The frequency of chromosomal abnormalities and the prenatal cytogenetic analyses for couples with recurrent abortions

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Between 1988-1998, cytogenetic analyses were performed for 1,476 couples and 162 women with recurrent abortions. We applied GTG-banding, high resolution-banding and FISH (fluorescent in situ hybridization) techniques in this study. The frequency of balanced translocations was 3.6% (112/3114). Of them, 74 cases (2.38%) were reciprocal translocations and 38 (1.22%) were robertsonian translocations. Chromosome aberrations were more frequent in women (80 cases) than in men (32 cases). No phenotypical abnormalities were found in all carriers who had experienced recurrent spontaneous abortions or experienced giving birth to malformed offsprings. Prenatal cytogenetic analyses were carried out on 40 subsequent pregnancies for carrier couples with balanced translocation. The fetal karyotypes showed that 13 cases (32.5%) were normal, 25 (62.5%) were balanced translocations, and two (6%) were unbalanced translocations. It is believed that the frequency of chromosomal abnormalities in patients with recurrent spontaneous abortion is higher than that of the normal population. Most of the fetal samples showed normal karyotypes or balanced translocations matching that of one of their parents. Although the incidence of chromosomal imbalance in the fetuses was relatively low in prenatal cytogenetic analysis, individuals with balanced translocations are predisposed to giving birth to malformed offsprings with partial trisomy or monosomy. Therefore, we recommend the cytogenetic and the prenatal cytogenetic analysis for those who experiences recurrent abortion as well as in case they become pregnant, to prevent the birth of offsprings with chromosomal abnormalities.

Keywords: Recurrent abortion, balanced translocation, prenatal cytogenetic study

INTRODUCTION

The frequency of chromosomal abnormalities is found to be higher in couples who have had recurrent abortion than couples in the general group (Mennuti *et al.*, 1978; Tóth *et al.*, 1984; Portnoi *et al.*, 1988). Of those chromosomal abnormalities, reciprocal balanced translocation is most frequent (Smith and Gaha, 1990; Uehara *et al.*, 1992). Most of the carriers of balanced translocation have normal phenotype and are generally healthy. As such, they are usually diagnosed as carriers after cytogenetic study to find the cause of their recurrent abortion, stillbirth or birth of malformed offsprings (Ohno *et al.*, 1989; Barisic *et al.*, 1996). We took such couples as the subjects of our cytogenetic study. The subjects of our study were referred to us after pregnancy for chromosome analysis of their fetus.

SUBJECTS AND METHODS

This study was conducted from 1988 to 1998 at the Genetic Research Laboratory of the Samsung Cheil Hospital with study subjects numbering 1,476 couples and 162 pregnant mothers whose husbands couldn't participate. These subjects had previous history of recurrent abortion, stillbirth and malformed offsprings with chromosomal abnormalities. We applied GTG-banding after lymphocyte culture for chromosomal analysis. For cases suspected of subtle chromosomal abnormality or to find more accurate breakpoint, we analyzed using high-resolution, R-banding and/or FISH. Among all the study subjects, karyotypes were analyzed in 20 cases of abortus and 4 cases with

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congenital malformation of balanced translocation carriers.

We performed the prenatal diagnosis using 34 cases amniotic fluid and 6 cases of chorionic villi samples from the 36 carriers. Statistical analysis was calculated by Chi-square and Fisher's exact test with Epi-info version 5.01. Results were considered significant at $p<0.05$.

RESULTS

Of the total 3,114 individual subjects of chromosomal study, we found 112 (3.6%) to be carriers of balanced reciprocal translocation. Of the 112, 74 had translocations in the autosomal chromosome. Two had translocations involving both autosome and sex chromosome. Remaining 38 had robertsonian translocations, with 2 having mosaicism of both translocated and normal cell line. The gender breakdown of the carriers appeared to be 80 (2.6%) females and 32 (1.0%) males. The rate of reciprocal balanced translocation between women and men was significantly different (p=0.03). The rate of robertsonian translocation between women (32 cases) and men (6 cases) was also significantly different (p=0.00) (Table 1). According to the history of the 112 carriers prior to referral, couples of 94 carriers had only experienced recurrent abortions never leading to a birth of a baby and it was most common. Couples of 10 carriers also experienced recurrent abortions but have had 1 or 2 healthy children. Of the couples of 8 carriers who had experienced giving birth to babies with malformation or growth retardation, 7 were reciprocal translocations and 1 was robertsonian translocation. The comparison of frequency of giving birth to normal or malformed offsprings between the reciprocal translocated and the robertsonian translocated couples was not statistically significant (p>0.05) (Table 2). Among the offsprings of balanced translocation carriers, we observed 19 cases of structural abnormalities with unbalanced translocation, 2 cases of numerical abnormalities and 3 cases of balanced translocation as same as the parents from 4 cases of blood sample from children with congenital malformation, mental and physical development retardation and 20 cases of abortus tissues (Table 3). During the 40 pregnancies of the 36 carriers of balanced translocation, they were referred to the genetic laboratory for the prenatal diagnosis. We found 13 cases of normal karyotype, 25 cases of balanced translocation carrier and 2 cases of unbalanced translocation carrier. In the fetal karyotype, the comparison of frequency of unbalanced fetus between the reciprocal translocated and the robertsonian translocated couples was insignificant (P>0.05) (Table 4).

Table 1. Types of balanced translocation carriers.

Translocation	No. of cases	Female	Male	P value[a]
Reciprocal	74	48	26	0.03
Robertsonian	38	32	6	0.00
Total	112	80	32	

[a] Chi-square test, α=0.05 (P=0.00)

Table 2. Reproductive data of 112 couples with translocation.

Translocation	Abortions[a] only	Abortions + normal liveborns[b]	Abortions + malformed offsprings[b]
Reciprocal	63	4	7
Robertsonian	31	6	1

[a] Abortions; history of recurrent spontaneous abortions more than 2 times

[b] Fisher's exact test,α=0.05 (P>0.05)

Table 3. Cytogenetic findings of abortuses and malformed offsprings in balanced translocation carriers.

	Numerical aberrations	Structural aberrations	
		Balanced	Unbalanced
Malfromed offsprings	0	2	2
Abortuses	2	1	17

Table 4. Prenatal cytogenetic diagnosis in translocation carriers.

Parental karyotype	No. of carriers	Fetal karyotype		
		Normal	Balanced	Unbalanced[a]
Reciprocal translocation	21	7	14	1
Robertsonian translocation	15	6	11	1
Total	36	13	25	2

[a] Fisher's exact test,α=0.05 (P>0.05)

DISCUSSION

It is known that the cause of the recurrent abortion, the

stillbirth and the birth of malformed baby is structural chromosomal abnormality of the parents (Tsenghi *et al.*, 1981). While in the general adult population, the frequency of balanced translocation carrier is 0.4%, higher frequency of balanced translocation carrier is found in those who had experience of recurrent abortion, stillbirth and giving birth to malformed babies (Diedrich *et al.*, 1983; Fortuny *et al.*, 1988; Gadow *et al.*, 1991; Uehara *et al.*, 1992). As we found the carrier frequency of 3.6% in these patient group, it is also higher than that of the general population. Futhermore, reciprocal translocation carriers were found to be higher than the robertsonian translocation carriers (Davis *et al.*, 1982; Fortuny *et al.*, 1988; Portnoi *et al.*, 1988). Our result shows concordance with the results of other similar studies. With the exception to the chromosome 19, reciprocity of translocation is found in all other chromosomes. The highest frequency of translocation, 14 cases were found for chromosome 6; 13 cases of reciprocal translocation of chromosome 11; 11 cases of chromosome 7; 9 cases of chromosome 2, 7, 8 and 22. However, frequency was low for chromosome 21 and chromosome 16, 2 cases. Also, in the sex chromosome, frequency of translocated with autosomes was low; 1 case for X and Y chromosome respectively. Even in the same chromosome translocation of the carriers, the break points in the cases showed great variability as well as translocation target chromosome. However, we were able to detect the 6 cases of reciprocal translocation carrier with translocation between chromosomes 11 and 22. This translocation is the most common (Delattre *et al.*, 1988; Soler *et al.*, 1993; Cohen *et al.*, 1996).

In the case of robertsonian translocation, of the 38 cases, we found 21 cases with translocation between the chromosomes 13 and 14 which has been reported as the most common (Page and Shaffer, 1997). Generally, chromosome abnormality is higher in females than males (Ohno *et al.*, 1989; Gadow *et al.*, 1991). In our study, we found higher frequency in females for both reciprocal and robertsonian translocation. In particular, the frequency of the robertsonian translocation was much higher in comparison to reciprocal translocation.

The result of chromosome analysis using first trimester abortus tissues in carrier shows that the major reason is the transmission of unbalanced chromosomal materials from the carrier parents to their offsprings. The structural abnormalities observed in the 16 cases from the abortus tissues and the offsprings with delayed development or malformation confirmed to be transmitted from their carrier parents (Uehara *et al.*, 1992). In two cases, trisomy 16 which is the most common in the spontaneous abortus tissues (Eiben *et al.*, 1990) was found. In one abortus and two offsprings with delayed development and congenital malformation, we found same balanced karyotypes with those parents.

In these cases, we can't rule out the subtle deletion or addition of chromosome which is difficult to distinguish by general GTG-banding. Subtle chromosomal abnormalities can lead to stillbirth or birth of malformed newborn (Gadow *et al.*, 1991). Accordingly, parents with experience of recurrent abortion or offsprings having congenital anomalies should receive chromosomal analysis (Shaffer *et al.*, 1996). In cases where translocated regions are extremely small or where patient of translocation take place between two heterochromatin or two euchromatin, high-resolution GTG-banding and FISH are useful for the detection of subtle deletion or translocated chromosome (Speleman *et al.*, 1992, Delaroche *et al.*, 1995). The two methods can be used for prenatal diagnosis for carriers with subtle translocated chromosome. High resolution GTG-banding can help in finding the subtle chromosomal deletion in reciprocal translocated patients with unknown congenital anomaly (Yang-Feng *et al.*, 1985). By high resolution GTG-banding and FISH, we were able to find the subtle translocated region in the terminal of chromosome 2 in a three-year-old girl with developmental delay and congenital malformation (Park *et al.*, 1996).

To find the origin of the girl's imbalanced translocation, we tested the parents and found the subtle balanced reciprocal translocation in the mother who was pregnant at the time. With the FISH and high-resolution GTG-banding, subtle balanced translocation between chromosome 2 and 12 was found; further test using amniotic fluid, and obtained the same result as the mother. When one of the couple is confirmed as a carrier of chromosomal abnormality, prenatal diagnosis is necessary should the wife become pregnant (Sachs *et al.*, 1985). In our study, most cases were found to be normal or carriers of balanced reciprocal translocation. Only 2 cases with unbalanced translocation were found. In one case, trisomy 21 of robertsonian translocation type was diagnosed by chorionic villi sampling during first trimester. In one case which was suspected of cerebellar abnormality by ultrasonogram during second trimester, partial trisomy of chromosome 22 was found. It was confirmed to be inherited from mother through parental karyotyping. In the prenatal diagnosis of balanced reciprocal translocation carriers, number of normal or balanced reciprocal translocation are higher than imbalanced translocations. The reason is that the majority of imbalanced translocationed fetuses were aborted at first trimester before amniocentesis (Uehara *et al.*, 1992).

As a case in point, the frequency of fetus with imbalanced translocation is higher at chorionic villi sampling and it is mostly found that the trisomy occurs in the small chromosome for cases of imbalance diagnosed after birth or imbalanced translocation found by amniocentesis after 15 weeks (Ueharar *et al.*, 1992; Takeyama *et al.*, 1995).

We confirmed through the chromosome analyses of the offsprings of carriers or abortus tissues that the unequal crossing over of malformation of abortion from carrier parents was transmitted to both malformed babies and miscarriages. Among the 40 cases of carriers which had gone through prenatal diagnosis, 38 cases were found to be normal or balanced translocation. In case of prenatal diagnosis balanced translocation carriers, inherited or normalcy were higher than unbalanced translocation.

Although the frequency of fetus with unbalanced translocation was lower, balanced translocation was higher in the couples who had experienced recurrent abortion or giving birth to malformed baby than general group. It shows chromosomal translocation is related to the abortion intrauterine fetal death and congenital abnormalities. Accordingly, a couple with previous history of two or more unknown abortion, or the birth of malformed baby should have chromosomal analysis done (Uehara *et al.*, 1992). A couple with balanced translocation can not only experience recurrent abortion but also have a high risk of malformed or mentally retarded offsprings with abnormal chromosome (Sider *et al.*, 1988).

Therefore, upon discovery of balanced translocation, chromosome analysis is necessary for other family members. For those found to be carriers, we believe parental diagnosis is absolutely necessary for their next pregnancy.

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