Demonstration of Robertsonian Fusion by Silver Staining in a 13/14 Translocation in Man

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Translocations, balanced or unbalanced, involving D/D, D/G and G/G are not uncommon in human populations. The incidence of D/D translocations is estimated to be 1 in 1000 live births (Hamerton *et al.* 1972). Most of these translocations are between non-homologous chromosomes of the D group with t (13q; 14q) being most frequent (Centerwall and Merrell 1975). The D/D and D/G type translocation Down's syndrome frequency is reported to be slightly less than three percent (Wright *et al.* 1967). Balanced translocation carriers such as the D/D, though phenotypically normal, have a high incidence of spontaneous abortions (Fernhoff *et al.* 1976).

Translocations can be a typical Robertsonian fusion where breaks occur in both non-homologous chromosomes and in the process of production of a bivalent chromosome a centromere is lost. Fusion between non-homologous chromosomes may occur with a break in only one chromosome, such as that reported for 7/22 translocation (Larson *et al.* 1977). Translocations between non-homologous chromosomes may occur also without apparent breaks resulting in fusion between telomere to telomere (T-T), telomere and centromere (T-C) and centromere to centromere (C-C) in mammalian species that may result in a functional and a latent centromere in the dicentric chromosomes (Hsu *et al.* 1975). These are specialized type of Robertsonian fusions according to Hsu's classification.

The short arms of human acrocentric chromosomes of the D and G groups can be subdivided into three regions: the C-positive centromeric region, the C-negative secondary constriction and the C-negative terminal satellite. The five pairs of acrocentric chromosomes with the secondary constriction regions are known to be involved in the production of nucleoli. Since the silver staining technique of Goodpasture and Bloom (1975) has now been greatly simplified (Lau *et al.* 1978), it is possible not only to identify the individual chromosomes involved in translocation but to determine if the nucleolar organizer regions (NOR) have been lost.

Materials and methods

A 26 year old mother who has a phenotypically normal 2½ year old girl had a second child that was stillborn with multiple congenital malformations. The abnormalities in the stillborn included, presence of extra digits, bilateral cleft palate and high interventricular septal defect. The parents were therefore advised to undergo cytogenetic examination.

Lymphocyte culture from whole blood was used for standard karyotype analysis of both the parents. Slightly modified G banding procedure of Seabright (1972) as described elsewhere (Larson *et al.* 1977) was utilized for identification of chromosomes. The C banding was a slight modification (Jalal *et al.* 1974) of the procedure by Arrighi and Hsu (1971). The simplified silver staining technique of Lau *et al.* (1978) was used to stain the nucleolar organizer regions (Ag-NOR). The procedure involved a pre-treatment of air dried slides, in borate buffer (0.1 M Na_2SO_4 , 0.005 M $\text{Na}_2\text{B}_4\text{O}_7$, pH 9.1) for 15–20 minutes followed by a brief rinse in distilled water and incubation in 50% AgNO_3 solution in a moist chamber for 18–22 hours at 55–60°C.

Fig. 1. G banded karyotype. Arrow indicates the t (13q; 14q) chromosome.

Results and discussion

Based on the analysis of 30–33 cells each the father had a normal diploid chromosome complement, and the mother with a 45 chromosome count seemed to have a balanced translocation. Five G-banded karyotypes confirmed the observation that the mother had a t (13q; 14q) translocation (Fig. 1). The C-band of the translocated chromosome revealed a single centromere, though dicentric chromosomes

are also known to occur in such translocations (Niebuhr 1972, Hsu *et al.* 1975).

Forty-five spreads were counted for nucleolar organizer regions (NOR) by silver staining. The number of NORs ranged from 3 to 8 with an average of 6.3 per cell (Table 1). The translocation product, t (12q; 14q) was devoid of any silver stain (Fig. 2) in all the cells examined. The break points must therefore be

Fig. 2. Silver stained NORs from 4 G and 3 D group chromosomes. Arrow indicated the two No. 3 and an unstained translocated chromosome.

Table 1. Frequency of silver stained nucleolar organizers in a 13/14 translocation

No. of cells	Ag-NOR/cell*
2	3
3	4
2	5
14	6
18	7
6	8

* Represents the total number of stained nucleolar organizers in the D and G group chromosomes.

in the centromeric regions so that the nucleolar organizers are lost from both chromosomes. Since the translocated chromosome seems to have a single centromere we conclude that this is a typical Robertsonian fusion. Another case of 13/14

Robertsonian type translocation has been reported by Hurley and Pathak (1977). Gosden *et al.* (1979) report a loss of slightly over 60% of satellite DNA (Sat. III) in a 13/14 translocation where both nucleolar organizers were deleted. It is known that the ammonical silver staining technique (Goodpasture and Bloom 1975) corresponds closely to the sites of ribosomal cistron determined by "in situ" hybridization procedures (Pardue and Hsu 1975, Hsu *et al.* 1975). It is interesting that complete loss of two nucleolar organizers does not interfere with normal development. This follows the pattern reported for the toad, (*Xenopus*), where a loss of 50% r DNA does not seem to interfere in normal differentiation (Brown and Gurdon 1964). The simplified silver staining technique along with C- and G-banding should help in documentation of the approximate break points, number of nucleolar organizer regions and the nature of such translocations for the D and G group chromosomes, in normal human populations. The risk where one parent is a balanced translocation (13/14) carrier and the other is normal would be expected to be 50% unbalanced translocations or monosomics resulting in spontaneous abortions, 25% balanced translocation carriers and 25% normal.

Summary

G banded karyotypic analysis of normal parents, whose second child was stillborn with multiple congenital abnormalities, revealed a normal 46, XY father and 45, XX, t (13q; 14q) mother. By a simplified silver staining technique it was possible to demonstrate that the entire secondary constriction of both chromosomes were lost in the process of translocation. C banding displayed the translocated chromosome to be monocentric. We therefore conclude that the translocation represents a typical Robertsonian fusion. Loss of two nucleolar organizers has seemingly no deleterious effect in development.

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