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Lateral Asymmetry in Human Constitutive Heterochromatin: Frequency and Inheritance

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INTRODUCTION

Chromosome 1 in man has long been recognized as having a heteromorphic segment in the long arm proximal to the centromere. Prior to banding techniques, this was known variously as the "secondary constriction" or "uncoiler region." However, with the introduction of techniques for staining constitutive heterochromatin in man [1], it was found that chromosomes 1, 9, and 16—all known previously to have "secondary constrictions"—in fact had large blocks of deeply staining material in these positions. By comparison with earlier studies on the chromosomes of the mouse [2], it seemed likely that these regions of constitutive heterochromatin in the human chromosome complement would contain large amounts of satellite DNA in which the base composition and degree of sequence repetition would be distinct from the rest of the genome. Subsequently, in situ hybridization studies of the four major satellites isolated from human DNA have shown that two of these satellite fractions are localized predominantly in the constitutive heterochromatin of chromosomes 1 and 16 (satellite II) and in chromosome 9 (satellite III) [3].

A new staining technique previously used to detect DNA synthesis fluorometrically [4] has recently been applied to the study of regions of constitutive heterochromatin in mammalian chromosomes. This technique uses the dye 33258 Hoechst, the fluorescence of which is modified when 5-bromodeoxyuridine (BrdU) is incorporated into chromosomal DNA. Lin et al. [5] first observed that if mouse cells were grown for 1 generation in BrdU and the preparations stained with Hoechst, the fluorescence of the two chromatids was asymmetric, one staining much more brightly than the other in the regions of constitutive heterochromatin.

Subsequently, the occurrence of lateral asymmetry was reported in certain human chromosomes. By applying the same technique, Latt et al. [6] demonstrated lateral asymmetry in the long arm of the Y chromosome and in the constitutive heterochromatin of chromosome no. 16. Kim [7], using a fluorescence plus Giemsa technique, found lateral asymmetry in chromosomes 1 and 16, and Angell and Jacobs [8] showed that

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lateral asymmetry could be demonstrated in chromosomes 1, 15, 16, and the Y. They also demonstrated that, whereas the asymmetrical staining in chromosomes 15, 16, and the Y is simple and confined to one arm, it is compound in chromosome 1 (i.e., differential staining is present in both sister chromatids, but at any one point lateral asymmetry is maintained). The pattern of compound lateral asymmetry varied from individual to individual but was constant in all cells from any one individual [8]. Therefore compound lateral asymmetry appears to be another heteromorphic feature associated with the variable region of chromosome 1. Angell and Jacobs' observations of asymmetry, both compound and simple, were confirmed by Galloway and Evans [9], who also reported finding lateral asymmetry in the C-banded regions of chromosomes 17, 20, 21, and 22.

The purpose of the present report is (1) to determine the relative frequencies and types of lateral asymmetry found in chromosomes 1, 9, 15, 16, and the Y with special reference to the pattern of compound lateral asymmetry in chromosome 1; (2) to determine whether or not there is a correlation between the type of pattern found in chromosome 1 and the size of its heterochromatic region; and (3) to determine the mode of inheritance of compound lateral asymmetry seen in chromosome 1.

MATERIALS AND METHODS

Peripheral blood lymphocytes were grown in Hams F10 (GIBCO medium) supplemented with 20% fetal calf serum and containing BrdU (Sigma, St. Louis, Mo.) at a final concentration of 30 μg/ml. Colcemid (final concentration of 0.8 μg/ml) was added for the last hour of the 48 hr culture period, the cells were harvested, and air-dried preparations were made in the standard way. The slides were stained in 33258 Hoechst at a concentration of 5.0 μg/ml in tap water for 1 hr, then rinsed and mounted in tap water, sealed with a coverslip, ringed with a rubber solution to prevent evaporation, and exposed to a UV light source. A suitable UV light source was found to be a 200W mercury vapor lamp. The slides were exposed at a distance of 15 inches from the bulb for 45 seconds. The coverslips were then removed, and the slides were placed in 2X SSC. at 60°C for 2 hr, rinsed in water, stained with 3% Giemsa (Gurr's) for 5 min, rinsed in water, dried, and mounted. This technique clearly differentiates between cells arrested in their first division in culture, in which the chromosomes appear uniformly stained with the exception of certain areas of highly repetitive DNA, and those in the second division, in which all the chromosomes have a characteristic harlequin appearance due to generalized differentiation of the sister chromatids. For the observations on lateral asymmetry, only those cells in their first division were considered and the fairly numerous cells in their second division and the very rare cells in their third division in culture were disregarded.

Lateral asymmetry was studied in cultures of peripheral lymphocytes from three different groups of people, namely: (1) a population unselected with respect to the nature of their constitutive heterochromatin, consisting of 19 mentally retarded individuals and five controls who were having their chromosomes examined as part of another project; (2) seven individuals selected because they were known to have a larger than average block of constitutive heterochromatin in the proximal region of the long arm of one or both no. 1 chromosomes; (3) 10 family groups consisting of two parents and one or more offspring who were selected either because of availability or, in the case of three families, because they were being studied for another reason [i.e., to investigate the inheritance of a pericentric inversion of chromosome 9 (families 3 and 7) or a reciprocal translocation between chromosomes 6 and 11 (family 8)].

Patterns of Compound Lateral Asymmetry and Scoring Techniques

The following method for describing the pattern of compound lateral asymmetry in the proximal part of the long arm of chromosome 1 was adopted. Since the total size of the darkly

staining regions on both arms was found to correlate with the size of the C-band region [8], the description starts with the size of the C-band using a scale of 1–5 (very small, small, average, large, and very large); this is followed in parenthesis by a description, based on visual examination on the microscope, of the proportion of darkly staining material on each arm expressed as a fraction of 10 irrespective of the size of the C-band, the block proximal to the centromere being described first. For example, if, in an average size C-band (3), the size of the part proximal to the centromere was estimated as 2/10 of the total, then the pattern would be 3(2, 8); if, in a large C-band, most of the darkly staining block was proximal to the centromere with only a small portion being distal, this would be 4(9,1); and if the pattern was simple rather than compound, it would be 4S. Examples of these patterns are illustrated in figure 1. This system was convenient as a visual description, but since the fraction of the chromosome under consideration approaches the limits of resolution, it was also practical to group the patterns into three classes: CLA I where the block of darkly staining material proximal to the centromere is smaller than the distal block (1,0; 2,8; 3,7); CLA II where the two blocks are approximately equal (4,6; 5,5; 6,4); and CLA III where the distal block was smaller than the proximal block (7,3; 8,2; 9,1).

Three minor problems of resolution were occasionally found in determining the pattern of compound lateral asymmetry in chromosome 1. First, in preparations in which optimally stained cells showed compound lateral asymmetry with a very small proximal or distal part, there were some cells where the finer details were lost and the pattern appeared as simple lateral asymmetry. Where the pattern was clearly seen to be compound in the best cells, it was recorded as compound. The second problem concerned resolution at the point where the chromatids converged at the centromere. We have suggested [8] that an apparent pattern of the type 1,9 might be simple lateral asymmetry and the compound appearance simply due to a symmetrically staining centromeric dot. However, further experience on improved preparations have shown us that such patterns are truly compound, and there is no evidence for a symmetrically staining region at the centromere. The third problem was found in chromosomes in which the C-banded region was small or very small where it was difficult to resolve the exact nature of the asymmetry. To minimize these problems, the pattern was assessed from at least 10 good cells, but nevertheless there may still be an overrepresentation of patterns classified as having simple lateral asymmetry.

Sister strand exchange within the region of lateral asymmetry, as noted by Kim [7], was seen only very rarely and presented no problem to the scoring of the basic type of lateral asymmetry, present in every cell.

RESULTS

Type and Frequencies of Patterns of Lateral Asymmetry

The frequencies of the different patterns of asymmetry found in the no. 1 chromosomes of 44 unrelated individuals (24 unselected individuals and the parents of the 10 families examined) are presented in table 1. Of the 88 no. 1 chromosomes, 71 have a pattern of compound lateral asymmetry and 17 simple lateral asymmetry, although, for reasons discussed above, there may be an overrepresentation of the latter group. The most frequent types of compound lateral asymmetry are those where the size of the distal part is equal to or larger than that of the proximal, there being far fewer of the type where the distal part is smaller.

Twenty of the 44 individuals were unequivocal heterozygotes based on differences in pattern or size or a combination of the two. An individual was classified as an unequivocal heterozygote when the pattern of asymmetry of the two homologues differed by more than two types scored in the initial observation (e.g., 1,9;4,6 and 2,8;5,5 are considered heterozygotes but 1,9;3,7 and 2,8;4,6 are not) or when the size

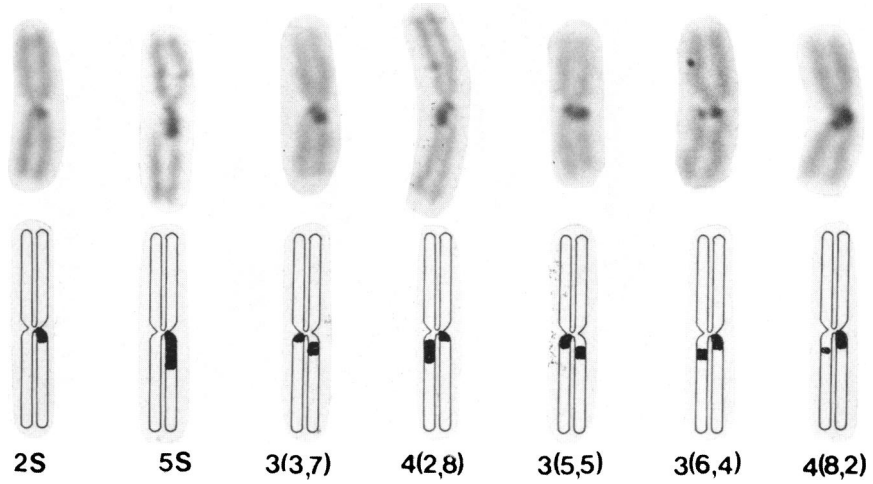

FIG. 1.—Seven no. 1 chromosomes illustrating different patterns of simple and compound lateral asymmetry.

of the C-band differed by more than one class. Only four of these 20 could be classified as heterozygotes on the basis of the C-band size alone. The staining intensity of the heteromorphic region of chromosome 9 and the Y is consistently less than chromosomes 1, 16, or 15. This makes the pattern in chromosome no. 9 more difficult to define (fig. 2). It was previously reported that the majority of no. 9 chromosomes stain symmetrically and that only a few had some degree of asymmetry [8]. However, further observations suggest that a large proportion of no. 9 chromosomes may have a complex and asymmetrical staining pattern. However the limitations of the present staining technique prevent any variation of their pattern being assessed accurately.

The heterochromatic region of chromosomes 16 and 15 almost invariably showed simple lateral asymmetry; only one example of possible symmetry was seen in a single chromosome 16.

Correlation between Type of Pattern and Size of Heterochromatic Region

An attempt was made in chromosome 1 to correlate the pattern of asymmetry with size of the heterochromatic region, and the observations are presented in table 2. They are based on data from 51 unrelated individuals; 44 whose patterns are shown in table 1,

TABLE 1

PATTERN OF LATERAL ASYMMETRY IN CHROMOSOME 1 FROM 44 UNSELECTED INDIVIDUALS

SIMPLE LATERAL ASYMMETRY	COMPOUND LATERAL ASYMMETRY								
S	CLA I			CLA II			CLA III		
0,10	1,9	2,8	3,7	4,6	5,5	6,4	7,3	8,2	9,1
17	10	14	10	9	20	3	2	3	...

TABLE 2

CORRELATION BETWEEN SIZE OF C-BAND REGION AND PATTERN OF LATERAL ASYMMETRY IN CHROMOSOME 1 FROM 51 UNRELATED INDIVIDUALS

SIZE OF C-BAND	SIMPLE LATERAL ASYMMETRY	COMPOUND LATERAL ASYMMETRY								
	S	CLA I			CLA II			CLA III		
	0,10	1,9	2,8	3,7	4,6	5,5	6,4	7,3	8,2	9,1
Very small or small (1–2)	9	1	2	3	...	3	...	1	...	...
Medium (3)	7	7	9	6	7	15	2	1	3	...
Large (4)	1	2	7	1	3	3	1	...	...	...
Very large (5)	5	2	...	...	...	1	...	...	...	...

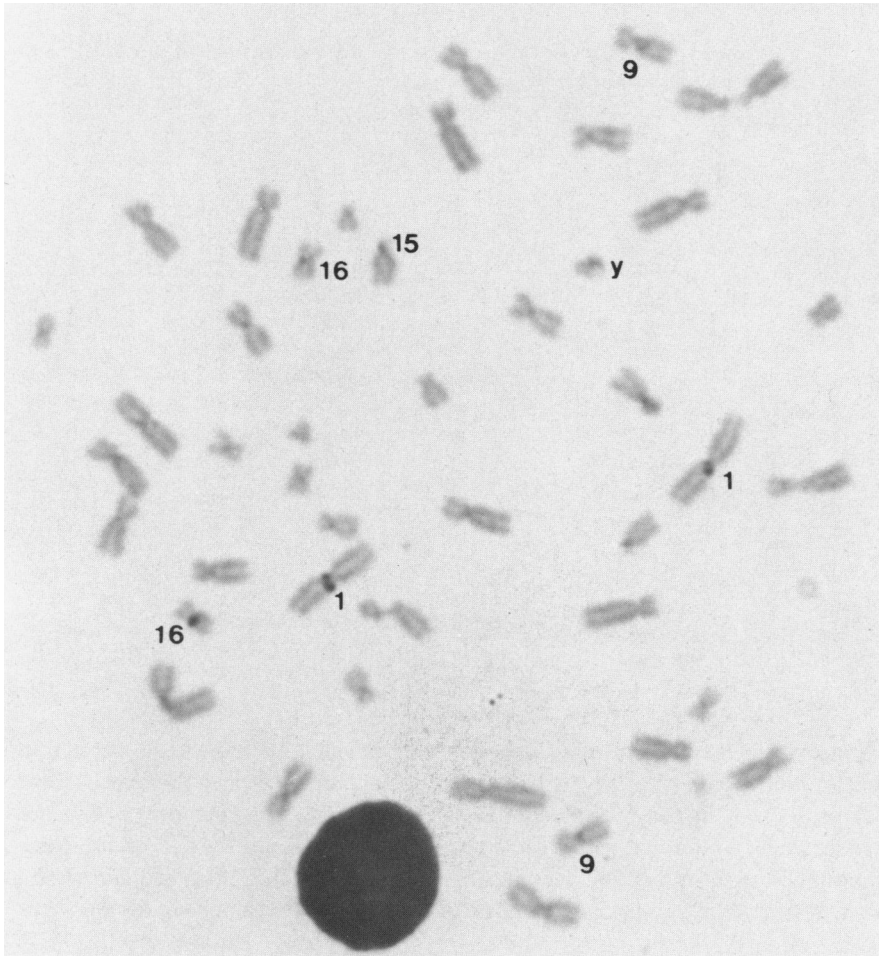

FIG. 2.—Representative cell showing lateral asymmetry.

and seven selected because they were known to have a large or very large C-band in chromosome 1. Inspection of the data suggests a correlation between large or very large C-bands and simple or compound lateral asymmetry type I. However when this was tested formally, excluding those with a small or very small C-band because of the problem of identifying the pattern accurately, no significant correlation between size and pattern was found.

Inheritance of Pattern of Lateral Asymmetry in Chromosome 1

The patterns of inheritance of the 10 families studied are presented in table 3. In every family, the size and pattern of compound lateral asymmetry in no. 1 chromosomes in the F_1 generation were compatible with their having been inherited in a Mendelian fashion, and there was no chromosome 1 in the F_1 which was different from

TABLE 3

INHERITANCE OF PATTERN OF COMPOUND LATERAL ASYMMETRY IN CHROMOSOME 1

FAMILY	PARENTS				F_1	
	Paternal		Maternal		Paternal	Maternal
1	2S	3(2,8)	2S	3(1,9)	3(2,8)	2S
2	2S	3(1,9)	3S	3(1,9)	3(1,9)	3S
3	3(2,8)	4(1,9)	2(3,7)	3(5,5)	3(2,8)	2(3,7)
4	2(3,7)	3(8,2)	2S	3(1,9)	3(8,2)	3(1,9)
5	2S	3(5,5)	2S	4(3,7)	1. 3(5,5) 2. 2S	4(3,7) 4(3,7)
6	3(3,7)	3(1,9)	2(5,5)	4(2,8)	1. 3(3,7) 2. 3(3,7)	4(2,8) 2(5,5)
7	3(5,5)	5S	4(5,5)	4(2,8)	1. 5S 2. 5S	4(5,5) 4(2,8)
8	3S	3(4,6)	2(2,8)	3(4,6)	1. 3(4,6) 2. 3S 3. 3(4,6)	2(2,8) 3(4,6) 2(2,8)
9	3(4,6)	3(2,8)	2(5,5)	3(4,6)	1. 3(2,8) 2. 3(4,6) 3. 3(4,6)	3(4,6) 2(5,5) 2(5,5)
10	3(2,8)	4(4,6)	3(3,7)	3(5,5)	1. 3(2,8) 2. 4(4,6) 3. 3(2,8)	3(3,7) 3(3,7) 3(5,5)

that in the parents. The results from family 7 in which all four of the parents' no. 1 chromosomes could be distinguished unequivocally are illustrated in figure 3. Both no. 1 chromosomes in the father could be identified from information on C-band size alone, but not in the mother whose chromosome 1 C-bands were the same size. The data suggest that compound lateral asymmetry is a stable inherited morphological characteristic which gives a high degree of resolution between chromosomes.

DISCUSSION

Our present results confirm our previous observations of lateral asymmetry in chromosomes 1, 15, 16, and the Y in the human chromosome complement. However, further observations on chromosome 9 suggest that our initial report of symmetry in the heterochromatic region might be erroneous and that the pattern on chromosome 9 may well be compound but is very difficult to resolve because it is poorly differentiated using the present technique. It is clear that the pattern of compound lateral asymmetry in chromosome 1 is a true heteromorphism: it has a wide range of different morphological expressions in the population, its expression is constant in different cells within the individual, and it appears to be inherited in a simple Mendelian way. In our population lateral asymmetry, even when heterozygotes were defined very conservatively, was about five times more efficient in discriminating between the two no. 1 chromosomes in an individual than C-banding alone. Therefore, this technique should be extremely useful in linkage analysis and in determining the origin of chromosome abnormalities involving chromosome 1.

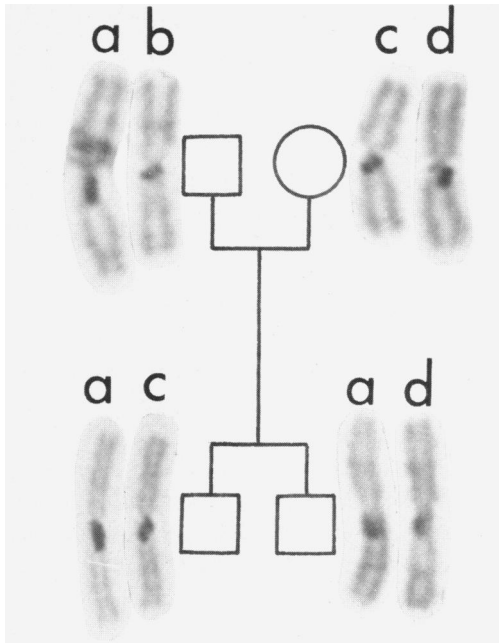

FIG. 3.—Inheritance of pattern of lateral asymmetry in chromosome 1 in family 7.

SUMMARY

The relative frequencies and types of lateral asymmetry found in chromosomes 1, 9, 15, 16, and the Y were determined. The pattern of asymmetry is simple in chromosomes 15, 16, and the Y but compound in 1 and possibly also 9. The pattern of compound lateral asymmetry is a stable heteromorphism inherited in a simple Mendelian way and is an efficient morphological discriminator between the members of the no. 1 chromosome pair.

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The 17th annual postgraduate course entitled "Clinical Genetics for the Pediatrician," sponsored by the Department of Pediatrics of Emory University School of Medicine, will be held April 3–5, 1978. The course will cover a fundamental review of human genetics, the genetics of common disorders, cytogenetics, and inherited metabolic disorders. Recent advances in treatment and control of heritable disorders will also be covered. Guest faculty will include: Dr. James Hanson, University of Iowa; Dr. Lewis B. Holmes, Harvard University; Dr. Arthur Robinson, University of Colorado, Dr. Leon E. Rosenberg, Yale University; and Dr. Louis J. Elsas, II, coordinator, Emory University. Direct queries to Postgraduate Education, Department of Pediatrics, Emory University School of Medicine, 69 Butler Street, S.E., Atlanta, Georgia 30303.

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