New C band markers of human chromosomes: C band position variants

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SUMMARY Although it is well known that variations in size are common in the centric heterochromatin of human chromosomes, it has not been appreciated that centric heterochromatin may be positioned either entirely on the short arm, centrally, or entirely on the long arm. Inspection of C banded karyotypes in which chromosomes were identified by previous Q banding showed C band position variants in all of the chromosomes of groups A, B, C, E, and F. These 'new' C band markers were followed in families and found to be heritable. By scoring chromosomes for both size and position of C bands it should be possible to distinguish the majority of homologous chromosomes in most people. The ease of scoring and high frequency of variation should make C band position variants extremely useful in linkage studies and population surveys.

Previous work has shown that in chromosome 9 the position of the heterochromatic secondary constriction with respect to the centromere is variable, so that in some subjects the 9h region is entirely on the long arm, in some it is on both sides of the centromere, and in others it is entirely on the short arm.¹ The frequency of subjects with an inverted 9h region varies in different human populations.² Less frequent but similar variations have been described for chromosomes 1 and 16.^{3,4} Although previous studies^{5,6} have shown that there is extensive variation in the size of the constitutive heterochromatin of many human chromosomes, variations in the position of the C band with respect to the centromere were not noted for chromosomes other than 1, 9, and 16. Evidence is presented in this paper that shows that the position of the centromere with respect to the centromeric heterochromatin is a polymorphic trait in the majority of chromosome pairs.

Materials and methods

Blood samples were obtained from subjects with sickle cell anaemia and their relatives.

Lymphocyte cultures were prepared by a modification of the method described by Moorhead *et al*⁷ with colcemid treatment of 2 to 4 hours. Air dried slides were prepared and stained with 0.5% atetrin and mounted in 70% sucrose in phosphate buffer for

fluorescence microscopy. Later, these slides were destained by soaking in fixative after removal of the coverslip and then treated to obtain the C bands. The C band method was a modification of that of Arrighi and Hsu⁸ in which slides were treated for 6 minutes with 0.07 NaOH in 2XSSC, rinsed and incubated overnight in 2XSSC at 65°C, and then passed through alcohol three times and stained with 10% Giemsa at pH 6.8. At least a dozen Q banded cells were photographed after C banding, so that a minimum of six double karyotypes with good C banding could be prepared. These were done in accordance with the Paris Conference,⁹ and the position of the C bands on each chromosome scored as p (short arm), pq (centre), and q (long arm). Different karyotypes on the same subject gave the same results with respect to C band position.

Results

The position of the C band was found to be variable in all of the chromosome pairs of the A, B, C, E, and F groups except for chromosome 16. The C band was either entirely on the short arm, entirely on the long arm, or centrally located. Homologous chromosomes which differ in C band position are shown in fig 1a and b. The acrocentric chromosomes are not considered in this paper since differences in the size and staining characteristics of the stalk and satellite regions on the short arms of these chromosomes are well known.

Received for publication 6 December 1979

FIG 1 (a) Homologous chromosomes which differ in C band position (chromosome pairs, 1, 2, 3, 4, 5, 6, and 7); (b) homologous chromosomes which differ in C band position (chromosome pairs 8, 9, 10, 11, 12, 17, 18, 19, and the X chromosome).

TABLE 1 Distribution of C band position variants in chromosomes from unrelated subjects*

Chromosome pair	Position of C band		
	p	pq	q
1	0.00	0.15	0.85
2	0.46	0.48	0.06
3	0.14	0.67	0.19
4	0.32	0.29	0.39
5	0.46	0.28	0.26
6	0.82	0.10	0.08
7	0.23	0.25	0.52
8	0.48	0.46	0.06
9	0.04	0.06	0.90
10	0.35	0.35	0.30
11	0.46	0.32	0.22
12	0.33	0.26	0.41
17	0.53	0.33	0.14
18	0.77	0.15	0.08
19	0.35	0.29	0.36
20	0.29	0.40	0.31
X	0.26	0.23	0.51

*Based on a sample of 52 chromosomes from 26 subjects.

The distribution of the C band position variants in the population studied is given in table 1 and varies considerably for different chromosome pairs. If chromosomes 1, 9, and 16 are excluded, the frequency of chromosomes with the C band entirely on the short arm varied from 15 to 82% for different chromosome pairs, and the frequency of chromosomes with the C band entirely on the long arm varied from 6 to 52%. Chromosome pairs which usually had the C band on the short arm in this population sample included chromosomes 2, 5, 6, 8, 11, 17, and 18. Chromosome pairs 7, 12, and X had the C band more often on the long arm. In the other chromosome pairs all three positions were common. Although the population sampled here was small ($n = 26$), it seems likely that there are real differences in the frequency of C band positions on the different chromosome pairs.

Inheritance of the C band position variants has been followed in several families. Table 2 shows the

TABLE 2 Inheritance of C band position variants in family 1

Chromosome pair	C band position on homologous chromosomes					
	Mother	Father	Child 1	Child 2	Child 3	Child 4
2	p, p	p, p	p, p	p, p	p, p	p, p
3	pq, q	p, pq	pq, pq	q, p	q, pq	q, pq
4	p, q	pq, p	p, p	q, p	q, p	q, pq
5	p, p	p, q	p, q	p, q	p, q	p, p
6	p, p	p, p	p, p	p, p	p, p	p, p
7	p, q	pq, q	pq, q	q, p	pq, q	q, q
8	p, p	p, pq	p, pq	p, p	p, p	p, pq
10	p, q	q, q	p, q	q, q	q, p	q, q
11	p, pq	p, p	pq, p	p, p	pq, p	pq, p
12	p, q	pq, q	p, q	q, pq	p, q	p, pq
17	p, pq	p, p	pq, p	p, p	p, p	pq, p
18	p, p	p, p	p, p	p, p	p, p	p, p
19	p, p	p, q	p, q	p, p	p, p	p, p
20	q, q	pq, q	q, pq	q, q	q, q	q, pq
X	p, q	q	p, q	p, q	p	q

C band positions on each chromosome for a family with four children. The results are consistent with Mendelian inheritance. It is interesting that out of the 15 chromosome pairs in the A, B, C, E, and F groups (excluding 1, 9, and 16), there were only two chromosomes pairs (6 and 18) for which the parents

had the same C band positions on their homologous chromosomes. Thus, for the majority of chromosome pairs, inheritance of the parental homologous chromosomes could be traced for one or both of the parents.

The inheritance of C band position variants for this family for chromosomes 5, 7, X, and 19 is shown in figs 2 to 5. As shown in fig 2, the father had the C band on 5p on one chromosome and 5q on the other. In the mother the C band was on 5p on both chromosomes. Three of the children inherited the 5p chromosome from the father, and one inherited the chromosome with the C band on 5p. All the children received a 5p chromosome from the mother. As shown in fig 3, the father had the C band on 7q on one chromosome and a large centrally located C band (7pq) on the other. The mother had the C band on 7p on one chromosome and 7q on the other. Children 1 and 3 inherited the 7pq from the father and the 7q from the mother. Child 2 inherited 7p from the mother and 7q from the father, and child 4 inherited 7q from both parents. For the X chromosome (fig 4) the mother had the C band on Xp on one chromosome and on Xq on the other

FIG 2 Inheritance of C band position variants on chromosome 5.

FIG 3 Inheritance of C band position variants on chromosome 7.

FIG 4 Inheritance of C band position variants on the X chromosome.

FIG 5 Inheritance of C band position variants on chromosome 19.

FIG 6 (a) Inheritance of C band position variants on the X chromosome in family 2; (b) inheritance of C band position variants on the X chromosome in family 3.

chromosome. The father had the C band on Xq. Among the children, one son inherited Xq from the mother and the other inherited the Xp. Both daughters inherited the Xp from the mother and the Xq from the father. For chromosome pair 19 (fig 5), the father had the C band on 19p on one chromosome and on 19q on the other. The mother had the C band on 19p on both chromosomes. The first child inherited the 19q from the father, while the other three children inherited the 19p from father. All the children inherited a 19p from the mother.

In the population studied, homologous X chromosomes could often be distinguished on the basis of C band position. Fig 6a and b show the inheritance of C band position on the X chromosome for two additional families. In fig 6a the mother had the C band on Xp on one chromosome and on Xq on the other. Of the three sons, the first son inherited the Xp and the second and third sons inherited the Xq. In fig 6b the mother had the C band on Xp on one chromosome and on Xq on the other. The father's C band was on Xp. The first son had the C band on

Xp and the second son on Xq. The daughter inherited Xp from the father and Xq from the mother.

Discussion

The position of the C band centric heterochromatin appears to be an extremely polymorphic trait. In the sample studied, all possible C band positions (p, q, and pq) were found for the 15 chromosome pairs in the A, B, C, E, and F groups excluding chromosomes 1, 9, and 16. The distributions of chromosomes with the C band on p, pq, and q were different for each chromosome pair. Larger samples will be needed to determine whether there is a characteristic frequency for each chromosome pair and how much it varies between different human populations. Preliminary results (unpublished observations) suggest that there are no large differences between North American black and white populations.

Since C band position variants are heritable, they will be especially useful for linkage studies. C band size variants have been used in linkage studies, but their usefulness is limited for chromosomes other than 1, 9, and 16, since it is difficult to measure accurately the size of C bands. Variation in relative size of the C band occurs at different stages of chromosome contraction, as well as variation in the apparent size with different degrees of staining. It is difficult to determine accurately the boundary between heterochromatin and euchromatin.¹⁰ Because of these difficulties, C band size variants have been most often used as markers for chromosome pairs 1, 9, and 16 which have large heterochromatic regions.¹¹ C band position is much easier to score, and when combined with the obvious size variants should enable the majority of homologous chromosomes to be distinguished in most people. This will make linkage studies much more efficient. The high frequency of C band position variants found on the X chromosome in the population sample studied is of special interest since significant variation in the size of the C band is relatively infrequent for this chromosome. Scoring for X chromosome C band position variants will have application in determining the origin of struc-

tural abnormalities of X chromosomes, ruling out maternal contamination in amniocentesis, and greatly facilitating the cytogenetic mapping of the X chromosome.

This project was supported in part by BRSG Grant 5 S07 RR07 181 01 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

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