Chromosome Analysis in Couples with Recurrent Abortions

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SOH, K., YAJIMA, A., OZAWA, N., ABE, Y., TAKABAYASHI, T., SATO, S., SOU, S. and SUZUKI, M. *Chromosome Analysis in Couples with Recurrent Abortions*. Tohoku J. exp. Med., 1984, **144** (2), 151-163 — Chromosome analysis using a G-Banding technique was performed in 35 couples (70 individuals) with a history of two or more spontaneous abortions of unknown cause. Among these individuals, 5 (7%) showed balanced translocations, all of whom were female. The outcome of 31 pregnancies of 10 balanced translocation carriers was as follows: Ten of the offspring had normal phenotypes (32%), 5 (16%) were born with chromosomal abnormalities and 16 (52%) were spontaneously aborted. — recurrent abortions; chromosome aberration; translocation carrier; amniocentesis

Spontaneous abortions account for 15-20% of the total number of clinically verified pregnancies, so that the frequency of abortions including cases in which abnormal fertilized ova fail to become implanted at the time of implantation is undoubtedly much higher. The cause of spontaneous abortion during the first trimester of pregnancy is often uncertain, but a recent study has indicated that some 50% of spontaneously aborted fetuses have chromosomal abnormalities (Kajii 1979). The majority of such abnormalities, which arise at the stage of gamete development or fertilized ova cleavage, are either cases of chromosomal nondisjunction or mutation and, consequently, the recurrence of such abnormalities is unlikely. In a very small percentage, however, it is thought that the gamete has the same chromosomal abnormality as that of one of the parents. In such a case, it often occurs that an unbalanced gamete is produced repeatedly, which may result in the recurrence of spontaneous abortions.

It is known that the most common chromosomal abnormality of parents of normal phenotypes is the so-called balanced translocation and it has been reported that such translocation carriers account for 0.3% of the normal population (Jacobs et al. 1972).

In recent years, several studies of the incidence of translocation carriers among couples experiencing recurrent spontaneous abortions have been made, but there has been little agreement on the actual incidence. Genest (1979) reported an incidence of 0% among 102 subjects, whereas Wisniewski et al. (1980) have reported the highest incidence of 50% among 12 subjects. The present paper reports the incidence of translocation carriers among couples experiencing either recurrent abortions or birth of offspring with congenital abnormalities, and the outcome of pregnancies of translocation carriers.

METHODS

Chromosome analysis of peripheral lymphocytes

Peripheral venous blood was obtained with a heparin-treated injection syringe from the parents of children with congenital anomalies or the couples experiencing recurrent abortions. RPMI 1640 supplemented with 20% fetal bovine serum (GIBCO) and 100 $\mu\text{g/ml}$ AB-PC was used for the culture of the lymphocytes. Two ml of this medium was added to sterilized plastic tubes together with 0.05 ml of PHA (phytohemagglutinin M-form). To this solution was dripped 0.1 ml of the sample blood and, after thorough mixing, the solution was kept for culture in a 37°C container containing 5% CO_2 . After 70 hr of culture, colcemid was added to obtain a final concentration of 0.2 $\mu\text{g/ml}$ and further 2 hr of culture then allowed.

The cultured cells were then centrifuged at 1,000 rpm for 5 min. After adding 4 ml of a hypotonic 0.075 M KCl solution to the sedimentated cells, careful pipetting was performed and the solution allowed to rest for 7–10 min. Again 5 min of centrifugation at 1,000 rpm was done and the supernatant was discarded. After careful stirring and attaining a floating state, 4 ml of the previously cooled Carnoy fixative solution (methanol to acetic acid in a ratio of 3 : 1) were added along the sides of the test tube and again the solution was left to mix well. Fixation entailed washing the sediment thrice for over 30 min. The supernatant was again discarded and 2 or 3 drops of the solution were put on a slide glass and dried in several seconds using a dryer.

The prepared samples were dried at room temperature for about 7 days and then treated at 37°C for about 30 sec in 0.05% trypsin-Hanks solution. They were then rapidly washed in 0.2% EDTA and distilled water and again dried. Finally, they were stained for 20 min in 2% buffered Giemsa solution (pH 6.8).

Under a microscope, the chromosome number was determined from more than 20 cells and the karyotype of the chromosomes was analyzed in photomicrographs.

Culture of amniotic fluid and chromosome analysis of fetal cells

In cases that either the patient or her husband was a balanced translocation carrier, the amniotic fluid was sampled from the abdominal region between the 16th and 20th weeks of pregnancy of the patient. F-10 (GIBCO) supplemented with 20% fetal bovine serum (GIBCO) and 100 $\mu\text{g/ml}$ AB-PC was used as the culture medium. Three ml of amniotic fluid were placed in Nunc plastic dishes with 3 ml of the culture medium. This solution was left to culture in a 37°C container holding 5% CO_2 for one week. One half of the culture medium was then replaced, and thereafter one half was replaced every three days.

After sufficient proliferation of fibroblasts, colcemid (at a final concentration of 0.3 $\mu\text{g/ml}$) was added and 4–6 hr of further culture allowed. The cells were then washed twice in Puck's saline A solution and detached from the culture dish using 0.05% trypsin EDTA solution (GIBCO). After 5 min of sedimentation at 800 rpm, the supernatant was discarded and the cells treated with a 0.075 M KCl hypotonic solution for 10–15 min. Fixation, sample preparation, staining and microscopy were performed as described for blood samples.

TABLE 1. *Chromosome studies in couples with recurrent spontaneous abortions*

Number of abortions	Couple(s) with no normal liveborn offspring	Couple(s) with normal liveborn offspring	Total
2	13	1	14
3	14	2	16
4	2	1	3
5	1	0	1
8	1	0	1
Total	31	4	35

TABLE 2. *Translocation carriers among patients with recurrent spontaneous abortions*

Case No.	Translocation type	Karyotype	Number of abortions
1	rob. (D/D)	45, XX, -13, -14, +t(13q14q)	2
2	rob. (D/G)	45, XX, -13, -22, +t(13q22q)	2
3	rob. (D/G)	45, XX, -14, -21, +t(14q21q)	5
4	rob. (G/G)	45, XX, -21, -21, +t(21q21q)	3
5	rep. (D/D)	46, XX, t(14; 15)(q24; q24)	3

Fig. 1. Karyotype of Case 1.
45, XX, -13, -14, +t (13q14q)

RESULTS

The incidence of translocation carriers among couples experiencing recurrent abortions

Chromosome analysis was performed in 35 couples (70 individuals) with a

Fig. 2. Karyotype of Case 2.
45, XX, -13, -22, +t (13q22q)

Fig. 3. Karyotype of Case 3.
45, XX, -14, -21, +t (14q21q)

history of two or more spontaneous abortions of unknown cause (Table 1). Among these individuals, 5 showed balanced translocations (Table 2), all of whom were female. There were four cases of Robertsonian translocation; that is, Case 1, t (13q14q) (Fig. 1), Case 2, t (13q22q) (Fig. 2), Case 3, t (14q21q) (Fig. 3), Case

Fig. 4. Karyotype of Case 4.
45, XX, -21, -21, +t (21q21q)

Fig. 5. Karyotype of Case 5.
46, XX, t (14; 15) (q24; q24)

TABLE 3. *Frequencies of chromosome abnormalities among couples with recurrent spontaneous abortions*

Number of abortions	Robertsonian translocation (couples)	Reciprocal translocation (couples)	Total (%)
2	2/14	0/14	2/14 (14)
3	1/16	1/16	2/16 (13)
4	0/ 3	0/ 3	0/ 3 (0)
5	1/ 1	0/ 1	1/ 1 (100)
8	0/ 1	0/ 1	0/ 1 (0)
Total	4/35	1/35	5/35 (14)

Fig. 6. Pedigree of Case 1.

* 45, XY, -13, -14, +t (13q14q) (Case 6)

† 45, XX, -13, -14, +t (13q14q) (Case 1)

Fig. 7. Karyotype of Case 6.

45, XY, -13, -14, +t (13q14q)

4, t (21q21q) (Fig. 4). The other one case was reciprocal translocation ; Case 5, t (14 ; 15) (q24 ; q24) (Fig. 5). In other words, the incidence among these 35 couples was 14% (5/35) — all 5 of the carriers being female.

Pedigree studies of the carriers

Pedigree study was possible in 2 of the 5 translocation carriers, Cases 1 and 5 of Table 2. As shown in Fig. 6, it was found that the father of Case 1 (that is, Case 6) was a translocation carrier of the same kind (Fig. 7). Pedigree study of Case 5 (Fig. 8) showed that the father (Case 7) had balanced translocation (Fig. 9), the younger brother had a normal karyotype and the younger sister had an unbalanced translocation.

Fig. 8. Pedigree of Case 5.

* 46, XY, t (14 ; 15) (q24 ; q24) (Case 7)

† 46, XX, t (14 ; 15) (q24 ; q24) (Case 5)

‡ 46, XX, der (15), t (14 ; 15) (q24 ; q24) pat

Fig. 9. Karyotype of Case 7.

46, XY, t (14 ; 15) (q24 ; q24)

TABLE 4. *Parental chromosome studies in three cases of chromosome abnormalities*

Child karyotype	Father karyotype	Mother karyotype
47, XX, +18, t(5; 18) (q15; q22)	46, XY, t(5; 18) (q15; q22) (Case 8)	46, XX
47, XY, +21, t(5; 12) (q13; q24)	46, XY	46, XX, t(5; 12) (q13; q24) (Case 9)
47, XX, +21	46, XY/46, XY, t(8; 11) (q24; q13) (Case 10)	46, XX

Fig. 10. Karyotype of Case 8.
46, XY, t (5; 18) (q15; q22)

The relationship between offspring with congenital chromosomal abnormalities and the parents' karyotype

Table 4 summarizes the findings concerning offspring with congenital defects and their parents' karyotype. It was found that one carrier (Case 8) was the father of a stillborn fetus with unbalanced translocation showing 3:1 segregation (Fig. 10). Another parent (Case 9) of a stillborn fetus which had 21-trisomy and chromosomal translocation was found to be a carrier of a similar translocation (Fig. 11). One parent (Case 10) of a free 21-trisomy child was found to be a mosaic of a normal karyotype and balanced translocation (Fig. 12).

The outcome of pregnancies of the balanced translocation carriers

Table 5 presents the outcomes of the 31 pregnancies of the 10 balanced translocation carriers (Cases 1-10). Ten of the offspring had normal phenotypes (32%), three of which were found to be balanced translocation carriers. Five of

Fig. 11. Karyotype of Case 9.
46, XX, t (5 ; 12) (q13 ; q24)

Fig. 12. Karyotype of Case 10.
46, XY, t (8 ; 11) (q24 ; q13)

the offspring (16%) were born with chromosomal abnormalities and 16 (52%) were spontaneously aborted.

TABLE 5. *Pregnancy outcome in parents with chromosome translocation*

Case No.	Karyotype	Liveborn without anomalies	Liveborn with anomalies	Stillborn with anomalies	Spontaneous abortions	Total
1	45, XX, -13, -14, +t(13q14q)	0	0	0	2	2
2	45, XX, -13, -22, +t(13q22q)	1	0	0	2	3
3	45, XX, -14, -21, +t(14q21q)	1*	1 ^a	0	5	7
4	45, XX, -21, -21, +t(21q21q)	0	0	0	3	3
5	46, XX, t(14; 15) (q24; q24)	1*	0	0	3	4
6	45, XY, -13, -14, +t(13q14q)	2 (1)†	0	0	0	2
7	46, XY, t(14; 15) (q24; q24)	2 (1)†	1 ^b	0	0	3
8	46, XY, t(5; 18) (q15; q22)	1*	0	1 ^e	1	3
9	46, XX, t(5; 12) (q13; q24)	1*(1)†	1 ^c	0	0	2
10	46, XY/46, XY, t(8; 11) (q24; q13)	1*	1 ^d	0	0	2
Total		10 (3) (32%)	4 (13%) (16%)	1 (3%)	16 (52%)	31

* Prenatal diagnoses.

† Number of balanced translocation cases in parenthesis.

^a 46, XY, -14, +t(14q21q)^b 46, XX, der. (15) t(14; 15) (q24; q24)^c 47, XY, +21, t(5; 12) (q13; q24)^d 47, XY, +21^e 47, XX, +18, t(5; 18) (q15; q22)

DISCUSSION

In recent years, there have been frequent reports of the incidence of translocation carriers among couples experiencing recurrent abortions (Table 6). Taking these statistics together, 4.5% (6.1% for the females, 2.9% for the males) of the individuals examined were found to be translocation carriers. In the present study we found an incidence of 7.1%, all of the translocation carriers being female.

In light of the fact that the incidence of balanced translocation carriers in population studies is quite low (0.3%), the incidence found among those experiencing spontaneous abortions may suggest a possible causal relationship between recurrent abortion and the translocation condition.

TABLE 6. *Frequency of balanced translocation carriers among parents with recurrent abortions*

Reference	Translocations detected		Total	Incidence (%)
	Wife	Husband		
Kim et al. (1975)	4/50	0/50	4/100	4.0
Schmidt et al. (1976)	2/39	1/35	3/74	4.1
Tsenghi et al. (1976)	1/77	4/77	5/154	3.2
Stenchever et al. (1977)	6/28	1/28	7/56	12.5
Byrd et al. (1977)	4/55	2/55	6/110	5.5
Mennuti et al. (1978)	1/34	4/34	5/68	7.4
Tho et al. (1979)	9/100	2/100	11/200	5.5
Genest (1979)	0/51	0/51	0/102	0.0
Matton et al. (1980)	4/96	4/96	8/192	4.2
Wisniewski et al. (1980)	5/6	1/6	6/12	50.0
Antich et al. (1980)	4/32	2/32	6/64	9.3
Subrt (1980)	6/115	3/115	9/230	3.9
Michels et al. (1982)	6/200	2/200	8/400	2.0
Davis et al. (1982)	5/100	3/100	8/200	4.0
Present report	5/35	0/35	5/70	7.1
Total	62/1,018 (6.1%)	29/1,014 (2.9%)	91/2,032 (4.5%)	

All parents had a history of two or more spontaneous abortions and with or without stillborn or anomalous liveborn infant.

All studies used the banding technique.

In the case of the female carriers, normally only one ovum matures each month, so that if the ovum is an unbalanced translocation gamete, then its fertilization will lead to the production of an abnormal zygote and most frequently to spontaneous abortion; only infrequently will offspring with congenital abnormalities survive.

In the case of male carriers, however, millions of sperms are released by every ejaculation, so that even when unbalanced translocation gametes are present, they will only infrequently fertilize the ovum and do not often cause the production of abnormal zygotes. This difference is thought to be the underlying factor which results in the higher incidence of female carriers detected than male carriers.

There is of course still the possibility of offspring with balanced or unbalanced translocations in which spontaneous abortions were caused by the male carrier, such as found in our Cases 6, 7, 8 and 10 (Table 5). Since translocation characteristics can be transmitted from father to offspring, it is essential that chromosome study of the male in cases of recurrent spontaneous abortion also be undertaken.

Habitual abortion has been defined as three or more incidents of abortion — and only those couples who are habitual aborters are generally thought to be

suitable for chromosome analysis. In the present study, however, we have found that 14% of the couples who had experienced only 2 spontaneous abortions included translocation carriers. It is, therefore, desirable that all couples with two or more spontaneous abortions undergo chromosome analysis.

Both on theoretical grounds and from clinical experience it is known that carriers of translocations of homologous chromosomes (Case 4) (Table 5) will produce 100% abnormal fetuses and that normal offspring cannot be expected. With regard to the children of carriers of translocations on non-homologous chromosomes, it is expected theoretically that the ratio of normal offspring (including translocation carriers) to abnormal offspring will be 1 : 2, provided that chromosomal crossing-over is disregarded. If all abortuses were abnormal offspring, the ratio of normal to abnormal fetuses in the present research would be 1 : 1.8 — or quite close to the theoretical value (excluding only Case 4 from Table 5). It should be noted, however, that the carriers of the present study were those with recurrent spontaneous abortion or the parents of congenitally abnormal offspring and their families—i.e., only those lineages which showed clinical symptoms. Consequently, it is thought that the pregnancy outcome showed a tendency which is less favorable than that in the general population. It remains unclear what the pregnancy outcome of symptomless translocation carriers might be. Particularly in light of the fact that chromosomal screening tests of newborn infants have been carried out on recent years at various institutions, it is worthwhile to investigate prospectively the outcome of pregnancies of the children of detected translocation carriers.

As noted above, analysis of the 31 pregnancy outcomes of the carriers in our study (Table 5) showed two 21-trisomy offspring (Cases 9 and 10); indicating an incidence of 6%—which is nearly 50-fold higher than the incidence of 21-trisomy in Japan (0.09–0.13%). This extremely high incidence of 21-trisomy is thought to indicate that, in addition to unbalanced translocations, the offspring of translocation carriers exhibit a chromosomal trisomy which is unrelated to the parental translocated chromosome. At the same time, this finding suggests that the parent of offspring with free 21-trisomy (which is thought to be a non-genetic condition) is a translocation carrier possibly.

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