

Further Evidence for Close Linkage of the Hb^{β} and Hb^{δ} Loci in Man*

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Nine kindreds have been reported previously which give evidence concerning the linkage relationships of the determinants of the β and δ chains of human haemoglobin. We wish to document a tenth kindred in which β and δ chain variants are segregating. The fact that no crossovers were observed among 8 offspring of a doubly heterozygous male provides further confirmation of the existing evidence for close linkage of the β and δ loci.

Methods

We performed haemoglobin typing and haptoglobin typing by starch gel electrophoresis, as described previously (Nance, 1968), and blood typing by the slide and tube methods using Ortho antisera. We quantified haemoglobin fractions after separation on starch gel by a modification of the method of Sunderman (1964), with a Technicon Autoanalyzer. With this technique the modal value for percentage Hb-A₂ among 272 otherwise normal subjects with sickle cell trait was 2.2; the values for 90% of the subjects tested fell between 1.6 and 3.2%.

Case Report

The proband was a 45-year-old Negro with hypertensive cardiovascular disease. During the routine screening of in-patients of African descent at the Nashville Veterans Administration Hospital for haemoglobin and glucose-6-phosphate dehydrogenase variants, he was found to have two major and two minor haemoglobin bands on starch-gel electrophoresis (Fig. 1). The major haemoglobin variant had the electrophoretic mobility of haemoglobin S; the identity of this haemoglobin was further confirmed by a positive sickle cell preparation. The minor haemoglobin variant migrated just in front of the origin at pH 8.7 in the tris-versene-borate buffer system employed for these studies. This variant is believed to correspond to HbB₂ (HbA₂), but the protein

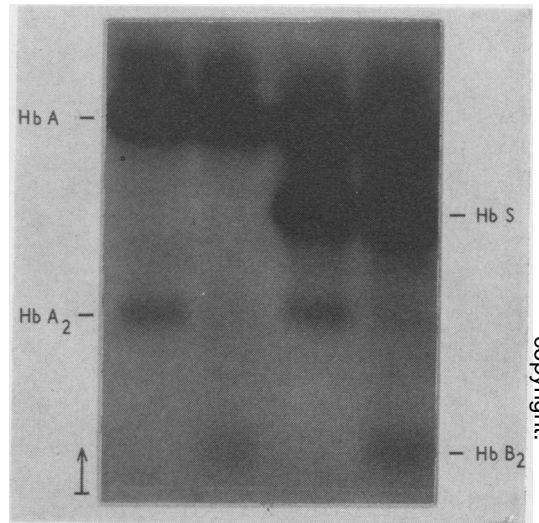


Fig. 1. Appearance of haemolysates following starch gel electrophoresis, benzidine stain. Proband (right), wife (left), two children (centre).

was not characterized further. Though the patient also had glucose-6-phosphate-dehydrogenase deficiency (GdA-), no history suggestive of anaemia or haemolysis could be elicited. Blood samples were obtained for study from the patient's wife, sister, and 10 children, who included two sets of fraternal twins. The results of haemoglobin and blood typing are shown in Fig. 2, while the haematological data are given in Table I.

Results

All but two of the proband's children carried one or the other of the haemoglobin variants. The two exceptional children carried neither paternal haemoglobin variant, and in both cases the blood typing results indicated a misrepresentation of the biological parents. Regarding the proband, the data are consistent with close linkage of the β and δ chain variants in repulsion: no crossovers were observed in 8

Received August 2, 1968.

* This work was supported by USPHS grants NB-06408 and HE-08195.

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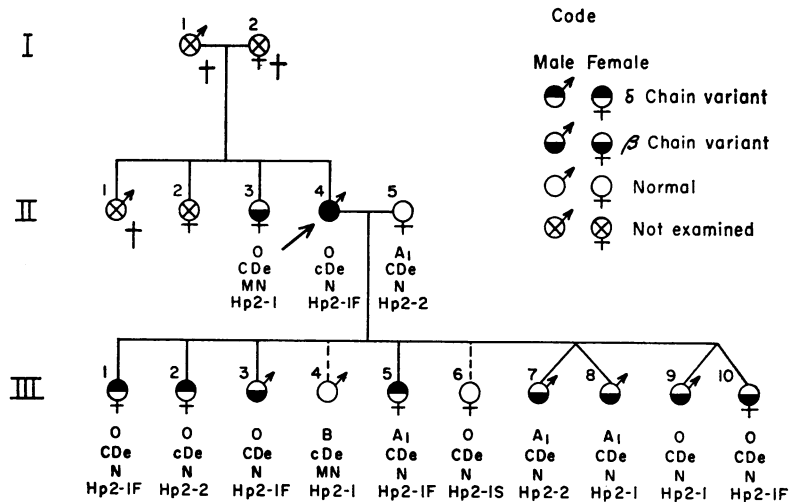


FIG. 2. Pedigree of family. III.4 is inconsistent in the ABO and MN system, while III.6 is inconsistent in the haptoglobin system.

TABLE I
HAEMATOLOGICAL DATA

Subject No.	Age (yr.)	Sex	Hb (g./100 ml.)	Red blood cells (10 ⁹ /cu. mm.)	Haematocrit (%)	Hb-A (%)	Hb-S (%)	Hb-A ₂ (%)	Hb-B ₂ (%)
II.3	50	F	12.7	4.72	38	58.7	38.8	2.5	—
II.4	45	M	14.1	5.95	44	65.5	30.5	2.5	1.5
II.5	43	F	14.9	5.55	44	97.3	—	2.7	—
III.1	17	F	13.5	4.62	42	97.2	—	1.5	1.3
III.2	15	F	13.1	4.83	41	97.0	—	1.6	1.3
III.3	13	M	14.4	5.34	44	62.7	34.2	3.1	—
III.4	12	M	13.6	5.26	42	97.3	—	2.7	—
III.5	10	F	12.5	4.46	39	97.2	—	1.6	1.2
III.6	9	F	13.5	4.48	41	97.6	—	2.4	—
III.7	7	M	12.8	5.54	39	63.5	33.8	2.7	—
III.8	7	M	12.9	4.97	41	61.8	33.8	4.4	—
III.9	6	M	12.6	4.78	39	62.8	34.5	2.7	—
III.10	6	F	11.8	5.32	38	64.5	32.6	2.9	—

TABLE II
 β AND δ CHAIN LOCI: SUMMARY OF LINKAGE DATA

Author	Family	Parental Haemoglobin Type		Offspring Phenotypes			
		Father	Mother	β^*/β / δ/δ	β/β / δ^*/δ	β^*/β^* / δ/δ	Not Tested
<i>Backcross Matings</i> Ceppellini (1959) Boyer <i>et al.</i> (1963)	2	A/S, A ₂ /B ₂	A/A, A ₂ /A ₂	4	2		
	A	A/S, A ₂ /B ₂	A/A, A ₂ /A ₂	2	4		
	B	A/C, A ₂ /B ₂	A/A, A ₂ /A ₂	3	7		
	C	A/A, A ₂ /A ₂	A/C, A ₂ /B ₂	1	5		
Pearson and Moore (1965) Schwartz, Smith, and Nathan (1967) Mishu and Nance (Present case) Ranney <i>et al.</i> (1963)	I.7	A/A, A ₂ /A ₂	A/S, A ₂ /B ₂	2	2		
	II.4	A/S, A ₂ /B ₂	A/A, A ₂ /A ₂	5	1		
	II.16	A/S, A ₂ /B ₂	A/A, A ₂ /A ₂	5	3		
	II.5	A/S, A ₂ /F1	A/A, A ₂ /A ₂	1	3		
Horton and Huisman (1963)	II.8	—	A/S, A ₂ /F1	1	1		
	II.8	—	A/S, A ₂ /F1	1	1		
	II.5	A/A, A ₂ /A ₂	A/S, A ₂ /A ₂	3	2		
	II.5	—	A/S, A ₂ /A ₂	1	2		
<i>Single Intercross Matings</i> Horton <i>et al.</i> (1961) Ranney <i>et al.</i> (1963)	II.5	A/A, A ₂ /B ₂	A/S, A ₂ /B ₂	1	4	2	2
	II.16	A/S, A ₂ /B ₂	A/S, A ₂ /A ₂	1	1		

* Indicates a variant from normal haemoglobin.

opportunities. The haematological studies showed no evidence of significant anaemia in any family member, and examination of peripheral blood smears showed no notable alterations of red cell morphology. In the 4 family members who carried the δ chain variant, the average percentage of Hb-B₂ was 1.3 and the percentage of Hb-A₂ was 1.8 for a total 3.1%. The average percentage of Hb-A₂ in family members who did not carry the δ chain variant, 2.9, did not differ significantly from this value ($t=0.63$, $p>0.5$).

Discussion

The available data concerning linkage between the β and δ loci are summarized in Table II. There have been no detected crossovers among 63 offspring from 14 probable backcross matings; if the families of Ranney *et al.* (1963) and Horton and Huisman (1963), in which the parental mating type is uncertain, are excluded, the total becomes 57. In the two single intercross matings, the untyped offspring and those who are heterozygous for the intercross factor provide no information about linkage. Since the parental linkage phases are known, however, these pedigrees do provide 4 additional non-crossover offspring. The pooled total of 61 non-crossover offspring leads to estimates of 4.9 and 7.5 as the maximum possible map distance between the two loci at the 0.05 and 0.01 confidence levels, respectively, under the assumption of a Poisson distribution. In contrast, 2 crossovers have been observed among 29 offspring of individuals doubly heterozygous for δ chain variants and ' β thalassaemia', or 7% (Pearson and Moore, 1965). Whether these results truly reflect a measurable difference in map distance between the δ locus and the β and ' β thalassaemia' loci remains conjectural. Our data support this view but also indicate how difficult it will be to settle the question by conventional linkage studies in man. In the present family, neither parent acknowledged non-paternity of the exceptional offspring; were it not for the blood group inconsistencies, 2 crossovers might have been claimed.

The identity of the δ chain variant observed in this family is not known with certainty, but it had the electrophoretic mobility of Hb-B₂ (Jones *et al.*, 1966). In keeping with previous observations, the

measured percentage of the variant haemoglobin in heterozygotes was less than that of the normal Hb-A₂. Furthermore, the ratio of normal to abnormal A₂ haemoglobin in the father, 1.6, was greater than the average ratio among his 3 carrier children, 1.2. If the ratios reflect differences in the rates of synthesis of the constituent haemoglobin chains (Boyer, Hathaway, and Garrick, 1964), the reduced activity of the father's β^s allele would not appear to have inhibited polypeptide chain production by the adjacent normal Hb $^{\delta}$ allele.

Summary

No crossovers were observed among 8 offspring of a man who was doubly heterozygous for δ and β chain variants of haemoglobin. A total of 61 non-crossover offspring of double heterozygotes has now been observed, providing strong evidence for close evidence for close linkage of the two loci.

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