Heterochromatic polymorphism in spontaneous abortions

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SUMMARY Since the advent of C-banding as a routine diagnostic procedure, the significance of heterochromatic polymorphism has been questioned. Some workers have considered variations in heterochromatin in chromosomes 1 and 9 to be associated with fetal wastage, recurrent abortions and abnormal phenotypes.

Over a 15-month period, this laboratory made a study of the diagnostic significance of heterochromatic variants in 50 couples with a history of recurrent abortions. A control group of 50 couples with at least two normal children and no miscarriages was investigated simultaneously.

The results indicated no significant difference in the heterochromatic regions between aborting and non-aborting couples.

It has long been known that the amount of DNA in higher organisms greatly exceeds that which is required to code for their necessary proteins, but in recent years it has become evident that much of this 'surplus' DNA is found in the constitutive heterochromatin in the form of repetitive polynucleotide sequences (McDermott, 1975).

The development of a simple stain for constitutive heterochromatin has brought localisation within the scope of the diagnostic laboratory. However, since the role of constitutive heterochromatin is still not completely understood, the significance of C-banding polymorphism has become a subject of speculation, research, and varied interpretation (Nielsen *et al.*, 1974).

Polymorphism, resulting from duplication, deletion, and inversion of heterochromatin appears to occur at a relatively high frequency in the general population.

The variability of heterochromatin in chromosome pairs 1 and 9 is said by some workers to bear a relationship to fetal wastage (Kunze and Mau, 1975), recurrent abortion (Tsengki *et al.*, 1976; Ford, 1977), and abnormal phenotypes (Gardner *et al.*, 1974; Halbrecht and Shabtay, 1976).

This laboratory, wishing to establish the diagnostic significance of C band polymorphism in reproductive failure, undertook a complete cytogenetic investigation of 50 aborting couples and, at the same time, of a control group of 50 normal couples.

Patient selection

Criteria for patient selection required the aborting couple to have had three or more spontaneous abortions. The 'normal controls' were required to have had at least two normal children and no miscarriages.

Methods and materials

Chromosome preparations were made of cultured lymphocytes using a modification of the method of Moorhead *et al.* (1960). G-banding was carried out using a PBS trypsin solution at 10°C (Scheres, 1972). C-banding of the heterochromatic region was obtained using acid hydrolysis followed by treatment with barium hydroxide and 2X SSC (Sumner, 1972).

MEASUREMENT OF C BANDS

Until now, the method of measurement of heterochromatin followed that of Muller *et al.* (1975), who assessed the amount of heterochromatin in relation to the size of the long arms of chromosome 21 in the same cell.

We found that problems arose with this procedure because of the difficulty in detecting accurately the length of the long arms of chromosome 21 and because of the distortion in the size of the chromosomes on the edge of the metaphase (Fig. 1).

The C band of chromosomes 1 and 9, therefore, was measured quantitatively and expressed as percentage of the overall length of the chromosome.

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as shown in Fig. 2. So that this could be done as accurately as possible, only those cells with long straight chromosomes and clearly stained C bands and terminal regions were used.

The normal range for heterochromatin size of chromosomes 1 and 9, measuring 600 chromosomes, was established for this method and laboratory. As shown in Table 1, the heterochromatin of chromo-

Fig. 1 *Distortion of chromosomes on the edge of the metaphase.*

Fig. 2. *Method used for heterochromatin measurement.*

some 1 occupies approximately 20% of its length and that of chromosome 9 approximately 33%. Heterochromatin was examined microscopically on 10 cells from each patient. Photographs and measurements were made of the chromosomes from two metaphases.

Results

Table 2 shows the distribution among aborting and control couples with heterochromatin above the normal range.

In 45.5% of the aborting couples, one parent had one chromosome 9 with increased heterochromatin (9qh+) compared with 57.5% in the control group. In 15% of the aborting couples, both parents had one chromosome 9 with increased heterochromatin, compared with 5% in the control group.

There was an increase in the heterochromatin in one of the pair of chromosomes 1 (1qh+) in 62% of the aborting group, compared with 60% in the control couples. In 2.5% of the aborting group, both parents had chromosome 1 with increased heterochromatin, compared with 10% in the control group. Statistical analysis with the application of the χ^2 test showed no significant difference between the two populations. These results appear to differ markedly from those obtained by another worker (Ford, 1977), who found that approximately 83% of aborting couples had at least one 9qh+. The control popula-

tion used in his investigation was a newborn population.

In this study the following factors were thought to be significant:

- (1) The normal control population should be equivalent to the population under investigation.
- (2) The obstetric history of the control group must be known or the comparison is meaningless.
- (3) The couples must be examined as a unit.

These factors could account for the difference between the two studies.

Our laboratory was interested in the significance of pericentric inversions in the aborting group, in view of the finding of Boué *et al.* (1975), who suggested a correlation between a complete pericentric inversion of the heterochromatin of chromosome 9 and infertility. In our study, 28% of the aborting couples were seen to have a pericentric inversion of chromosome 9, compared with 22% in the control couples. It was decided that a pericentric inversion was said to occur when at least one third of the heterochromatin was in the short arm. Using our measurements, there was no significant difference in its occurrence in the two groups.

There was an unexpectedly high percentage of major chromosomal abnormalities in the aborting group of 50 couples. The four cases found are as follows.

- (1) 47,XXX/46,XX,80%:20%.
- (2) 45,XX,t(13q;14q).
- (3) 45,XX,t(13q;14q).
- (4) 46,XX,t(13;22) (q34;q12).

No abnormalities were found in the control group.

Abnormally large heterochromatic variants were found in this survey, but, surprisingly, only in the control couples.

Fig. 3 shows the enlarged heterochromatin of chromosome 1 (seen in one patient) and the marked increase in the acrocentric heterochromatin of a D group chromosome (seen in two patients). Obviously neither of these heterochromatic variants were having any deleterious effect on their phenotype or reproductive ability.

Conclusion

From the present evidence, our laboratory now feels that the size of the heterochromatic region, when measured quantitatively as described, bears no relation to poor reproductive history. However, because of the relatively high percentage of major chromosomal abnormalities seen, we feel that cytogenetic studies play an important part in the investigation of couples who miscarry.

Table 1 Normal range for heterochromatin in chromosomes 1 and 9.

9 qh $\bar{x}$	:	29%
SD	:	6.0
NR	:	23-35%
1 qh $\bar{x}$	:	18.5%
SD	:	3.0
NR	:	15.3-21.5%

Table 2 Percentage distribution of findings.

	% of population		χ^2	Significance
	Aborting (50 couples)	Control (50 couples)		
1 $\times$ 9 qh+ Maternal or paternal	45.5%	57.5%	1.00	NS
1 $\times$ 9 qh+ Maternal and paternal	15.0%	5.0%	1.95	NS
1 $\times$ 1 qh+ Maternal or paternal	62.5%	60.0%	<0.01	NS
1 $\times$ 1 qh+ Maternal and paternal	2.5%	10.0%	1.60	NS

(a)

Fig. 3 (a) *Enlarged heterochromatin in chromosome 1.*

(b)

Fig. 3(b) *Enlarged heterochromatin in a large acrocentric chromosome.*

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