balanced translocations normally do not cause abnormal phenotypes, but unbalanced translocations can cause miscarriages, stillbirths, or multiple anomalies, developmental delay and mental retardation.

Segmental aneuploidies are a significant cause of morbidity and mortality in children, with an estimated incidence of 0.7–1 in 1,000 births. Most cases reported in the literature are of familial origin, in which the aneuploidy is the result of an unbalanced segregation of a parental balanced translocation.

Reciprocal chromosome translocations are one of the most common chromosomal structural anomalies in humans [6, 9, 20]. Meiotic malsegregation of the chromosomes involved in chromosome translocation may lead to an unbalanced karyotype of the progeny, which can have clinical effects [14]. The clinical effects (miscarriages, stillbirths, malformed offspring at birth and at prenatal diagnosis, and early newborn deaths) depend on the survival rate of the unbalanced embryo/fetus/child [6, 9, 20]. The pattern of unbalanced embryo/fetus/child and the survival rate

observed in families of carriers with chromosome translocation may facilitate prediction of how a balanced rearrangement will segregate. There is a great number of pedigrees (1120 pedigrees) elaborated by Stengel-Rutkowski et al. (1988) [17] for carriers of reciprocal chromosome translocation but specific empiric data are not available so far for all reciprocal translocations. Therefore empirical, clinical and cytogenetic data should be collected and evaluated further [10, 11, 17].

At approximately 4.5% of couples with recurrent abortions, one or both partners have a balanced chromosome abnormality. A high percentage of duplications or deletions are present in gametes produced by individuals carrying balanced translocations.

There are studies indicating that in spermatogenesis, there is a strict checkpoint in gametogenesis which stops meiosis when unbalanced chromosome complements are detected, leading to a reduced number of altered spermatozoa [15] although the arrest may be overcome and result in the production of diploid or aneuploid sperm. In oogenesis the anaphase checkpoint is not so stringent so altered oocytes can be produced [14]. Taking into account this, the frequency of chromosome abnormalities observed in embryos could be different depending on the sex of the rearrangement carrier.

Cytogenetic analysis of spontaneous abortions provides valuable information to establish the cause of fetal losses, essential to provide accurate reproductive and genetic counseling to the couple. Nearly half of pregnancies are aborted spontaneously, even before the first appearance of clinical symptoms [18]. 10-15% of clinically obvious pregnancies end with spontaneous abortion before the end of the first quarter. 50-70% of early abortions are due to chromosomal abnormalities;

the most common abnormality is represented by trisomy. Cytogenetic analysis for spontaneous abortion can bring valuable information to determine the cause of recurrent abortions. Risks for carriers of a balanced translocation are: recurrent abortions, offspring with chromosomal abnormality due to deletions and/or duplications, risk of recurrence of balanced translocation. Prenatal diagnosis is therefore recommended.

Direct or semi-direct analysis of chorionic villi is performed for first trimester prenatal cytogenetic diagnosis and it has been successfully applied for the study of spontaneous abortions [12]. This is a rapid method that allows the detection of all kind of chromosome abnormalities in a single analysis.

The aim of the present study was to evaluate the karyotypes of the members of a family and to reveal the implications of familial chromosomal translocations.

MATERIALS AND METHODS

A couple presented for genetic counseling after an ultrasound examination that showed that the foetal heart rate was not perceived and the suspicion that the pregnancy stopped growing was raised. The couple's partners were 30 years, respectively 36 years old, with no relationship of consanguinity and the record indicated the couple had two miscarriages first at 11 weeks gestational age and the second at 8 weeks of pregnancy.

Chorionic villi were collected in warmed RPMI1640 medium supplemented with Penicillin and Streptomycin. CVS samples were delivered immediately to the laboratory of Medical Genetics at the University of Medicine and Pharmacy Timișoara. Samples were examined under the microscope and were released from maternal deciduas and blood clots. They were divided into fragments of 3–5 mg, which were processed independently. A semi-direct method of culture was performed, as CVS were incubated 24 hours to obtaining G-banded metaphase chromosome preparations. In addition, two long-term monolayer cultures were established by finely chopping approximately 15 mg villi, followed by a 15- min collagenase digestion at 37°C. Monolayer cultures were harvested at 10 days post sample receipt for karyotyping using standard cytogenetic techniques.

Cytogenetic analysis of the foetus was performed. Metaphase slides were prepared from chorionic villi cultures. Chromosome analysis was performed according to routine methods and GTG-banding technique. Resolution patterns were obtained for 550 bands/haploid sets. Breakpoint position identification was performed according to the ISCN guidelines [16].

Cytogenetic analysis for the parents was performed from peripheral blood lymphocytes.

Fluorescence in situ hybridization (FISH) was also performed. Metaphase chromosome spreads from lymphocyte cultures were prepared as follows: the slides were incubated for 10 min at 37°C in 0.001 mol HCl containing 5% pepsin and washed in phosphate buffered saline for 5 min and air dried. Then the slides

were dehydrated in ethanol series, and denatured in 70% formamide, 2×SSC at 72°C for 3 min. Telomere probes for chromosomes 10 and 11 were used according to standard procedures for their hybridization and detection. The hybridization mixture, which contained 1 ml of each probe, 1 ml purified water and 7 ml of hybridization buffer, was applied to each slide, covered with a 20 mm x 20 mm coverslip, and sealed with rubber cement. After 16 hours of hybridization at 37°C, slides were washed for 2 min at room temperature in 0.4x saline–sodium citrate buffer (Sigma-Aldrich) with vigorous shaking, followed by 2 min at 72°C. The slides were wiped dry with a tissue and Dapi II solution (Vysis) was applied to the area of sample. The specimen was covered with a 20x20-mm glass coverslip.

RESULTS

A number of 30 metaphases prepared from chorionic villi cultures were analyzed and a deletion of the q arm of chromosome 11 was established (Fig. 1).

Peripheral blood samples were obtained from parents and karyotype was performed according to standard protocols. For each genitor, a number of 30 metaphases were analyzed. Father's karyotype was normal, 46,XY. Mother's karyotype revealed a balanced translocation between chromosomes 10 and 11: 46,XX,t(10;11)(10qter→10p14::11q21→11qter,11pter→11q21::10p14→10pter) (Fig. 2).

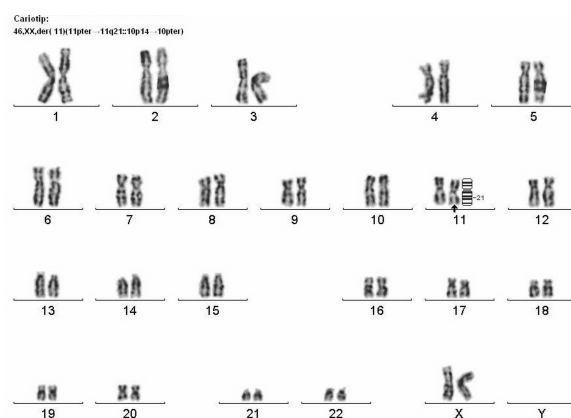


Figure 1. Foetus karyotype showing 11 q deletion.

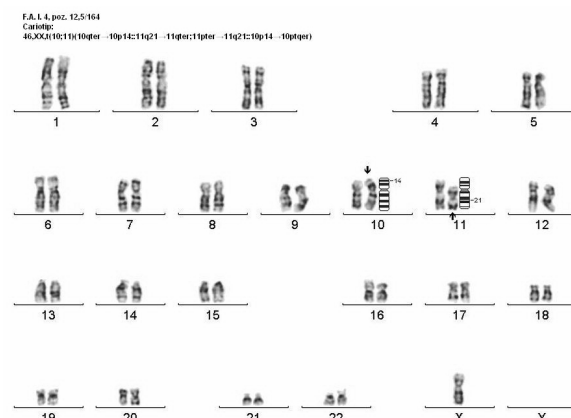


Figure 2. Mother's karyotype showing a balanced translocation between chromosomes 10 and 11.

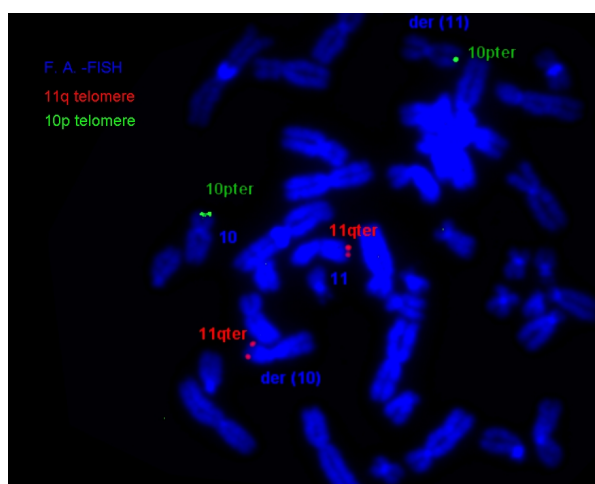


Figure 3. FISH using telomeric probes for the short arm of chromosomes 10 and the long arm of chromosome 11.

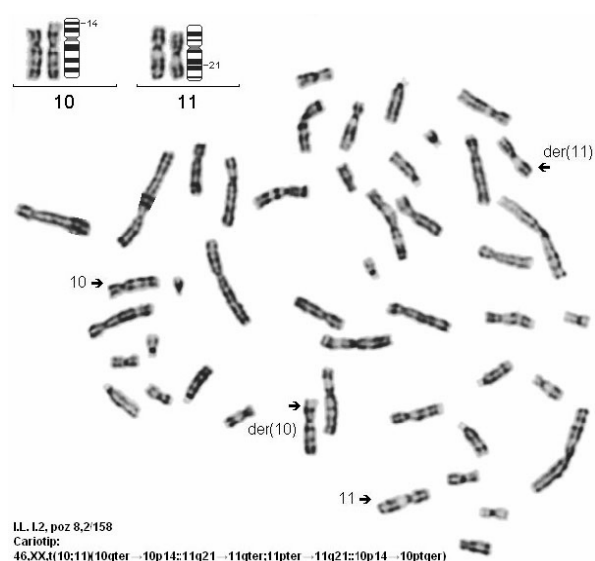


Figure 4. Maternal mother metaphase and partial karyotype showing a balanced translocation between chromosomes 10 and 11.

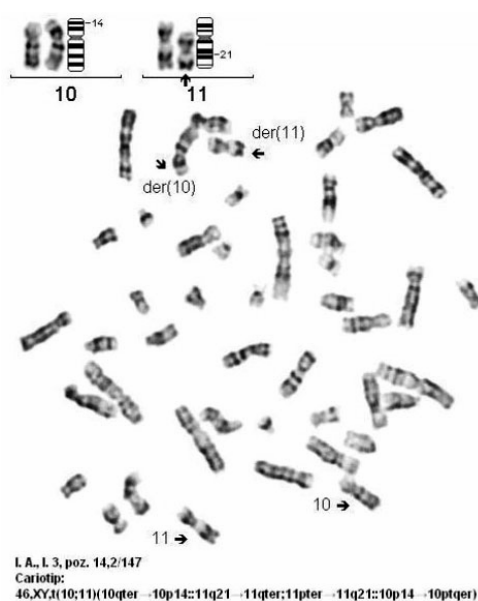


Figure 5. Genitor's brother metaphase and partial karyotype showing a balanced translocation between chromosomes 10 and 11.

Fluorescence in situ hybridization (FISH) using telomere probes for the short arm of chromosomes 10 and the long arm of chromosome 11 confirmed the balanced state in the mother (Fig. 3). Slides were viewed under a fluorescence microscope. A total of 50 metaphases were analyzed.

Fluorescence in situ hybridization (FISH) analyses together with parental karyotypes demonstrated that the fetal unbalanced chromosomal abnormality originated from the malsegregation of a maternal balanced translocation involving chromosomes 10 and 11. Performing cytogenetic and FISH analysis for the mother enabled to establish the following karyotype 46,XX,der(11)(11pter→11q21::10p14→10pter) for the foetus.

Consequently the cytogenetic investigation of the genitor family (parents and brother) was done. The brother and the mother were identified having the same balanced structural abnormality. It is important to mention that the brother is partner in a couple with a history of reproductive failure.

Genitor's mother karyotype (Fig. 4): 46,XX,t(10;11)(10qter→10p14::11q21→11qter,11pter→11q21::10p14→10pter).

Genitor's brother karyotype (Fig. 5): 46,XY,t(10;11)(10qter→10p14::11q21→11qter,11pter→11q21::10p14→10pter).

DISCUSSIONS

Segregation of chromosomes can occur in multiple ways. When 2:2 and 3:1 segregations are considered, there are 14 different segregation products of a reciprocal translocation carrier. Of these 14, one is normal, one is balanced translocation, and 12 are unbalanced. Some of the unbalanced segregants can result in viable fetuses and survive to mid trimester or to term.

Of the multiple ways that balanced translocated chromosomes can line up and divide, 2 are most common - about 50% have what is called an alternate 2:2 segregation and 50% have an adjacent 2:2 segregation. Of these two types of segregation, 6 different types of gametes can be produced. 4 of the 6 gametes produced will be unbalanced (this is a result of adjacent 2:2 segregation), 1 of 6 is balanced and 1 of 6 is normal (these last 2 are a result of alternate 2:2 segregation).

Segregation 3:1, 4:0 lead to the formation of gametes with anomalies incompatible with reproduction [1, 2]. The following factors are thought to favor 3:1 meiotic segregation of reciprocal translocations: involvement of an acrocentric chromosome, at least one break close to the centromere, and the very unequal size of the participating chromosomes. The symmetry of quadrivalent and occurrence of chiasmata appear to play a significant role.

Inherited translocations are passed through generations in a codominant manner. One copy of each chromosome remains normal, thus, both parent and descendent with such a translocation are heterozygous or balanced carriers. Half of their gametes will include

one copy of each gene, either on the translocated chromosomes or their normal homologs. The other halves are unbalanced, with some combination of translocated and normal homologs. The result is that the gamete has two copies of some genes, but no copies of other genes, from the translocated chromosomes [8]. This is an unbalanced gamete and if it takes part in fertilization, often disrupts development quite severely and the individual does not survive to be born. If the number of unbalanced genes is low, descendants may be born, but often they have growth defects and mental retardation. Couples with recurrent spontaneous abortions may have one partner carrying a balanced translocation. Thus, gene copy number determines the specific phenotypes associated with a translocation, or with any chromosome aberration [4].

Carriers of balanced translocations may have a recurrence risk of having another child with an unbalanced translocation of 15% if a familial translocation has been detected through an individual with an unbalanced translocation and the recurrence risk is 1-2% if it was not diagnosed by finding an individual with an unbalanced translocation. There is a risk for spontaneous abortions of 25% in both situations [19].

Small deletions of the terminal region of chromosome 11 are associated with Jacobsen syndrome, but terminal deletions extending proximal to 11q23.3 generally are not observed. An exception is the case of an infant whose karyotype was in mosaic 46,XY/46,XY,del(11)(q21qter) and presented holoprosencephaly, cyclopia, and arrhinencephaly, among other major malformations [7]. He died several minutes after birth. A possible explanation for the survival of this infant until 35 weeks may be the mosaic status. Terminal deletions extending proximal to 11q23.3 are considered to be associated with in utero lethality.

Duplications of different extent of the short arm of chromosome 10 have been reported in the literature as pure trisomy or as being part of a complex aneuploidy [3, 5], but without lethal evolution.

In the context of a prenatal diagnosis, it appears that the cytogenetic analysis of chorionic villi obtained through CVS before evacuation may be considered as the more comprehensive, cost-effective and reliable method for detecting a large spectrum of chromosome abnormalities in spontaneous abortions. It allows providing an explanation for the pregnancy wastage, identifying couples at risk for further unbalanced chromosome rearrangements and permit to establish an accurate genetic counseling not only to the couple but also to their relatives at risk.

CVS karyotyping is the method that detects the broadest spectrum of chromosome abnormalities in spontaneous abortions with the greatest reliability of the fetal origin of the sample. The development of molecular cytogenetic technologies has increased the possibilities to detect chromosomal aberrations and it is useful for a better evaluation of chromosomal anomalies. The availability of prenatal diagnosis can restore reproductive confidence for many couples at risk of transmitting a genetic disease in starting a

pregnancy and thereby increasing their prospect of having a healthy child. For this couple it was recommended *in vitro* fertilization followed by pre-implantation genetic diagnosis (PGD) that allows selection of embryos without chromosomal anomaly. FISH technique is also useful in identifying partial monosomies or trisomies of chromosomes that are common in embryos from patients with reciprocal translocations. By not transferring those embryos, it is possible to reduce the chances of miscarriage or pregnancy with an affected fetus.

In the previously reported cases, either an acrocentric chromosome was involved in the translocation or there were breakpoints close to the centromere, and the meiotic configuration was particularly asymmetric [13]. In our case, no acrocentric chromosome is involved, but there is a breakpoint close to the centromere, and the chromosomes derived from the complex rearrangement are very unequal in size. Our case presented an unbalanced translocation and a large terminal deletion of long arm of chromosome 11 as well as duplication of short arm of chromosome 10.

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