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Chromosomal Localization of the Heterochromatic Region 16qh(.76) linked to α-Haptoglobin in Man

THE structural gene locus for the alpha chain of haptoglobin (α-Hp) in man seems[1] to be on the long arm (q) of chromosome 16 linked[2] to a fragile heterochromatically staining region (h). We here report measurements localizing this region, which we designate 16qh(.76).

16qh(.76) was found to be segregating in a large family[2]. From thirty-one of the family members heterozygous for 16qh, 1,709 lymphocyte metaphases were analysed microscopically and 801 metaphases photographed. Both chromosomes No. 16 were relatively straight and free of overlap by other chromosomes in 193 metaphases. Eighty of these metaphases showed 16qh clearly and were therefore selected for measurement.

Using photographic prints with a final enlargement of ×3,840, we measured the No. 16 chromosomes with a specially designed, manually operated wheel on which a millimetre scale was directly engraved (unpublished results of H. W., W. J. K., and F. H.). Both chromatids were measured and the results averaged. We measured the length of the short arm (p) from each chromosome No. 16, q from the normal homologous 16 ($q^{homol.}$), and the euchromatic parts of q proximal ($q^{prox.}$) and distal ($q^{dist.}$) to 16qh.

We compared the length of $q^{prox.}$ to $q^{homol.}$ in each cell and found the mean length of $q^{prox.}$ to be 0.759 of $q^{homol.}$ (s.e.= 0.011). Thus, the fragile heterochromatic site is approximately 76% of the distance along the long arm of No. 16. Since other heterochromatic regions may be located on 16q, we have designated this site 16qh(.76).

Table 1 One Way Analysis of Variance of the Length of the Distal Segment of the 16qh Chromosome

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Between person	31.0	0.6631	0.0213	
Within person	48.0	0.7215	0.0150	
Total	79.0	1.3846		1.422

We then tested whether 16qh(.76) detectably affected the length of euchromatin on that chromosome, using a paired observation t test. The mean length of p from the involved No. 16 was not significantly different from the mean length of p from the homologue ($t=1.68$, df$=79$, $P>0.05$). The sum of the means of $q^{prox.}+q^{dist.}$ was, however, 2.6% greater than the mean of q from the homologue ($t=2.17$, df$=79$, $0.05>P>0.02$).

Finally, we tested for heterogeneity between persons in the length of $16q^{dist.}$ and found none. The F ratio (between person/within person variance) was 1.42 (Table 1), which is not significant.

The foregoing cytological data should not be interpreted to mean that the structural gene for α-Hp is necessarily located 76% of the distance from centromere. α-Hp could be cytologically at some distance from h(.76) but seem to be closely linked, if the heterochromatic region tended locally to suppress crossing-over. The data also cannot reveal if α-Hp is between the centromere and 16qh(.76) or distal to 16qh(.76).

Concurrent segregation analysis of chromosome markers (for example, 16qh(.76)) and gene markers (for example, α-Hp) permits the testing of map assignments deduced from studies with chromosome rearrangements[1] and the localization of specific genes to specific chromosome regions, as in the present study. The efficiency of chromosome marker work will predictably increase as chromosome variants are recognized more frequently. In this family we have recently observed the segregation of three other potentially useful variants (3 inv, 17qh and Ginv), as will be reported in more detail.

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Interpretation of Linkage in Somatic Cell Hybrids

Kao and Puck[1] have measured the linkage between human genes by studying their association in cell hybrids with Chinese hamster cells which carried mutations homologous to the human genes. Such studies are made possible by the extensive loss of human chromosomes from such hybrids. If, however, one of the human genes is retained owing to the operation of specific selection, the presence or absence of an unselected gene gives a measure of linkage between them.

In one hybrid studied by Kao and Puck the human genes were inositol independence (*inos*$^+$) and proline independence (*pro*$^+$). Among forty-two clones selected for *inos*$^+$, only seven (one sixth) were also *pro*$^+$. In another hybrid the human genes were glycine independence (*gly*$^+$) and *pro*$^+$. Among fifteen clones selected for *gly*$^+$ only five (one third) were also *pro*$^+$. Both these experiments were interpreted as evidence of non-linkage between the pair of marker genes. I submit that such associations, though well below the 50% expected between unlinked genes in sexual hybrids, are nevertheless greater than would be expected from random association of the marked chromosomes in cell hybrids. The first problem is to determine what would be the extent of random association between two unlinked genes (that is, genes on separate chromosomes). This would depend on the number of human chromosomes still retained in the hybrid. In the first hybrid, because of the method of selection one chromosome must carry *inos*$^+$. If a second chromosome is present the probability that it will not carry *pro*$^+$ is 43/45. The chance that a third and fourth chromosome will also be free of *pro*$^+$ will be 42/44 and 41/43 respectively. In general terms, if a hybrid clone has n human chromosomes of which one carries *inos*$^+$, the chance of *pro*$^+$ being also present will be $1-\frac{43!\,..\,(45-n)}{45!\,..\,(47-n)}$. When $n=2$ the association of *inos*$^+$ and *pro*$^+$ will be 0.044 and increases