Linkage relationships between β - and δ -structural loci and African forms of β thalassaemia

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Summary. Five families are described in which there have been matings between individuals doubly heterozygous for β thalassaemia and the δ -chain variant haemoglobin A₂' to normal persons. In all there were 24 informative offspring. There were no crossovers between the β -thalassaemia and δ -chain loci; in three of the families the genes were linked in *cis* and in two families the genes were found in *trans*. Together with previously reported families there have now been 58 opportunities for crossing over between the β -thalassaemia and δ -chain loci and there have been two possible and one highly probable crossovers. Of the total of 9 families reported to date 4 have had the genes for β thalassaemia and Hb A₂' in *cis* and 5 in *trans*. These findings are contrasted with the findings in families where a β -chain structural variant and Hb A₂' have been observed together and these genes have always been found in *trans* and never in *cis*. The reasons for linkage disequilibrium of this type are discussed. It is concluded tentatively that the distance between the δ -structural and β -thalassaemia loci is greater than that between the δ -structural and β -structural loci. To date this conclusion can only be applied to the β^+ -thalassaemia and β^0 -thalassaemia genes as found in the African population, since this is the only population with a high incidence of δ -chain mutants which allows linkage analysis of this type to be carried out.

The β thalassaemias are a heterogeneous group of inherited disorders of β -chain synthesis which fall into two major types characterized by either diminished β -chain production (β^+ thalassaemia) or a total absence of β -chain synthesis (β^0 thalassaemia) (Weatherall and Clegg, 1972). Though the defect in these disorders has yet to be elucidated at the molecular level, there is strong evidence that in all types of β thalassaemia there is a diminished amount or total absence of mRNA for β globin chain production (reviewed by Weatherall and Clegg, 1975), indicating that defective regulation of β gene transcription is a probable cause in most if not all these conditions. Since so little is known about the precise mechanisms involved in these processes

in mammals some formal genetic evidence regarding the chromosomal location of the β -thalassaemia genes would be an extremely useful adjuvant to current studies involving gene localization in β thalassaemia by DNA/DNA hybridization (Ottolenghi *et al.*, 1975).

Studies of $\delta\beta$ and $\gamma\beta$ fusion haemoglobins (reviewed by Weatherall, Pembrey, and Pritchard, 1974) indicate that the non α -chain genes of the human haemoglobins are closely linked with a probable order $\alpha\gamma\text{-}\delta\beta$, though there is still some doubt about the precise number of γ -chain genes. If the β -thalassaemia genes are caused by mutations at the β -structural locus, they should show close linkage or allelism to it. Thus the offspring of individuals heterozygous for both β thalassaemia and a structural β -chain mutant should have children with either one or other defect but no

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normal or doubly affected children. Published data on nearly 200 such offspring are available (reviewed by Weatherall and Clegg, 1972; Wasi, 1973) and no unequivocal exceptions to this expectation have been observed.

The existence of a δ -chain structural haemoglobin variant (Hb A₂') at a relatively high frequency in the African Negro population (Ceppellini, 1959; Huisman, Punt, and Schaad, 1961) offers an opportunity to study linkage between the δ - and β -structural loci and the δ -structural and β -thalassaemia loci in that population. Mishu and Nance (1969) have reviewed the findings in 61 offspring of individuals heterozygous for a β -structural variant and Hb A₂'. There was no example of crossing over between the β - and δ -structural loci and the genes were always observed in the *trans* position in the doubly heterozygous parents. Weatherall (1967) in reviewing matings between individuals heterozygous for β thalassaemia and Hb A₂' was able to find 31 offspring and noted at least one possible crossover. Furthermore the β -thalassaemia and Hb A₂' genes were observed in *cis* in at least two of the families in which these offspring had been studied. This observation suggested that the β -thalassaemia gene might not be as close to the δ -structural locus as is the β -structural gene.

We report here findings on five more families

where matings between individuals doubly heterozygous for thalassaemia and Hb A₂' to normal persons had occurred. In all there were 24 informative offspring. Furthermore, in three of the families the genes for β thalassaemia and Hb A₂' were found in *cis*.

Methods

Routine haematological methods were used (Dacie and Lewis, 1970). Red cells were counted with an electronic cell counter. Haemoglobin electrophoresis was carried out in starch gel at pH 8.4 (Weatherall and Clegg, 1972), fetal haemoglobin was measured by alkali denaturation (Betke, Marti, and Schlicht, 1959), and Hb A₂ and Hb A₂' (B₂) were quantified by DEAE-Sephadex chromatography (Dozy, Kleihauer, and Huisman, 1968) or by elution from cellulose acetate strips following electrophoresis (Weatherall and Clegg, 1972). Serum iron was measured by a routine method modified from that of Beale, Bostrom, and Taylor (1962).

Results

Families A, B, and C are of mixed racial background, predominantly Negro, and live in Jamaica, West Indies. Families D and E are located in England and are also Negro of Jamaican origin.

TABLE I
RED CELL INDICES, SERUM IRON, PROPORTION OF Hb F, A₂, AND A₂' IN RELEVANT MEMBERS OF FAMILIES A, B, AND C

	Age (yr.)	Hb (g/dl)	PCV (%)	RBC ($\times 10^6/\mu$ l)	MCHC (g/dl)	MCH (pg)	MCV (fl)	Fe (μ g/dl)	TIBC (μ g/dl)	Satn (%)	Hb F (%)	A ₂ (%)	A ₂ ' (%)	Haemoglobin Electrophoresis
Family A														
I.1	30	12.3	39	5.3	31	23	75	161	386	42	0.4	3.1	2.9	A, A ₂ , A ₂ '
I.2	26	13.1	39	4.5	33	29	86	55	370	15	0.3	3.0	—	A, S, A ₂
II.1	6	12.8	36	4.3	35	29	83	98	332	30	0.4	2.6	—	A, A ₂
II.2	5	11.7	35	4.2	33	28	84	83	358	23	0.6	3.2	—	A, S, A ₂
II.3	4	8.6	28	4.4	30	20	65	162	353	25	8.4	2.6	2.4	F, S, A ₂ , A ₂ '
II.4	1	8.3	30	4.9	28	17	63	48	496	9	2.6	1.8	2.0	A, A ₂ , A ₂ '
Family B														
I.1	?	11.2	38	5.9	29	19	65	40	328	12	0.3	4.2	2.8	A, A ₂ , A ₂ '
I.2	?	12.2	37	—	33	—	—	—	—	—	—	—	—	A, S, A ₂
II.1	12	8.7	30	4.1	29	21	76	136	335	41	8.3	5.6	—	F, S, A ₂
II.2	10	8.0	27	3.8	30	21	71	73	293	25	4.7	5.5	—	F, S, A ₂
II.3	7	12.1	34	4.2	36	28	84	—	—	—	1.0	2.0	1.7	A, A ₂ , A ₂ '
II.4	6	9.4	32	5.1	29	18	65	—	—	—	3.8	6.9	—	A, A ₂
II.5	5	10.0	34	5.2	29	19	64	105	325	32	9.6	5.7	—	A, A ₂
II.6	1	—	—	—	—	—	—	—	—	—	—	—	—	A, S, A ₂ '
Family C														
I.1	70	9.9	35	4.7	28	21	75	—	—	—	1.1	4.7	—	A, A ₂
I.2	65	11.1	35	4.1	31	27	86	—	—	—	0.2	1.2	1.1	A, S, A ₂ , A ₂ '
II.1	42	12.2	35	4.0	35	31	88	—	—	—	—	—	—	A, S, A ₂
II.2	38	10.3	34	5.3	30	19	64	9*	406	2	0.4	2.6	2.8	A, A ₂ , A ₂ '
III.1	19	8.8	28	3.8	31	23	74	123	374	33	6.4	4.6	—	F, S, A ₂
III.2	18	7.6	23	3.3	32	23	71	120	295	41	7.0	4.7	—	F, S, A ₂
III.3	17	12.5	36	3.8	35	33	94	111	348	32	0.3	1.3	1.3	A, A ₂ , A ₂ '
III.4	15	9.8	32	5.0	30	20	65	72	323	22	0.8	6.5	—	A, A ₂
III.5	9	10.2	33	5.0	30	20	67	58	289	20	1.1	5.8	—	A, A ₂
III.6	7	7.9	25	3.4	32	23	73	115	307	37	6.8	4.4	—	F, S, A ₂
III.7	0.5	11.4	34	5.7	33	30	62	—	—	—	21.3	4.2	—	A, F, A ₂

* One week post partum.

TABLE II
RED CELL INDICES AND PROPORTIONS OF Hbs A, A₂, A₂', AND F IN FAMILIES D AND E

	Age (yr.)	Hb (g/dl)	PCV (%)	RBC ($\times 10^6/\mu\text{l}$)	MCHC (g/dl)	MCH (pg)	MCV (fl)	Hb F (%)	Hb A (%)	Hb A ₂ ' (%)	Haemoglobin Electrophoresis
Family D											
I.1	45	15.6	46	5.0	34	31	91	< 1.0	2.5	—	A, A
I.2	34	13.2	41	6.5	32	20	63	1.8	3.5	1.6	A, A ₂ , A ₂ '
II.1	19	13.2	40	6.5	33	20	63	0.4	3.8	2.0	A, A ₂ , A ₂ '
II.2	17	12.8	38	6.5	33	20	64	1.0	3.7	2.0	A, A ₂ , A ₂ '
II.3	16	11.6	36	5.9	32	20	63	0.4	2.6	1.6	A, A ₂ , A ₂ '
II.4	14	14.0	40	4.6	35	30	88	< 1.0	2.0	—	A, A ₂
II.5	7	13.2	40	6.5	33	20	63	0.4	3.7	1.7	A, A ₂ , A ₂ '
Family E											
I.1	46	13.7	43	6.0	32	22	71	0.9	3.4	2.5	A, A ₂ , A ₂ '
I.2	52	14.2	43	4.9	33	29	87	0.6	2.4	—	A, A ₂
II.1	19	14.7	44	5.1	33	28	85	0.7	2.1	1.0	A, A ₂ , A ₂ '
II.2	16	16.5	47	5.2	35	32	91	1.2	1.8	1.1	A, A ₂ , A ₂ '

FIG. 1. Family pedigrees showing linkage between the δ locus and β thalassaemia. In families A, B, and C the thalassaemia gene interacts with Hb S and is expressed as sickle cell- β^0 -thalassaemia.

Relevant haematological and haemoglobin analytical data are listed in Tables I and II and the pedigrees are shown in Fig. 1 and 2. Full blood group analysis data on families A, C, D, and E are available on request to the authors; there were no incompatibilities.

Family A (Fig. 1)

The father (I.1) was found to be heterozygous for both Hb A₂' and β thalassaemia. The thalassaemia gene is of the β^0 -thalassaemia type in his son (II.3)

who has Hb S/ β^0 thalassaemia. On DEAE-Sephadex chromatography, and on electrophoresis, Hbs S, A₂, and A₂' were seen but no Hb A (Fig. 3a). One daughter (II.4) has β thalassaemia and Hb A₂'. This 1-year-old child was iron deficient but also clearly has a β -thalassaemia trait. The Hb A₂, A₂', and red cell indices were remeasured after a short course of oral iron but they had not changed. Two other children (II.1 and II.2) have neither Hb A₂' nor β thalassaemia, but one has the sickle-cell trait. In this family the δ -chain variant and the β^0 -thalassaemia gene appear to be linked in *cis*. Un-

fortunately the parents of I.1 were dead and all attempts to locate sibs were unsuccessful.

Family B (Fig. 1)

In this family a Hb-A₂' gene is in *trans* to a β -thalassaemia gene in the father (I.1). His wife

(I.2) has sickle-cell trait. Two daughters (II.4, 5) have β^0 -thalassaemia trait without Hb A₂', and two (II.1, 2) have Hb S/ β^0 thalassaemia without Hb A₂'. The other two daughters (II.3, 6) do have Hb A₂', one with Hb S, but show no evidence of β thalassaemia.

FIG. 2. Family pedigrees of families D and E.

Family C (Fig. 1)

In this family the Hb A₂' gene is in *trans* to a β^0 -thalassaemia gene in the second and third generation. I.1 has Hb A₂' and Hb S, which must be in *trans* because some of her children have received Hb S and others Hb A₂', but none has both. One of her children (II.9) has Hb S/ β^0 thalassaemia. Another (II.2) has received the β -thalassaemia trait from her father and Hb A₂' from her mother, and so is doubly heterozygous with the two genes in *trans*. This woman has seven children; five of them have received the β^0 -thalassaemia gene and two have received Hb A₂', but none have both. Three of the children also received Hb S from their father (II.1), and so have Hb S/ β^0 thalassaemia (Fig. 3b). II.7 was only 6 months old when the study was completed.

FIG. 3. Starch gel electrophoresis, pH 8.6 (Benzidine stain). From left to right: (a) Family A, I.1, I.2, II.1, -2, -3, -4. (b) Family C, II.1, II.2, III.1, -2, -3, -4.

Family D (Fig. 2)

In this family individual I.2 has the β -thalassaemia and Hb A₂' genes in *cis*. Of the five children, four have both Hb A₂' and β thalassaemia while one (II.2) is normal. It is not possible to determine whether the β -thalassaemia gene is of the β^+ or β^0 variety.

Family E (Fig. 2)

The propositus (I.1) has β thalassaemia and Hb A₂'. Both his sons have Hb A₂' but no evidence of thalassaemia. Thus it appears that the β -thalassaemia and Hb-A₂' genes are in *trans* in the propositus. It is not possible to determine the variety of β thalassaemia in this family.

Discussion

We have described five families where 24 offspring of matings between individuals heterozygous for both β thalassaemia and Hb A₂' to normal individuals or those with the sickle-cell trait were available for study. There is strong genetic evidence that in three of these families the genes for β thalassaemia and Hb A₂' were in *cis* while in the other two they were in *trans*. No crossing over between the β -thalassaemia and δ -chain loci were observed. Four similar families have been reported previously (Huisman *et al*, 1961; Weatherall, 1964; Pearson and Moore, 1965; Thompson, Odom, and Bell, 1965). In three of these families possible crossing over between the β -thalassaemia and δ -structural loci were observed; these families are summarized in Fig. 4. In the family of Weatherall

(1964) there were two normal children born of a mating between an individual heterozygous for β thalassaemia and Hb A₂' and a normal individual, but in one of these cases paternity was excluded by blood grouping, though this was not possible for the other individual. The family described by Thompson *et al* (1965) (Family 2, Fig. 4) also contains a possible crossover though it is also possible in this case that a crossover had occurred between the δ -structural locus and the β -structural locus. This family is not suitable for linkage analysis of this type, therefore. However, in the family of Pearson and Moore (1965) a crossover occurred between the β -thalassaemia and δ -structural loci and this could not be excluded by extensive blood grouping. Thus in 58 opportunities for crossing over between these loci there have been three possible and one very highly probable crossover (Table III). It should be noted also that of the nine families that make up this series, four have had the genes for β thalassaemia and Hb A₂' in *cis* and five in *trans*. A further family showing β thalassaemia and Hb A₂' in *cis* has been reported by Friedman *et al* (1974).

These results are particularly interesting when examined in the light of findings in families in which offspring of individuals heterozygous for both β - and δ -structural haemoglobin variants have been studied (reviewed by Boyer *et al* (1963) and more recently by Mishu and Nance (1969) and Weatherall and Clegg (1972)). To date data are available on 14 back cross-matings between 11 double heterozygotes of this type and normal partners, who have produced 63 offspring. The genes have always been in *trans* and there have been no crossovers.

FIG. 4. Family pedigrees reported in the literature in which a crossover between a δ -structural variant with a β -thalassaemia gene has been observed. In each family the recombinant is indicated by an asterisk. 1, Weatherall (1964). 2, Thompson *et al* (1965). 3, Pearson and Moore (1965).

TABLE III
REPORTED OFFSPRING OF INDIVIDUALS HETEROZYGOUS FOR β THALASSAEMIA AND Hb A₂'

Mating	β -thal./ HbA ₂ ' Cis or Trans	β -thal. Type	Offspring								Total	Author	
			AA A ₂ A ₂	AA A ₂ A ₂ '	A thal. A ₂ A ₂	A thal. A ₂ A ₂ '	AS A ₂ A ₂	AS A ₂ A ₂ '	S thal. A ₂ A ₂	S thal. A ₂ A ₂ '	β thal. A ₂ A ₂ '		
A thal. A ₂ A ₂ ' × normal	cis	?	2	0	0	6	0	0	0	0	0	8	Huisman <i>et al</i> (1961)
A thal. A ₂ A ₂ ' × normal	cis	?	1	0	0	2	0	0	0	0	0	3	
A thal. A ₂ A ₂ ' × normal	cis	?	0	0	0	2	0	0	0	0	0	2	
A thal. A ₂ A ₂ ' × ?	cis	?	0	0	0	1	0	0	0	0	0	1	
A thal. A ₂ A ₂ ' × normal	trans	?	2*	1	3	0	0	0	0	0	0	6	Weatherall (1964)
A thal. A ₂ A ₂ ' × ASA ₂ A ₂	trans	?	0	1	0	1	0	0	2	0	0	4	Pearson and Moore (1965)
A thal. A ₂ A ₂ ' × normal	trans	?	0	0	3	0	0	0	0	0	0	3	
A thal. A ₂ A ₂ ' × normal	trans	?	0	2	2	0	0	0	0	0	0	4	
A thal. A ₂ A ₂ ' × ?	trans	?	0	0	3	0	0	0	0	0	0	3	
A thal. A ₂ A ₂ ' × ASA ₂ A ₂	cis	β^0	1	0	0	1	1	0	0	1	0	4	Present study
A thal. A ₂ A ₂ ' × ASA ₂ A ₂	trans	β^0	0	1	2	0	0	1	2	0	0	6	
A thal. A ₂ A ₂ ' × ASA ₂ A ₂	trans	β^0	0	1	3	0	0	0	3	0	0	7	
A thal. A ₂ A ₂ ' × normal	cis	?	1	0	0	4	0	0	0	0	0	5	
A thal. A ₂ A ₂ ' × normal	trans	?	0	2	0	0	0	0	0	0	0	2	
Total			7	8	16	17	1	1	7	1	0	58	

* Paternity excluded in 1 case.

In addition there have been three single cross-matings (including individual I.2, Family C of this report) in which the genes are in *trans*. Though the δ -chain mutants have been recognized since 1959, there still have been no reports of δ - and β -structural variants occurring in *cis*. Thus it seems likely that the chances of a new mutation occurring at the normal loci of a chromosome carrying either a β -thalassaemia gene or a δ -structural-variant gene are far less than the observed incidence of these genes in *cis*. The *cis* relationship is, therefore, most likely to be the result of crossing over.

The findings that there have been no families in which the β -structural and δ -structural variants have been linked in *cis* and that to date half the families that have been observed to have both β -thalassaemia and δ -structural genes have been in *trans* and half in *cis* is open to several explanations. First, of course, it is possible that this is still a chance observation since the number of families is still relatively small. A second possibility is that the distance between the δ locus and the β -structural locus is less than that between the δ locus and the β -thalassaemia locus. This would allow more crossing over to occur between the δ locus and the β -thalassaemia locus than the β -thalassaemia locus and the β -structural locus and hence would allow more cases of linkage in *trans* to be observed. Indeed, Boyer *et al* (1963) suggested that the absence of linkage

between the δ -structural locus and β -structural locus in *trans* might have resulted from the gene for the δ -chain mutation having come into the population relatively recently and because of the close linkage between the loci there has been no time for linkage equilibrium in *cis* and *trans* to be reached. It follows from this argument that the finding of several families with the genes for β thalassaemia and a δ -structural mutation in *trans* suggests that there has been opportunity for a considerable amount of crossing over between the β -thalassaemia and δ -structural loci. However, there are other reasons for the apparent lack of linkage equilibrium of this type. For one thing we do not know where these genes arose and when they came into the African population. It is possible that the β -thalassaemia and δ -chain mutants have been in the population longer than the sickle mutation and, therefore, the former have had more time to achieve equilibrium though this seems extremely unlikely. Another explanation which has to be considered seriously is the problem of natural selection. Assuming that two mutants are present in the population in repulsion, crossing over between them should, in the course of time, other things being equal, mean that they occur equally commonly in *cis* and *trans*. One of the main factors that might stop this process and cause linkage disequilibrium to persist is selection. If the combination of the two mutants together in

the same individual is particularly deleterious then the *cis* chromosome will be at a greater disadvantage than the other combinations and, therefore, will be proportionately rarer than the *trans* chromosome. Thus if it were the case that a δ -chain mutant combined with β thalassaemia is less deleterious than the combination of a sickle mutation and a δ mutant there would be more of the former in *cis* even if the crossover values were identical. With our present state of knowledge about the functions of haemoglobin A₂ it is extremely difficult to envisage a situation where an individual with a δ -chain mutant and either β -thalassaemia or sickle-cell trait would be any better or worse off than an individual with these traits alone though this is an outside possibility. Therefore, selection of this type seems to be an extremely unlikely basis for the apparent linkage disequilibrium which has been observed in these families.

The finding of a distinct discrepancy between the *cis/trans* distribution of δ -chain mutants in association with β -structural as compared with β -thalassaemia mutations, taken together with the likely crossovers which have been observed between the β -thalassaemia gene and the δ -structural locus, suggest that the β -structural locus is closer to the δ locus than is the β -thalassaemia locus. When assessing the significance of this observation it should be remembered that there is very good genetic evidence that the δ locus lies between the γ and β loci in the γ^A - γ^A - δ - β gene cluster. This being so, these observations suggest that the β^+ - and β^0 -thalassaemia mutations in the Negro population may occur at the extremity of this complex in areas involved in termination of the β -chain gene transcription or that code for RNA sequences involved in maintaining β -mRNA stability. These suggestions may not hold for β thalassaemia in other racial groups, however, since it has been pointed out recently that β thalassaemia in the African population is very different from that observed in the Mediterranean and Oriental populations (Friedman *et al*, 1974; Wilcox, Weatherall, and Clegg, 1975). Indeed, the condition seems to be very much milder and there is strong genetic and clinical evidence that β thalassaemias in Africa are different entities from those observed in other racial groups.

Current evidence suggests that in all the β thalassaemias there is either a partial or total absence of β -chain mRNA in the red-cell precursors, but there is no indication as to how this abnormality has arisen. Clearly we badly need more genetic evidence of the type described in this report to

augment the haemoglobin biosynthetic studies currently being carried out to elucidate the basic molecular abnormality in β thalassaemia.

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