

# ROLE OF CHROMOSOMAL TRANSLOCATIONS IN RECURRENT SPONTANEOUS ABORTION

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## REZUMAT

**Introducere:** Implicarea anomaliilor cromozomiale ca și cauze de avorturi spontane și infertilitate a fost stabilită fără echivoc. Aproximativ 15%-20% dintre sarcini se termină prin avorturi spontane de prim trimestru. **Obiectiv:** S-a examinat relația dintre avorturile spontane și prezența translocațiilor cromozomiale la părinți, acestea determinând reducerea fertilității cuplului. **Material și metode:** Au fost investigate din punct de vedere citogenetic 260 cupluri care au avut două sau mai multe avorturi spontane consecutive. S-a efectuat analiza cromozomială la ambii parteneri, din culturi de limfocite din sânge periferic. Pentru fiecare caz au fost evaluate 30 metafaze și cel puțin 5 au fost cariotipate. **Rezultate:** Incidența translocațiilor a fost de 2,88%, identificându-se șase translocații Robertsoniene (1,15%) și nouă translocații echilibrate (1,73%). Prevalența translocațiilor la sexul masculin a fost de 1,53 %, iar la sexul feminin a fost 4,23%. **Concluzii:** Pentru majoritatea femeilor pierderea sarcinii reprezintă un eveniment sporadic, dar cazurile cu avorturi spontane repetate trebuie atent investigate. Este necesară efectuarea sistematică a analizei citogenetice la cuplurile cu infertilitate, deoarece anomaliile cromozomiale, inclusiv translocațiile, se întâlnesc cu o frecvență relativ crescută la aceste cazuri. Detectarea lor permite geneticienilor să stabilească riscul de recurență pentru o sarcină ulterioară și să ofere cuplului sfatul genetic adecvat.

**Cuvinte cheie:** avorturi spontane recurente, anomalii cromozomiale, translocație reciprocă, translocație robertsoniană

## ABSTRACT

**Introduction:** The involvement of chromosomal abnormalities as causes of pregnancy loss and infertility has been unequivocally established. About 15% to 20% of all recognized pregnancies end in a first- trimester spontaneous abortion. **Objective:** The aim of this study was to examine the relationship between spontaneous abortions and the presence of chromosomal translocations in parents, these eventually leading to decreased fertility. **Material and methods:** A total of 260 couples who had two or more consecutive spontaneous abortions were cytogenetically analyzed. Chromosome analysis was carried out on both partners, from peripheral lymphocyte cultures. In every case, 30 metaphases were counted, and at least 5 karyotyped. **Results:** The overall incidence of the translocations was 2.88 %, with 6 Robertsonian translocations (1.15 %) and 9 balanced translocations (1.73%). The prevalence of translocations in males was 1.53 % and in females was 4.23 %. **Conclusions:** Reproductive wastage is a sporadic event for most women, but cases with recurrent spontaneous abortions need to be carefully investigated. Systematically performing chromosome analysis in infertile couples is required, because the frequency of chromosomal abnormalities, including translocations, is relatively high in this population. Their detection enables geneticists establish the recurrence risk for a future pregnancy and offer the couple the adequate genetic counseling.

**Key Words:** recurrent spontaneous abortion, chromosomal abnormality, reciprocal translocation, robertsonian translocation

## INTRODUCTION

Chromosomal aberrations leads to reduced fertility in both men and women. About 15% to 20% of pregnancies end in spontaneous abortion, mostly in the first trimester, the most frequent cause being represented by chromosomal abnormalities. Their

incidence in these abortions is as high as 50%.<sup>1</sup> In spontaneous abortions, the majority of chromosomal anomalies (95%) are numerical. About 60% are trisomies, 20% are represented by X monosomy and another 15% by polyploidy, especially triploidy.<sup>2</sup> In the case of a numerical chromosomal aberration in the fetus, parental chromosomes are usually normal, so cytogenetic analysis of the parents is not indicated. The recurrence risk for a chromosomal abnormality following the diagnosis of trisomy in a pregnancy is estimated at about 1%. Prenatal diagnosis of the fetus for a future pregnancy may be considered. As well as these numerical changes, structural aberrations of the chromosomes can also be the cause of pregnancy loss and infertility. If a structural chromosomal anomaly is found in a fetus, parental karyotyping is required.<sup>3</sup> The presence of a balanced chromosomal rearrangement

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in a parent results in an increased recurrence risk for structural chromosomal defects in future pregnancies. An important proportion of spontaneous abortions is caused by the presence of a balanced chromosomal aberration in one of the parents, who is at risk of having gametes and embryos with unbalanced chromosome sets. All these changes can be identified by chromosome analysis. The clinical consequences of such abnormal gametes include sterility, repeated spontaneous abortions, or giving birth to malformed children.<sup>4</sup>

It is estimated that in about 7% of couples with at least two spontaneous abortions, one parent carries a balanced chromosome rearrangement.<sup>5</sup> The most frequent is a reciprocal translocation in which a segment of a chromosome has exchanged places with a segment of another nonhomologous chromosome. In these cases, the chromosomes have difficulties pairing up and dividing during meiosis. As consequence, gametes will have unbalanced chromosomal aberrations (duplications and/or deletions). Usually, these imbalances are lethal to the developing embryo or fetus, causing spontaneous abortion. Sometimes, the pregnancy continues to term, leading to the birth of an infant with multiple congenital anomalies and mental retardation. When a parent carries a balanced chromosome rearrangement, the chance of having a live birth with an unbalanced chromosome complement is usually about 1% to 15%. The exact risk depends on the specific chromosomes involved, size of the segment involved in the rearrangement, genes contained in the segment, sex of the transmitting parent, family history, and mode of ascertainment. It is estimated that the medium risk is 12% if the translocation is present in the female and 5% if it is present in males. When one parent carries a chromosome rearrangement, the chance of spontaneous abortion is usually 25% to 50%. Empirical and/or hypothetical data are available for predicting the risk of adverse pregnancy outcome for various rearrangements.<sup>3,6</sup>

Identifying the presence of a rearrangement in a parent is useful because it provides not only an explanation for the miscarriages, but also information about the risk for a child to be born with severe anomalies, as well as the risk for future miscarriages, availability of prenatal diagnosis in a future pregnancy and information for the family members who may be at risk and may wish to undergo chromosome testing.<sup>7</sup> Couples in which one partner has a chromosomal rearrangement may benefit from genetic counseling. Counseling includes an explanation of the findings, associated risks for miscarriages and live birth with

congenital anomalies and a discussion of reproductive options, including prenatal diagnosis. Genetic counseling is best provided before the next pregnancy, so all options may be discussed and explored.<sup>8</sup>

## **MATERIALS AND METHODS**

This study included 260 couples with reproductive failure who were referred for cytogenetic studies at the Medical Genetics Department from the Victor Babes University of Medicine and Pharmacy, Timisoara, between October 2003 and June 2007. The obstetrical history of couples was either recorded on the request form or retrieved from the files of patients. Cytogenetic analyses of peripheral blood lymphocytes in these infertile couples were performed. All cases were ascertained to have had two or more spontaneous abortions.

For standard cytogenetic analysis, peripheral blood was incubated in complete lymphocyte culture medium. Metaphases were harvested by adding colcemid, followed by hypotonic KCl treatment and fixation, using standard 3:1 methanol-acetic fixative. For each individual, a minimum of 30 metaphases were counted and at least five cells were karyotyped after standard G-banding. FISH method was used in one case with a translocation between chromosomes 4 and 21 to confirm that the broken fragment belonged to chromosome 4. TelVision™ DNA Probes were used, DAP Orange for the short arm of chromosome 4 and DAP Green for the long arm of chromosome 21.

## **RESULTS**

A total of 260 couples with history of repeated abortions were examined. The age of the wives ranged from 20 to 43 years. The number of previous abortions varied from 2 to 10 abortions.

The overall incidence of the translocations was 2.88 %, with 6 Robertsonian translocations (1.15 %) and 9 balanced translocations (1.73%). The prevalence of translocations in males was 1.53 % and in females was 4.23 %. These abnormalities included 9 balanced translocations and 6 Robertsonian translocations. (Table 1) Examples of the encountered chromosomal abnormalities are shown in Figures 1, 2, 4 and 5.

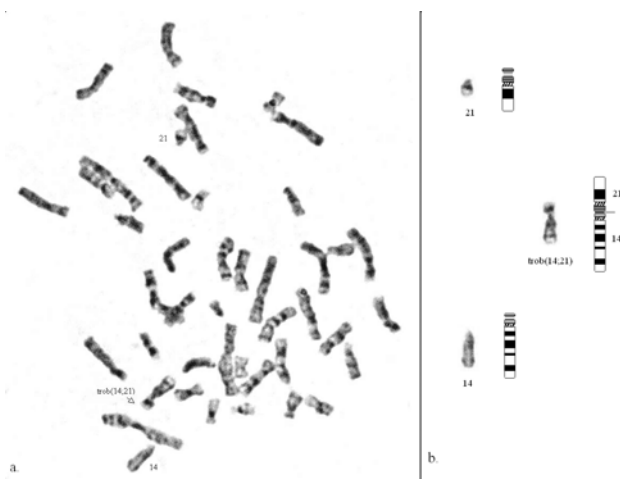
Among cases with abnormal karyotypes, having a translocation, the mean maternal age was 30.4 and the mean paternal age was 32.3. The mean number of abortions was 2.5 per couple.

Karyotype of one case revealed the presence of

**Table 1.** Cytogenetic findings, number of abortions and maternal age in cases with translocations.

No.	Karyotype	No. of abortions	Maternal age	Paternal age
1	46, XY, t(4;21) (q13.3;q22.3)	3		36
2	46, XX, t(4;21) (q13.3;q22.3)		24	
3	45, XX, -22,-22, rob(22;22)	4	38	
4	45, XX, -14,-21, rob(14;21)	2	28	
5	46,XX(90%)/45,X,-8,-14,+8p; t(8q;14q)(5%)/47,XX, t(8q;14q)	2	35	
6	45, XX, -13,-14, rob(13;14)	2	24	
7	45, XX, -13,-15, rob(13;15)	4	43	
8	46,XX,(88%)/46,XX,t(7;14) (p22;q22) (12%)	4	32	
9	45, XY, -13,-14, rob(13;14)	3		31
10	45, XX, -13,-14, rob(13;14)	3	22	
11	46, XY/46, XY, t(4;16) (p13.3;q4.35)	2		30
12	46, XX,t(Xq;4q) (q28;q31)	2	32	
13	46, XX,t(7;13) (q36;q12)	2	26	
14	46, XY, t (2;6) (q21.3;q26)	2		
15	45, XX, -21, t(X;21) (q28;q11)	2		29

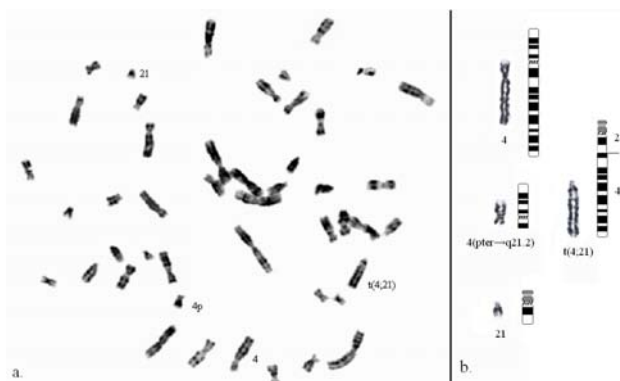
a Robertsonian translocation t(14;21). (Fig. 1) This couple had two spontaneous abortions in the first trimester and a child with Down syndrome.



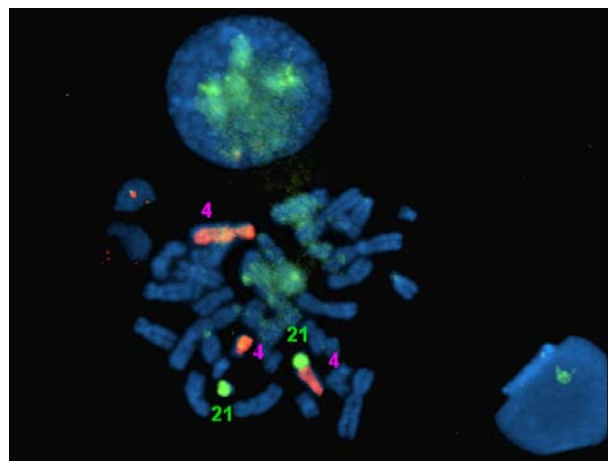
**Figure 1.** Metaphase (a) and partial karyotype (b) showing a Robertsonian translocation between chromosomes 14 and 21: 45, XX, -14,-21, rob(14;21).

In another couple referred for cytogenetic analysis because they already had three spontaneous abortions, the karyotype revealed that the husband had a translocation between chromosomes 4 and 21: 46, XY, t(4;21) (q13.3;q22.3). (Fig. 2) FISH technique was used in order to confirm the translocation between the two chromosomes and that the broken fragment

belongs to chromosome 4. (Fig. 3) Chromosomal investigation of the family members revealed that his sister also carried this translocation, thus her karyotype was: 46, XX, t(4;21) (q13.3;q22.3). This woman was the only case included in the study that did not have a history of spontaneous abortions, but the cytogenetic investigation proved that this is a familial translocation, as she also carries it. Being informed about the fact that she carries this translocation is very important for her future pregnancies.



**Figure 2.** Metaphase (a) and partial karyotype (b) showing a translocation between the long arms of chromosomes 4 and 21: 46, XY, t(4;21) (q13.3;q22.3).

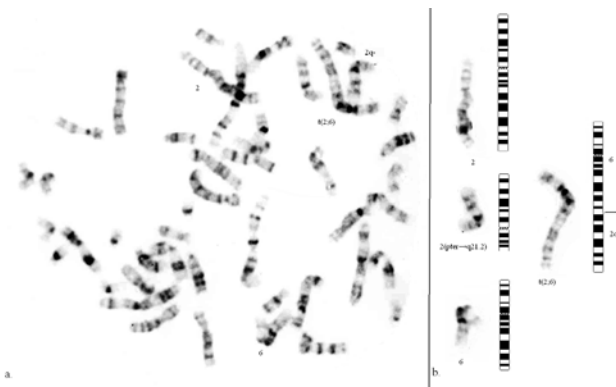


**Figure 3.** FISH analysis revealing the presence of the translocation between the long arms of chromosomes 4 and 21.

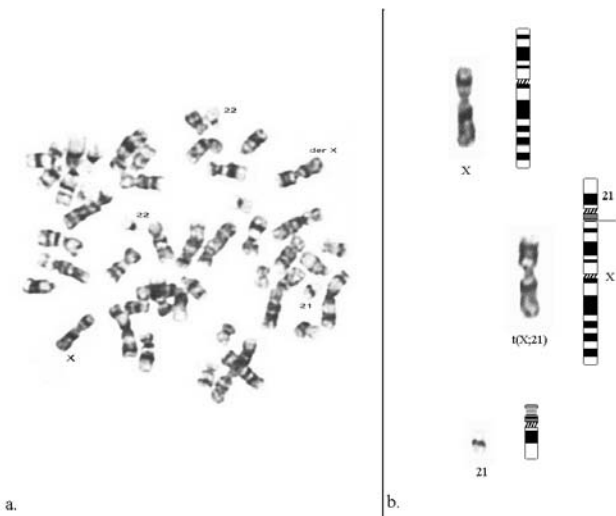
One of the investigated men, aged 33 years, had a translocation between chromosomes 2 and 6: 46, XY, t (2;6) (q21.3;q26). (Fig. 4) His wife had two spontaneous abortions. The last case we present is a 29 year old woman, who had two spontaneous abortions. Her constitutional karyotype was: 45, XX, -21, t(X;21) (q28;q11). (Fig. 5)

## DISCUSSIONS AND CONCLUSIONS

It is estimated that 60% of all spontaneous abortions in early pregnancy are caused by chromosomal aberrations in the embryo. The majority



**Figure 4.** Metaphase (a) and partial karyotype (b) showing a translocation between chromosomes 2 and 6: 46, XY, t(2;6)(q21.3;q26).



**Figure 5.** Metaphase (a) and partial karyotype (b) showing a translocation between chromosomes 21 and X: 45, XX, -21, t(X;21)(q28;q11).

of pregnancy losses or deaths after birth are caused by numerical chromosomal disorders (frequently trisomies of chromosomes 13, 15, 18, 16, 21 and 22).

Nondisjunctions of chromosomes in embryos are usually newly occurring events. Structural abnormalities are responsible for about 5 - 10% of early miscarriages. If structural anomalies of the chromosomes are present in one of the parents, they can lead to a considerably higher risk of having children with chromosomal disorders. Therefore, cytogenetic analysis should be performed on the parents to determine future risk.<sup>9</sup>

The incidence of chromosomal abnormalities among couples with a history of recurrent spontaneous abortion varies in different studies, from none to 21.4%. The differences between these studies may be due to variations in the size of the sample, criteria used for ascertainment of cases and the cytogenetic technique, but it is also possible that different populations vary in the incidence of carriers of chromosomal aberrations.<sup>10</sup>

Translocations are of two main types: reciprocal and Robertsonian. Reciprocal translocation represents the exchange of blocks of chromatin between two nonhomologous chromosomes. The process requires breakage of the involved chromosomes, with repair in an abnormal arrangement. Its incidence in neonates is estimated at about 1/1000 - 2/1000. A balanced translocation does not necessarily lead to an abnormal phenotype. However, translocation, like inversion, can lead to the formation of unbalanced gametes and therefore carry a higher risk of abnormal offspring. Normal humans differ from balanced translocation carriers in two pairs of homologous chromosomes. The homologous chromosomes pairs in normal humans form 23 bivalents, while in the latter they form 21 bivalents and 1 quadrivalent.<sup>11</sup>

It was reported that abortion was mostly caused by balanced translocation carriers. But in our study, Robertsonian translocations were seen in six cases, suggesting we must pay attention to this possibility. The incidence of Robertsonian translocation in neonates is 1/1000. It involves two acrocentric chromosomes, which fuse at the centromeric region and lose their short arms.

A carrier of a balanced Robertsonian translocation has 45 chromosomes including the translocated chromosome. Robertsonian translocations occur either by mutation or by segregation in the offspring of a balanced carrier. A carrier of a Robertsonian translocation has normal phenotype, but is at risk of producing unbalanced gametes and therefore unbalanced offsprings.<sup>12</sup>

Our data show that a high number of infertile couples is affected by chromosomal aberrations. Chromosomal aberrations in recurrent abortions are mostly structural ones. The structural aberrations of either sex or autosomal chromosomes were found.

The translocations that we encountered were divided into balanced chromosomal translocations (9/14) and Robertsonian translocation (6/14). Balanced chromosomal rearrangements have been found at an increased frequency in couples with pregnancy wastage, especially recurrent spontaneous abortions, compared with the general population. We found three cases with a Robertsonian translocation between chromosomes 13 and 14, but it is known that this type of translocation is the most common in humans.<sup>13</sup>

In general, the incidence of chromosomal abnormalities is higher in females than that in males.<sup>14</sup> In our study, the incidence was also higher in females, but paternal chromosomal abnormality may

also have a role in the pathogenesis of spontaneous abortions.<sup>15</sup> We found that 11 women and four men had chromosomal abnormalities, which was a ratio of 2.75:1. An almost similar male to female ratio has been found in most of the reported studies.

Other studies also revealed that chromosome aberrations are found with a higher incidence in couples who had recurrent spontaneous abortions than in the general population.<sup>16</sup> Of these, many are proved to be translocations. The results add credibility to the argument that chromosome aberration may play a role in the causal background of recurring abortions.

In cases of repeated abortion, cytogenetic examination of both partners is routinely justified and identification of chromosome aberrations offer valuable data that serve as a basis for genetic counseling. Regarding the relation of the obstetric history to the frequency of chromosomal aberrations, no statistically significant correlation was found between the number of deliveries and the occurrence of chromosomal abnormalities. A statistically significant correlation was found between the number of previous abortions and the occurrence of chromosomal abnormalities ( $p = 0.005$ ).

This study revealed that the incidence and distribution of chromosomal abnormalities among the investigated couples with repeated fetal loss is comparable to that reported worldwide. Physicians should bear in mind that in at least 5% of the couples they examine, chromosomal abnormality is the cause of abortions.<sup>17,18</sup> Those cases have to be detected as early as possible to arrange for adequate genetic counseling and to allow parents to make an informed reproductive decision regarding subsequent pregnancies. Prenatal diagnosis should be offered to these couples in the case of future pregnancies.

Cytogenetic analysis should be performed in all couples with more than two spontaneous abortions as it is an important part of the etiological investigation. In some cases combined use of classical and molecular cytogenetics is necessary for delineating the chromosome regions involved in specific rearrangements.

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