Am J Hum Genet 32:549–554, 1980

Cytogenetic Studies in 100 Couples with Recurrent Spontaneous Abortions

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SUMMARY

The reported incidence of translocations in couples with recurrent spontaneous abortions ranges from 3% to 31% (average 9.3%). We report 100 couples in whom no reciprocal translocation carriers were ascertained.

INTRODUCTION

The cause of recurrent early pregnancy wastage is often unknown, but has been associated with uterine abnormalities, hormonal imbalance, immunological factors, and infectious diseases [1]. An increased incidence of chromosomal translocations in couples who are "habitual aborters" has been reported [2–9]. This incidence ranges from 3% to 31% (average 9.3%) and has suggested to some that cytogenetic analysis be utilized as a primary tool in the assessment of recurrent pregnancy wastage [7]. Of the 100 couples we examined for a history of "habitual abortion," chromosome variations were noted in six individuals. However, in none of these was there a reciprocal translocation, and in only one (45,X/46,XX) can the karyotype be called definitely "abnormal."

MATERIALS AND METHODS

The couples included in this study were either involved in a gynecological evaluation or had completed the evaluation and were referred to the Genetics Clinic for a genetic investigation, including blood group study for possible incompatibility and chromosome analysis. All couples had a history of two or more consecutive miscarriages. Patients were not excluded from the study if a normal pregnancy preceded or followed a series of miscarriages, but were excluded if the

Received August 17, 1979; revised September 27, 1979.

This study was supported in part by grants from The Henry J. Kaiser Family Foundation, The Boettcher Foundation, and the Genetic Foundation.

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product of a third-trimester pregnancy was noted to have multiple anomalies and this was the reason for referral.

The couples under investigation were divided into three groups (table 1): those who had a history of recurrent first-trimester miscarriages (group I), those who had first- and second-trimester loss (group II), and those with a history of recurrent loss with one or more successful pregnancies (group III).

Whole blood cultures were established on all 200 individuals by the technique of Arakaki and Sparkes [10]. Metaphase spreads were prepared by a modified Hungerford technique [11]. All chromosome preparations were G-banded by a modification of the method of Seabright [12]. An average of 25 metaphases was counted on each individual, five metaphases were microscopically analyzed, and two karyotypes were prepared.

The control group consisted of 184 individuals of childbearing age (17 to 40) referred for reasons other than multiple miscarriages.

RESULTS

Among the 100 couples involved in this study, no balanced translocation carriers were ascertained. Six of 200 patients (table 2) had karyotypes that deviated from usual. Three of these had polymorphic variants, including one with prominent satellites on chromosome 15 and two with pericentric inversion of chromosome 9 (p11q13). One patient had a pericentric inversion of chromosome 2 (p11q13), another had a fragile 16, and one was an X-chromosome mosaic. The control group revealed two individuals with a pericentric inversion of chromosome 9 (p11q13); all other individuals were normal.

In both the experimental and control groups, sporadic cells were observed with chromosomal rearrangements. One of the controls had one cell with a 7/14 translocation, and another had one cell with a 12/18 translocation. Four patients had one cell with a reciprocal translocation between chromosomes 7 and 14, one had one cell with a 7/12 translocation, and another had one cell with a 10/18 translocation. These sporadic chromosomal rearrangements accounted for less than 1% of total cells analyzed.

TABLE 1

REPRODUCTIVE HISTORY OF COUPLES WITH MULTIPLE MISCARRIAGES

	No. couples		
	Two miscarriages	Three or more	Total
Group I (first-trimester loss)	15	41	56
Group II (first- and second-trimester loss)	1	14	15
Group III (multiple first- and second-trimester loss with successful pregnancies)	2	27	29
Total	18	82	100
Group III breakdown:			
Term pregnancy prior to multiple miscarriages	1	9	10
Term pregnancy after miscarriages*	1	10	11
Term pregnancy interspersed with multiple miscarriages	...	8	8

* These individuals were evaluated prior to the successful pregnancy.

TABLE 2

CHROMOSOME VARIANTS IN PATIENTS WITH MULTIPLE MISCARRIAGES

Reproductive history	No. losses	Karyotype	Comments
Group III	4	46,XX,15ps+	Prominent satellites in all metaphases
Group I	3	46,XX,inv(9)(p11q13)	Confirmed with C-banding
Group I	3	46,XX,inv(9)(p11q13)	Confirmed with C-banding
Group I	5	46,XX,inv(2)(p11q13)	...
Group III	3	46,XX	Fragile 16 in 8/60 cells, breakpoint at 16q22; not present in fibroblasts
Group III	3	45,X/46,XX	5/100 cells with 45,X

DISCUSSION

Our results differ significantly from the eight previous reports of chromosomal abnormalities in couples with recurrent pregnancy wastage [2–9] which suggest that chromosomal translocations are found at a rate of from 3% to 31% (average 9.3%) in these couples. This is a 30-fold increase in translocations over the frequency in newborns which is reported to be 0.3% [13]. However, the above data include couples who have had a congenitally abnormal child, which we have specifically excluded. Among the 100 couples reported here, no individual had a balanced translocation in a significant proportion of cells.

Three patients had structurally abnormal chromosomes. One patient was noted to have a pericentric inversion of chromosome 2, which has been reported to have no effect on reproduction [14]. However, if a recombinant chromosome were formed, it would include such a large portion of the genome that the resulting pregnancy would probably be nonviable.

An association of pericentric inversions of the heterochromatic secondary constriction of chromosome 9 and reproductive failure has been suggested [15–16]. The rate of pericentric inversions of chromosome 9 in our study (1.0%) is the same as that reported for the general population [14] and for the rate observed in the control group (1.1%). Therefore, within our sample of patients, there is no apparent relationship between recurrent pregnancy wastage and inversion 9.

X-chromosome mosaicism has been described in women with multiple miscarriages [17–19]. Although the case we report has a low degree of mosaicism in peripheral lymphocytes, it might account for the recurrent pregnancy wastage. The significance of the fragile chromosome 16 in the lymphocytes, but not in the fibroblasts, of one patient and prominent satellites on chromosome 15 in another is unknown.

The occurrence of 7/14 translocations in occasional cells of cultured human lymphocytes has been previously reported [20]. The meaning of sporadic cells with 7/14 or other translocations is as yet unknown, and, hence, the apparent increase of such cells in the patients is of uncertain significance.

These five chromosomal variants account for 2.5% of individuals (5.0% of couples) involved in this study. We are unable to explain the discrepancy between our findings and those of others [2–9]. We have possibly selected against balanced translocations in the parents by the majority of our couples having had three or more miscarriages

TABLE 3

SUMMARY DATA FROM LITERATURE

REFERENCE	TOTAL	GROUP A* KARYOTYPE Normal	GROUP A* KARYOTYPE Abnormal	GROUP B† KARYOTYPE Normal	GROUP B† KARYOTYPE Abnormal	GROUP C‡ KARYOTYPE Normal	GROUP C‡ KARYOTYPE Abnormal	No. with abnormal karyotype and 3+ miscarriages
Kim et al. [2]	50	12	4	34	0	...	...	...
Stenchever et al. [3]	28	5	7	...	...	16	0	0
Tsenghi et al. [4]	77	...	...	...	...	75	2	2
Schmidt et al. [5]	22	...	...	...	...	20	2	2
Byrd et al. [6]	55	8	3	...	...	41	3	3
Mennuti et al. [7]	34	...	...	6	1	23	4	3
Kajii and Ferrier [8]	73	...	...	...	...	71	2	0
Neu et al. [9]	30	...	...	...	...	29	1	1
Total	369	25	14 (35.9%)	40	1 (2.5%)	275	14 (4.8%)	11

* Group A: couples with a history of spontaneous abortion plus congenital abnormalities in offspring.
† Group B: couples with a history of spontaneous abortion plus normal offspring.
‡ Group C: couples with a history of spontaneous abortion without offspring.

(82/100). Only 27 of these couples carried a baby to term. Since a parent with a balanced reciprocal translocation has a significant chance with each pregnancy of having a child with a normal karyotype, and since the risk of such a parent having an offspring with an unbalanced translocation is low [21], one might expect couples who are balanced translocation carriers to have at least one successful pregnancy prior to multiple (three or more) miscarriages. Hence, since the majority of our patients had three or more miscarriages and no term pregnancies, there may have been selection against parents who carried translocations. The data in the eight studies referred to [2–9] do not permit determination of the proportion of patients who had only two spontaneous abortions. However, of the total of 369 couples reported, 14 had karyotypic abnormalities and no congenitally defective children (4.8%) (table 3). Three of this group had only two spontaneous abortions. Hence, the expectation that balanced translocations would be more frequently found in couples with only two spontaneous abortions does not seem likely.

The relatively low level of chromosomal variants plus the lack of translocations suggest that chromosomal analysis should not be considered as a primary tool for the evaluation of multiple miscarriages. Chromosome analysis may, however, yield useful information after other causes of recurrent pregnancy wastage have been ruled out. Moreover, in [2–9], there were 39 couples with multiple miscarriages and the birth of a congenitally defective child. Fourteen of these (36%) did reveal one parent with a karyotypic abnormality (table 3). Cytogenetic analysis is, therefore, indicated in this relatively small group.

REFERENCES

1. GLASS RH, GOLBUS MS: Habitual abortion. *Fertil Steril* 29:257–265, 1978
2. KIM HJ, HSU LYF, PACIUC S, CRISTIAN S, QUINTANA A, HIRSCHHORN K: Cytogenetics of fetal wastage. *N Engl J Med* 293:844–847, 1975
3. STENCHEVER MA, PARKS KJ, DAINES TL, ALLEN MA, STENCHEVER MC: Cytogenetics of habitual abortion and other reproductive wastage. *Am J Obstet Gynecol* 127:143–150, 1977
4. TSENGHI C, METAXOTOU-STAVRIDAKI C, STRATAKI-BENETOU M, KALPINI-MAVROU A, MITSANIOTIS N: Chromosome studies in couples with repeated spontaneous abortions. *Obstet Gynecol* 47:463–468, 1976
5. SCHMIDT R, NITOWSKY HM, DAR H: Cytogenetic studies in reproductive loss. *J Am Med Assoc* 236:369–373, 1976
6. BYRD JR, ASKEW DE, MCDONOUGH PG: Cytogenetic findings in fifty-five couples with recurrent fetal wastage. *Fertil Steril* 28:246–250, 1977
7. MENNUTI MT, JINGELESKI S, SCHWARZ RH, MELLMAN WJ: An evaluation of cytogenetic analysis as a primary tool in the assessment of recurrent pregnancy wastage. *Obstet Gynecol* 52:308–313, 1978
8. KAJII T, FERRIER A: Cytogenetics of aborters and abortuses. *Am J Obstet Gynecol* 131:33–38, 1978
9. NEU RL, ENTES K, BANNERMAN RM: Chromosome analysis in cases with repeated spontaneous abortions. *Obstet Gynecol* 53:373–375, 1979
10. ARAKAKI DT, SPARKES RS: Microtechnique for cultured leukocytes from whole blood. *Cytogenetics* 2:57–60, 1963
11. HUNGERFORD DA: Leukocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. *Stain Technol* 40:333–338, 1965

12. SEABRIGHT M: A rapid banding technique for human chromosomes. *Lancet* 2:971–972, 1971
13. JACOBS PA, FRACKIEWICZ A, LAW P: Incidence in mutation rates of structural rearrangements of the autosomes in man. *Ann Hum Genet* 35:301–319, 1972
14. LEONARD C, HAZAEL-MASSIEUX P, BOCQUET L, LARGET-PIET L, BOUE J: Inversion péricentrique inv. (2) (p11q13) dans des familles non apparentées. *Humangenetik* 28:121–128, 1975
15. BOUE J, TAILLEMITE JL, HAZAEL-MASSIEUX P, LEONARD C, BOUE A: Association of pericentric inversion of chromosome 9 and reproductive failure in ten unrelated families. *Humangenetik* 30:217–224, 1975
16. DE LA CHAPELLE A, SCHRODER J, STENSTRAUD K, ET AL.: Pericentric inversions of human chromosomes 9 and 10. *Am J Hum Genet* 26:746–766, 1974
17. PREDESCU V, CHRISTODORESCU D, TAUTU C, CNOVIRNACHE M, CONSTANTINESCU E: Repeated abortions in XO/XX mosaicism. *Lancet* 2:217, 1969
18. HSU LYF, GARCIA FP, GROSSMAN D, KUTINSKY E, HIRSCHHORN K: Fetal wastage and maternal mosaicism. *Obstet Gynecol* 40:98–103, 1972
19. LUCAS M, WALLACE I, HIRSCHHORN K: Recurrent abortions and chromosome abnormalities. *J Obstet Gynecol Br Commonw* 79:1119–1127, 1972
20. HECHT F, MCCAW BK, PEAKMAN D, ROBINSON A: Non-random occurrence of 7-14 translocations in human lymphocyte cultures. *Nature* 255:243–244, 1975
21. JACOBS PA: Structural rearrangements of the chromosomes in man, in *Population Cytogenetics*, edited by HOOK EB, PORTER IH, New York, Academic Press, 1977, pp 81–97