

# Giemsa banding of chromosome 1qh+ and linkage analysis

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**Summary.** A four-generation transmission of a 1qh+ chromosome was ascertained by routine chromosome analysis of a mildly dysmorphic and retarded 6½-year-old female. Concordance between synophrys and the 1qh+ marker was the only consistent phenotypic relationship. The variant chromosome did not appear uncoiled, and Giemsa centromeric staining (C-bands) revealed an increased width of the heterochromatin commensurate with the increased length of the long arm. Giemsa banding of the entire chromosome (G-bands) revealed two heterochromatin bands, identical in appearance, in the centromeric region with the remainder of the chromosome showing normal banding. The distribution of Duffy blood groups in the pedigree was consistent with the locus being on chromosome No. 1.

Variant No. 1 chromosomes with an increase in the length of the long arm are reported fairly frequently. Data from the New Haven newborn survey (Lubs and Ruddle, 1971) show this variant to occur in about one of every 400 Caucasian births. This type of variant has been reported in both abnormal and normal individuals (Cooper and Hernits, 1963; Yunis and Gorlin, 1963; Donahue *et al*, 1968; Ying and Ives, 1968).

Further characterization of these chromosomes is now possible by means of the fluorescent and Giemsa banding techniques (reviewed by Pearson, 1972). Lobitz, McCaw, and Hecht (1972) have reported a three-generation pedigree segregating for a 1q+ variant showing an increased number of bands in the centromeric region of the long arm. Holzer, Rosenkranz, and Glatzl (1972) have described a family with a segregating 1qh+ chromosome and noted that four individuals have the variant in a homozygous state. Osztovics *et al* (1973) and Kim (1973) also reported 1qh+ chromosomes.

We have investigated a four-generation pedigree segregating for a similar variant chromosome No. 1. We have shown this chromosome to have an in-

creased length due to an increase in the heterochromatic region and have studied the linkage of this marker with three traits.

## Methods and materials

Slides were made from cultures of human lymphocytes and centromeric banding (C-bands) and individual banding patterns (G-bands) were produced as previously described (Howard, Stoddard, and Seely, 1973). Determination of the Duffy and Rhesus blood group phenotypes was done by standard techniques.

## Case report

The proposita, a part American Indian female, was referred at 6½ years of age because of poor school performance and dysmorphic features. Phenotype included a prominent synophrys, mild hypertelorism, epicanthal folds, upturned nose with a wide philtrum, a high narrow palate, and a small, downturned mouth. Other features included generalized hypertrichosis, brachydactyly with broad great toes, clinodactyly of the little fingers, and abnormal dermatoglyphics. Radiographic evaluation revealed anomalies of the lumbar spine and malrotation of the right kidney. Psychometrics showed a dull normal intellectual function.

The phenotype of the proposita's father was normal; her mother had significant dysmorphic features which

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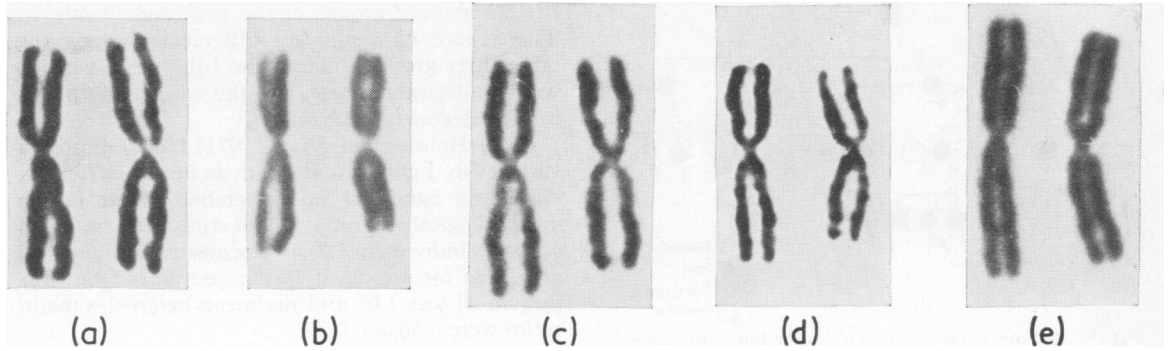


FIG. 1. Chromosome No. 1 pairs from five cells. Pair (b) is from a skin fibroblast cell; the others are from lymphocytes.

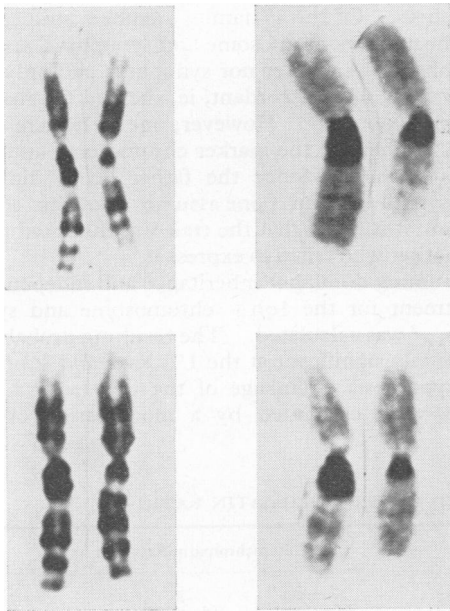


FIG. 2. The G-band (left) and C-band (right) patterns found in the 1qh+ chromosome and its normal homologue.

included facial asymmetry, hypertelorism, synophrys, and a high broad palate. The mother's left ear consisted of a rudimentary skin tag, absent ear canal and deafness; the right ear was low set but normally formed. Intellectual function was grossly normal.

## Results

**Chromosome studies.** Routine chromosome analysis on the proposita revealed asymmetry of the No. 1 pair with one being longer and less meta-centric. Chromosome analyses of the parents revealed the father's karyotype to be normal while the

mother had a similarly appearing non-metacentric No. 1. There was no evidence found in the karyotypes to indicate the occurrence of a translocation. The 1qh+ chromosome did not look uncoiled, and the chromatids usually did not show an area of diminished separation as has been reported in other pedigrees. In over 97% of the examined cells, the marker chromosome appeared as illustrated in Fig. 1 (b-e) and not like the pair shown in Fig. 1 (a).

The C-band and G-band patterns of the 1qh+ chromosomes and its normal homologue are shown in Fig. 2. An increase in size of the heterochromatin region (h) is evident in the C-band pattern. This pattern was found in all cells examined from an individual having the 1qh+ chromosome and, likewise, in cells grown from a skin biopsy of the proposita.

The normal G-band pattern was present in the short arm and also in the long arm below the first faint band as can be seen by comparing the marker chromosome to its normal homologue. However, in the area between the centromere and the first faint band, the marker chromosome had an additional band. In some cells, like those shown in Fig. 2, the heterochromatin appeared to be duplicated or doubled.

Family members from four generations were available for study (Fig. 3). The 1qh+ chromosome was transmitted to both male and female offspring and, assuming a segregation ratio of 0.5, the deviation from that expected was not statistically significant. All family members except the proposita and her mother had a normal phenotype. Thus, the marker chromosome most likely was not aetiologically related to the phenotype of the proposita.

The morphological arm ratio of chromosome No. 1 was calculated by dividing the length of the long arm (q) by the length of the short arm (p).

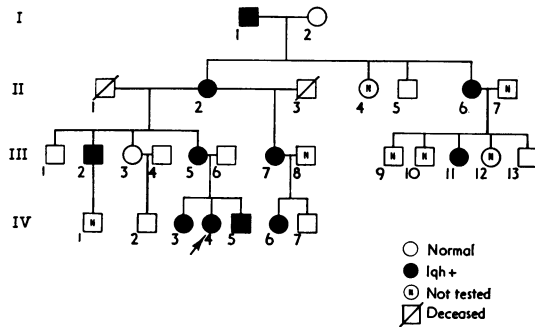


FIG. 3. Pedigree of the family in which the  $1qh+$  chromosome is segregating.

Measurements and calculations were done on chromosomes from family members both with and without ('at-risk') the  $1qh+$  chromosome and controls. An analysis of variance was performed on the angularly transformed data, and the individual means were compared by the Kramer modification (Kramer, 1956) of Duncan's new multiple range test (Table I). The  $1qh+$  chromosome data differed significantly from the other three groups at the 1% level; all other comparisons were non-significant.

Long arms and heterochromatic areas of the No. 1 pair were also measured in C-banded cells. The heterochromatin ratio was calculated as  $h/(q-h)$  (Hoehn and Martin, 1972). Analysis of variance and comparison of the individual means by the method previously described showed that the  $1qh+$  chromosome data were significantly different from

the other three groups at the 1% level (Table I). There were no significant differences among the other three groups. Thus, the  $1qh+$  chromosome was significantly longer, and the added length was in the heterochromatic region.

Craig-Holmes and Shaw (1971) have reported a variant No. 1 chromosome with an increased heterochromatin band but no detectable change in the morphological arm ratio. This study also contained one such individual (I.2). The mean morphological arm ratio for a total of 14 chromosomes from this individual was 1.05 and the mean heterochromatin ratios were 0.56 and 0.37.

**Linkage studies.** The  $1qh+$  chromosome was not associated with any consistent physical phenotypic expression with the possible exception of synophrys. Of the 17 family members studied, 10 had the marker chromosome and synophrys, six did not inherit the marker nor synophrys and only one member, II.6, is discordant, i.e., she had the marker but not synophrys. However, one of her children (III.11) inherited the marker chromosome and also had synophrys. Since the father (II.7) did not have synophrys, and if one assumes dominant transmission, it is likely that the trait was inherited from the mother who failed to express it.

Assuming dominant inheritance and independent assortment for the  $1qh+$  chromosome and synophrys,  $\chi^2$  was calculated. The resulting probability was highly significant at the 1% level and supports the hypothesis of linkage of the two traits. Lod scores were calculated by a modification of the

TABLE I  
MEAN AND STANDARD ERRORS FOR ARM AND HETEROCHROMATIN RATIOS

Category	Arm Ratio = $q/p$			Heterochromatin Ratio = $h/(q-h)$		
	Number Measured	$\bar{x}$ (decoded)	$\bar{x} \pm s_{\bar{x}}$ (coded)	Number Measured	$\bar{x}$ (decoded)	$\bar{x} \pm s_{\bar{x}}$ (coded)
$1qh+$ chromosome	65	1.24	119.03 $\pm$ 0.70	44	0.56	48.59 $\pm$ 0.78
Homologue	65	1.04	101.40 $\pm$ 0.71	44	0.32	34.25 $\pm$ 0.78
'At-risk' members	48	1.04	101.36 $\pm$ 0.82	10	0.36	36.97 $\pm$ 1.63
Controls	66	1.04	101.81 $\pm$ 0.70	40	0.32	34.60 $\pm$ 0.81

TABLE II  
LOD SCORES FOR RECOMBINATION BETWEEN  $1qh+$  MARKER AND SPECIFIC LOCI

Trait	Scorable Offspring	Recombination Fraction ( $\theta$ )					
		0.00	0.05	0.1	0.2	0.3	0.4
Synophrys	12	$-\infty$	+0.884	+0.956	+0.775	+0.456	+0.142
Duffy	11	+2.107	+1.863	+1.615	+1.103	+0.596	+0.177
Rhesus	8	$-\infty$	-0.940	-0.612	-0.286	-0.116	-0.028

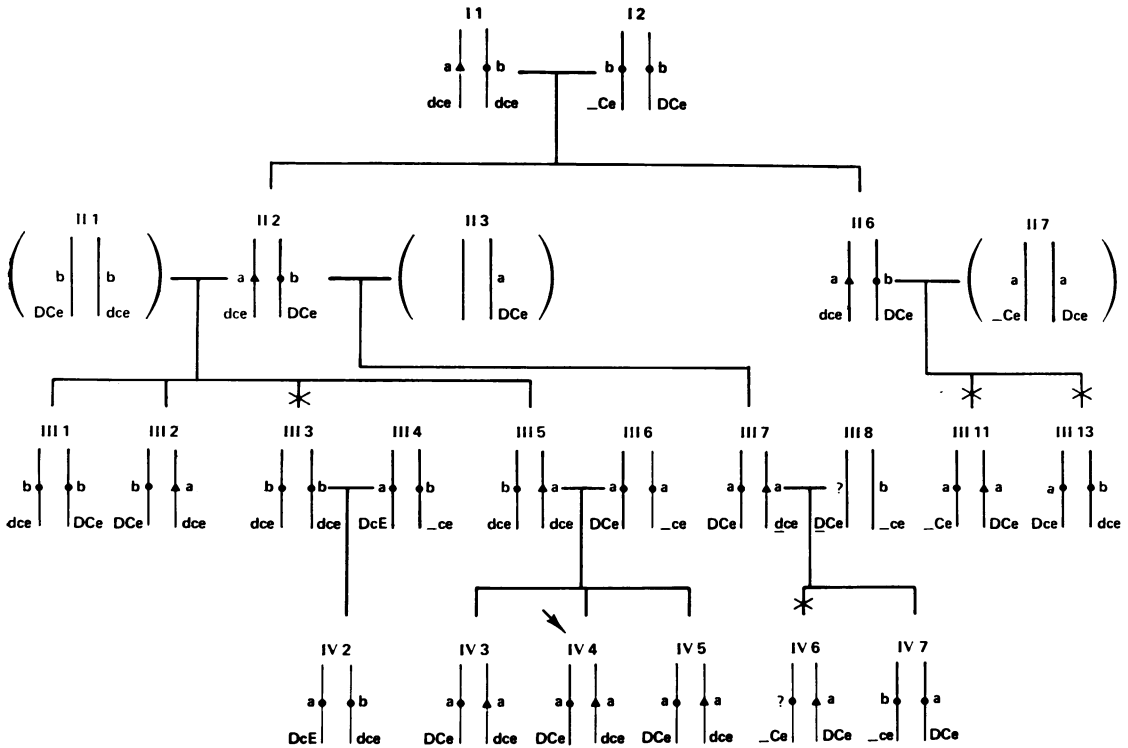


FIG. 4. Diagram showing the phase of 1qh+ /Duffy/Rh linkage. The 1qh+ is symbolized by (▲); individuals recombine for Rh are marked (X); brackets denote individuals unavailable for study and their genotype is deduced from their offspring. The paternal chromosome is shown on the left and the maternal on the right for generations II-IV.

method of Morton (Maynard-Smith, Penrose, and Smith, 1961); synophrys was treated as a dominant. The values (Table II) were consistent with loose linkage for the marker and synophrys.

A number of studies (reviewed by Ruddle *et al*, 1972) have provided good evidence that the Duffy blood group is on the No. 1 chromosome as was initially suggested by Donahue *et al* (1968). In this study, there was complete concordance between the 1qh+ marker and the Fy<sup>a</sup> gene (Fig. 4) with a total of 12 chances for crossover and none detected. The lod scores are given in Table II. Although the peak value was below +3, the data were suggestive of close linkage of these two traits as the peak value was at 0.00. Expansion of the scores between 0.00 and 0.05 (Smith, 1968) showed the peak value to still be at 0.00.

The New Haven Conference (1973) on gene mapping located the Rhesus linkage group (Rh, EL<sub>1</sub>, 6PGD, Pep C, PGM<sub>1</sub>) on chromosome No. 1. Fig. 4 shows the phase of the 1qh+ /Rh linkage. There were four recombinant individuals (denoted by X) out of a total of nine chances for recombina-

tion. Lod scores for the Rh locus (Table II) did not reach a value of -2 at which point recombination fractions are commonly excluded; however, our data do not support linkage.

### Discussion

The variant 1q+ chromosomes are thought to occur by one of three possible mechanisms; namely, (1) uncoiling in the area of the secondary constriction, (2) a translocation, or (3) a partial duplication. The addition of material as proposed in our case could occur by either of the last two mechanisms. The simplest explanation of the 1qh+ chromosome based on its banding pattern would be a partial or complete duplication of the heterochromatic region. One mechanism by which this could have occurred is unequal crossing-over during meiosis.

It has been postulated (Craig-Holmes and Shaw, 1971; Jones and Corneo, 1971; Lubs and Ruddle, 1971) that the heterochromatic region contains redundant DNA sequences. The duplication of such regions would be consistent with the absence of phenotypic effects as seen in this and other studies.

Our data for linkage of the Fy gene and the 1qh+ marker add support to the assignment of the Fy locus to chromosome No. 1. The data are not positive for linkage of the Rh locus to the marker and, hence to chromosome No. 1. Two possible conclusions to draw from this are that the Rh locus is not on chromosome No. 1; or, alternately, the Rh locus is on chromosome No. 1, but its distance from the 1qh+ region is too far to measure with this type of analysis. Since both family and somatic cell hybrid studies favour the location of the Rh linkage group on chromosome No. 1, the latter possibility seems more likely and is consistent with the proposed mapping (Merritt *et al*, 1973; Cook *et al*, 1973/1974).

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