$(\text{A}\gamma\delta\beta)^\circ$ -Thalassaemia in Blacks is due to a deletion of 34 kbp of DNA

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Received 26 March 1984; accepted for publication 1 July 1984

SUMMARY. DNA from members of 10 Black families with conditions considered to be $\text{G}\gamma(\delta\beta)^\circ$ -thalassaemia or $\text{G}\gamma(\delta\beta)^\circ$ -HPFH were studied by using restriction enzyme analysis. One or more affected members from each family were shown to have the same deletion of 34 kbp of DNA in the human β -globin gene cluster. A clone spanning the deletion was isolated from the DNA of one such person and studied in detail. The deletion removed part of the $\text{A}\gamma$ and all of the $\psi\beta$, δ and β -globin genes and is different from the four previously identified deletions which caused a condition presently known as $(\text{A}\gamma\delta\beta)^\circ$ -thalassaemia.

Hereditary persistence of fetal haemoglobin (HPFH) and $(\delta\beta)^\circ$ -thalassaemia (thal) are heterogeneous conditions which are characterized by absent or decreased synthesis of the δ and β globin of haemoglobins (Hb) and an increased synthesis of the γ chain containing fetal Hb. In the heterozygote, an anaemia is usually absent but a mild microcytosis may exist. Homozygotes for $(\delta\beta)^\circ$ -thal have a thalassaemia intermedia, while HPFH homozygotes might have a slight microcytosis but lack anaemia or clinical consequences of their anomaly (for review and references, see Weatherall & Clegg, 1981). Most examples of HPFH and $(\delta\beta)^\circ$ -thal thus far analysed are characterized by deletions of DNA in the β globin gene cluster which differ in size and location (Orkin *et al*, 1979; Mears *et al*, 1978; Ottolenghi *et al*, 1979, 1981; Fritsch *et al*, 1979; Tuan *et al*, 1979, 1980; Bernards *et al*, 1979, 1980; Jones *et al*, 1981a, b; Kutlar *et al*, 1984).

$\text{G}\gamma$ types of $(\delta\beta)^\circ$ -thal and $\text{G}\gamma$ types of HPFH have been observed in various populations and are characterized either by absent or decreased synthesis of β and δ chains, increased synthesis of $\text{G}\gamma$ chains, and a nearly normal haematology with mild microcytosis in the heterozygote. Several families with heterozygotes for one of these conditions have been observed by some of us among the Black populations of the southeastern U.S.A. Some have

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been labelled as $G_\gamma\text{-}\beta^+\text{-HPFH}$ (Huisman *et al*, 1975), and others as $G_\gamma\text{-(}\delta\beta\text{)}^\circ\text{-thalassaemia}$ or $G_\gamma\text{-HPFH}$ (Altay *et al*, 1977; Boyer *et al*, 1977). In order to determine whether or not there is a molecular basis for any phenotype differences between such individuals we have initiated a detailed investigation of many of these families using gene mapping and related techniques. The results of this study with individuals previously classified as having $G_\gamma\text{-(}\delta\beta\text{)}^\circ\text{-thal}$ or $G_\gamma\text{-}\beta^\circ\text{-HPFH}$ (Altay *et al*, 1977) show that all affected subjects have lost their G_γ , δ and β -globin genes as a consequence of the deletion of the same 34 kbp of DNA.

MATERIALS AND METHODS

Blood samples. Thirty-one (31) Black subjects from 10 families participated in this study. From each subject, 10–30 ml of blood were collected with EDTA as anticoagulant and transported in ice to the laboratory. Informed consent was obtained prior to collection.

Haematological and haemoglobin analyses. Complete blood counts were obtained with a Coulter Model S cell counter or with a Sysmex CC-720 cell counter. Evaluation of a possible microcytosis and hypochromia was made through examination of blood smears, while the distribution of Hb F was studied with the method of Kleihauer *et al*, as described in Huisman & Jonxis (1977). Globin chain synthesis studies were performed as previously described (Huisman & Jonxis, 1977). The presence of Hb F and Hb S in red cell lysates was demonstrated by cellulose acetate electrophoresis at alkaline pH and citrate agar electrophoresis at acid pH (Huisman & Jonxis, 1977). Hb F was quantitated by alkali denaturation (Betke *et al*, 1959), and Hb A₂ either by micro- or macro-DEAE-cellulose column chromatography (Abraham *et al*, 1976; Schleider *et al*, 1977). Hb F was isolated by DEAE-cellulose chromatography (Schroeder & Huisman, 1980) and its γ chain composition determined by high performance liquid chromatography (HPLC) (Huisman & Wilson, 1980).

DNA analyses. DNA was prepared from peripheral blood using the method of Poncz *et al* (1982). Restriction enzyme mapping of genomic DNA was done by previously described methods (Kutlar *et al*, 1984; Vanin *et al*, 1983) using the probes γ IVS and pRK28. The γ IVS probe is a 457 bp Bam HI-Pvu II fragment from the A_γ globin gene large intervening sequence. The pRK28 probe is a 1.1 kbp Eco RI-Sal I fragment from the plasmid pRK28, kindly provided by Dr Russell Kaufman. This probe is located approximately 16 kbp 3' to the β -globin gene. The relative number of α -globin genes were determined by digesting DNA with Xba I and Bgl II, and hybridizing with an α -globin probe derived from plasmid JW101, as described previously (Felice *et al*, 1982).

A 7.8 kbp Eco RI fragment detected in the DNA of patient B.A. using the γ IVS probe was cloned into phage Ch3A Δ lac by using a size fractionated Eco RI digest (6.0–9.0 kbp) of genomic DNA from patient B.A. and the procedures of Blattner *et al* (1978). The resulting phages were screened without amplification with the γ IVS probe. The 7.8 kbp insert from a recombinant phage was subcloned into the Eco RI site of plasmid p010 ori (Maeda *et al*, 1983). The resulting recombinant plasmid and the plasmid pRK20 were mapped with restriction enzymes used under conditions recommended by the manufacturers. The plasmid pRK20 (which contains a 9.3 kbp Bam HI-Eco RI insert of DNA from the region 3' to β) was also kindly provided by Dr Russell Kaufman.

RESULTS

The patients

Table I lists appropriate data for the 31 Black subjects belonging to 10 families. Twenty-four of the subjects were heterozygotes for a condition characterized by variable elevations of Hb F,

Table I. Haematological and haemoglobin data

	Family and case	Sex age	Hb type	Hb (g/dl)	PCV (l/l)	RBC ($10^{12}/l$)	MCV (fl)	MCH (pg)	A ₂ (%)	F _{AD} (%)	α /non- α ratio	% G _γ	α genes
W	W.W.	M, 50	AF	11.3	0.345	4.83	71	23.4	2.55	10.9	1.68	93.8	— α / $\alpha\alpha$
	L.W.	F, 25	SF	12.8	0.376	4.75	79	26.9	2.3	17.3	n.d.	89.8	$\alpha\alpha$ / $\alpha\alpha$
	S.W.	F, 24	AF	13.2	0.400	5.86	68	22.5	2.25	11.6	1.73	98.5	— α / $\alpha\alpha$
	B.W.	M, 21	AF	13.7	0.403	6.63	61	20.7	2.45	6.3	1.93	90.0	$\alpha\alpha$ / $\alpha\alpha$
	Ba.W.*	F, 19	SF	12.8	0.376	4.75	79	26.9	1.8	17.3	0.88	89.8	— α / $\alpha\alpha$
	J.W.	M, 17	AF	15.1	0.431	6.11	71	24.7	2.0	14.6	1.35	91.6	— α / $\alpha\alpha$
	A.W.*	F, 12	SF	11.4	0.352	4.74	75	24.1	2.0	21.0	1.71	98.9	$\alpha\alpha$ / $\alpha\alpha$
A	M.A.	F, 40	AF	10.6	0.337	3.46	91	30.6	1.8	14.5	1.85	98.8	n.d.
	B.A.*	F, 17	SF	9.3	0.285	3.39	83	27.4	2.3	21.6	1.93	94.8	$\alpha\alpha$ / $\alpha\alpha$
	H.A.*	F, 16	SF	9.2	0.273	3.55	76	25.9	2.0	19.2	1.92	95.3	$\alpha\alpha$ / $\alpha\alpha$
I/M	R.I.*	F, 45	AF	9.8	0.373	3.89	80	25.2	2.75	16.5	n.d.	92.8	— α / $\alpha\alpha$
	M.I.*	M, 17	AF	12.1	0.375	4.26	88	28.4	3.1	11.9	1.09	86.8	— α / $\alpha\alpha$
	J.I.*	M, 15	AF	12.6	0.405	5.13	79	24.6	3.25	8.4	1.15	86.2	— α / $\alpha\alpha$
	N.I.*	F, 13	AF	11.1	0.325	3.84	85	28.9	3.05	15.1	n.d.	86.1	$\alpha\alpha$ / $\alpha\alpha$
	F.M.	F, 19	SF	10.3	0.315	3.76	82	27.4	3.1	19.8	n.d.	92.8	n.d.
	J.S.	F, 37	SF	9.4	0.292	3.14	91	29.9	2.5	18.3	n.d.	90.3	n.d.
M/C	W.B.L.*	F, 35	AF	11.7	0.375	5.07	74	23.4	2.3	9.3	n.d.	95.8	— α / $\alpha\alpha$
	J.M.*	M, 15	AF	14.7	0.433	6.12	72	24.0	2.0	12.3	0.81	91.2	— α / $\alpha\alpha$
	W.C.C.*	M, 14	AF	13.3	0.395	6.16	66	21.6	2.2	4.0	1.38	94.5	— α / $\alpha\alpha$
	J.L.C.	M, 25	AF	14.2	0.445	6.14	74	23.1	2.15	5.2	n.d.	92.8	n.d.
	Jl.M.	M, 12	AF	14.3	0.423	6.00	73	23.8	2.1	11.8	n.d.	94.0	n.d.
S	W.S.*	M, 42	AF	14.0	0.418	6.36	66	22.0	1.8	9.2	n.d.	89.9	$\alpha\alpha$ / $\alpha\alpha$
	M.S.	M, 19	AF	13.6	0.424	5.56	76	24.5	1.7	8.5	n.d.	91.9	n.d.
T-1	G.T.*	F, 20	AF	11.8	0.365	4.50	81	26.2	2.6	16.2	n.d.	94.6	— α / $\alpha\alpha$
	A.T.*	M, 18	AF	14.5	0.443	5.90	75	24.6	2.5	13.3	n.d.	95.0	$\alpha\alpha$ / $\alpha\alpha$
W	B.W.*	F, 29	AF	12.2	0.375	5.12	73	23.8	2.5	12.7	1.25	87.0	$\alpha\alpha$ / $\alpha\alpha$
G	L.G.*	F, 21	AF	10.8	0.320	3.94	81	27.4	2.5	15.0	1.34	94.9	$\alpha\alpha$ / $\alpha\alpha$
T-2	M.T.*	F, 31	AF	10.7	0.360	5.16	70	20.7	2.0	8.4	n.d.	100.0	$\alpha\alpha$ / $\alpha\alpha$
	T.T.*	F, 11	AF	11.0	0.390	5.69	69	19.3	2.05	10.0	n.d.	95.3	$\alpha\alpha$ / $\alpha\alpha$
	Te.T.*	F, 9	AF	11.6	0.375	5.77	65	20.1	1.8	11.1	n.d.	96.4	$\alpha\alpha$ / $\alpha\alpha$
L	l.L.*	M, 20+	AF	14.5	0.475	5.72	74	25.3	2.6	11.9	1.18	100.0	$\alpha\alpha$ / $\alpha\alpha$

* The $(\Lambda\gamma\delta\beta)^0$ -thalassaemia was identified in this person through analysis of the DNA.

microcytosis and unbalanced globin synthesis. Seven relatives were also heterozygous for Hb S and produced no normal Hb A. Some data on members of three families have been presented before, namely for families A and L, both being previously classified as having $\text{G}_\gamma\text{-(}\delta\beta\text{)}^\circ\text{-thalassaemia}$ (Altay *et al*, 1977; Huisman *et al*, 1974), and for family I/M, one member of which was considered to have a $\text{G}_\gamma\text{-}\beta^\circ\text{-HPFH}$ (Altay *et al*, 1977; this person did not participate in the present study). The molecular defect, to be discussed below, was determined for at least one (and often several) member(s) of each of the 10 families presented in the table.

All 24 simple heterozygotes were healthy with only mild haematological abnormalities, notably a mild anaemia among older women and a microcytosis which varied greatly from one person to another (see Table I). The MCV and MCH values were nearly normal for members of the I/M, T and G families, whereas a marked microcytosis was observed in several other patients. The Hb A₂ values were on the lower side of normal (average 2.3%) while the Hb F level (%F_{AD}) varied from 4.0% to 16.5% (average 11.2%). Several subjects have been studied repeatedly and those with low Hb F percentages (W.C.C. and J.L.C. of the M/C family) consistently had low values. The % G_γ in the Hb F of the 24 heterozygotes ranged from 86.1% to 100% (average 93.2%). The difference between the values observed and the expected 100% may be due to contribution from the A_γ globin gene *in trans*.

The seven heterozygotes who also inherited Hb S have the Hb S-($\text{A}_\gamma\delta\beta\text{)}^\circ\text{-thalassaemia}$ condition which is associated with a haematologically and clinically mild variant of sickle cell disease. One of these patients, B.W. of family W, had an associated $\alpha\text{-thal-2}$ heterozygosity. Apart from a lower $\alpha/\text{non-}\alpha$ value (see below) her haematological values and Hb composition data were not different from those of the six other patients. Their Hb levels ranged from 9.2 to 12.8 g/dl and the MCV varied from 71 to 91 fl. The average Hb A₂ value was 2.3%. The level of fetal Hb (average 19.2%) were higher than those of the heterozygotes without Hb S, but the G_γ value of 93.1% was approximately the same.

In vitro synthesis data were obtained on three AF patients with the $\alpha\alpha/\alpha\alpha$ genotype who had an average $\alpha/\text{non-}\alpha$ value of 1.50 (range 1.25–1.93); seven AF patients with the $-\alpha/\alpha\alpha$ genotype who had an average $\alpha/\text{non-}\alpha$ value of 1.31 (range 0.81–1.73); three SF patients with the $\alpha\alpha/\alpha\alpha$ genotype who had an average $\alpha/\text{non-}\alpha$ value of 1.85 (range 1.71–1.93) and one SF patient with the $-\alpha/\alpha\alpha$ genotype who had an $\alpha/\text{non-}\alpha$ value of 0.88.

Detailed mapping of the deletion in patient B.A.

DNA samples from peripheral blood lymphocytes of B.A. (family A) and from a normal fibroblast culture were digested with various restriction enzymes and analysed by agarose gel electrophoresis. Blot hybridization studies employed either a $\gamma\text{IVS II}$ probe (γIVS) or a probe for the region 17 kbp 3' to the β gene (pRK28). Representative autoradiograms for the γIVS probe are shown in Fig 1. Band sizes obtained with both probes are given in Table II. The patient's DNA showed normal-size bands of normal intensity (from both the affected and normal chromosomes) and of half intensity (from the normal chromosome only), as well as bands of abnormal sizes (from the affected chromosome only).

These data permit the derivation of a map of restriction sites in the γ -gene region of the ($\text{A}_\gamma\delta\beta\text{)}^\circ\text{-thalassaemia}$ chromosome, if one assumes a simple deletion and not other

Fig 1. Autoradiograms from genomic mapping of total DNA. 'C' is DNA from a normal fibroblast cell line and 'P' is DNA prepared from whole blood of the affected patient B.A. The γ IVS2 probe was used while the restriction enzymes are indicated below the lanes. The sizes of the new bands appearing in the digests of the DNA from the patient are indicated in kbp.

chromosomal arrangements. For example, the presence of abnormal-size fragments detected with the γ IVS probe for Bam HI, Bgl II, Eco RI, Hind III, Sst I, Bcl I and Xba I shows that the following sites are absent in the affected chromosome: the Bam HI site in the δ -globin gene, the Bgl II site 6 kbp 3' of γ , the Eco RI site in exon 3 of γ , the Hind III site 1 kbp 3' to γ , the Sst I site in exon 3 of γ , the Bcl I site 3' to γ , and the Xba I site 7.5 kbp 3' to γ . All other sites are intact, as shown by the presence of normal-size fragments.

Fig 2 shows this map for the affected chromosome (Fig 2c), which includes data obtained using the cloned abnormal 7.8 kbp Eco RI fragment from this chromosome. Also shown are the maps of the normal chromosome in the γ -gene region (Fig 2b) and in the region 3' to the β -gene (Fig 2d). A comparison of the map of the affected and normal chromosomes localizes the 5' end of the rearrangement to between the Hpa I site in IVS II of γ (which is intact in B.A.) and the Sst I site in exon 3 of γ (which is absent in B.A.). Note that this argument assumes that the Sst I is deleted in the affected chromosome and not absent merely as the result of genetic polymorphism.

The location of the 3' end of the deletion is suggested by the fact that the map of the affected chromosome shows an arrangement of restriction enzyme sites similar to that found within the 11 kbp Eco RI fragment which lies 5 kbp 3' to the β -globin gene (Kaufman *et al*, 1980). This is indicated by data obtained with the γ IVS and the pRK28 probes, as both detected the same abnormal fragments for Eco RI, Bam HI, Hind III and Xba I (Table II); these data also show that the deletion in the affected chromosome lies entirely within the region

a. β -Globin Cluster

b. Normal 5' DNA

c. Deletion Chromosome

d. Normal 3' DNA

e. β -Globin Cluster

Table II. Fragment sizes from genomic mapping of control and affected (patient B.A.) DNA using the probes γ IVS and pRK28. Sizes are in kbp.

Restriction enzyme	γ IVS probe		pRK28 probe	
	Control	B.A.	B.A.	Control
• Bam HI	16.5, 5.0	(16.5), 14.0*, 5.0	(18.0) ⁺ , 14.0*	27.0 ⁺
Bcl I	19.0	(19.0), 13.5	4.5, 4.2	4.5, 4.2
Bgl II	13.0	(13.0), 12.0	n.d.	n.d.
• Eco RI	7.2, 2.7	7.8*, 7.2, (2.7)	(11.0), 7.8*	11.0
Hinc II	4.1, 2.7	4.1, 2.7	5.7	5.7
• Hind III	7.7, 7.2 ⁺ , 3.4	12.0*, 7.7, (3.4)	(17.5) ⁺ , 12.0*	16.0
Hpa I	24.0, 5.0	24.0, 5.0	25.5	25.5
Hpa I- Bgl II	5.1, 3.1	5.1, 3.1	1.0	1.0
Pst I	5.0, 4.0, 0.9	5.0, 4.0, 1.9, (0.9)	n.d.	n.d.
Pvu II	0.5	0.5	n.d.	n.d.
Sst I	3.9, 2.7	5.8, (3.9), 2.7	7.2	7.2
• Xba I	7.9, 5.4, 4.1	(7.9), 6.2*, 5.4, 4.1	6.2*	6.2

Values in parentheses indicate a normal fragment which gives a half-intensity band in the affected heterozygous patient. Values in italics indicate a new band in the affected individual. n.d. = not done.

* Bands detected in both probes.

† Fragments whose size are affected by previously described polymorphic restriction enzyme sites (Jeffreys, 1979; Kan *et al*, 1980; Tuan *et al*, 1983).

between the sequences complementary to these two probes in normal DNA (Fig 2). Comparison of Fig 2(c), the map of the affected DNA, to Fig 2(d), the map of normal DNA 3' to the β -globin gene, allows localization of the 3' end of the deletion to within 1.2 kbp of the Pst I site marked with an asterisk.

Fig 2. (a) A map of the human β -globin gene cluster, including the linked ϵ , γ , δ and β -globin genes. The vertical arrows below the line indicate the locations of the deletion ends in normal DNA. (b) A restriction enzyme map of a portion of the normal β -globin cluster—enlarged from the indicated portion of Fig 2(a). These sites are arranged according to previous sequence and mapping data (Shen *et al*, 1981; Fritsch *et al*, 1979). The stippled boxes below the line show the γ IVS probe used for genomic mapping. Only sites pertinent to the present mapping with this probe are shown. (c) A map of the deletion chromosome. The solid line indicates DNA derived from the 5' side of the deletion (b above) and the dashed line indicates DNA derived from the 3' side of the deletion (see line d below). Sites displayed above the line were derived from genomic mapping data. Sites below the line are additional sites mapped from cloned DNA. (d) A map of a portion of the normal β -globin cluster—enlarged from the indicated portion of part e of this figure. The stippled box above the line shows the human DNA insert in the plasmid pRK28, which was used as a probe in mapping. The map is derived from mapping of the plasmid pRK20 (Kaufman *et al*, 1980; Shafit-Zagardo *et al*, 1982; Henthorn, unpublished data). (e) A map of the human β -globin gene cluster, including the linked ϵ , γ , δ and β -globin genes. The vertical arrows below the line show the locations of the deletion ends in normal DNA. The 34 kbp of DNA between these arrows has been deleted in affected (deletion) chromosomes.

In comparing these maps we note that there is a Kpn I site in the normal DNA which is not present in the affected chromosome. All other mapped sites on the 3' side of the Kpn I site in normal DNA are also present in the affected DNA. By aligning these remaining restriction sites of the affected DNA with the sites in the 3' portion of normal DNA, a more precise location of the 3' end of the deletion can be determined. It is within approximately 200 bp, but on the 3' side of the Kpn I site. The argument for this localization is as follows. We determined above that the 5' end of the deletion is located between Hpa I and Sst I sites in the γ gene. These sites are separated by only 62 bp of DNA. In the deleted chromosome the DNA beyond 62 bp on the 3' side of the Hpa I site must come from the relevant region 3' to β . The next 3' mapped site in the deleted chromosome, that is also present in the normal 3' DNA, is the asterisked Pst I site in Figs 2(c) and 2(d). It is 1.2 kbp from the Hpa I site; therefore approximately 1.1 kbp (1.2 kbp to 62 bp) of DNA in that interval is of 3' origin. In the normal 3' DNA the Kpn I site, which is absent in the affected chromosome, is located approximately 1.2 kbp 5' to the asterisked Pst I site. Thus, the 3' end of the deletion is very close to, but on the 3' side of, the Kpn I site (assuming that its absence in the affected DNA is not due to DNA-sequence polymorphism). We put it within 200 bp so as to allow for measurement errors in mapping and possible polymorphic variation. It is thus estimated that the total amount of DNA deleted in the affected chromosome is 34 kbp.

DNA analyses on the additional patients

DNAs from 19 individuals (shown with asterisks in Table I) were analysed using the restriction enzymes Pst I, Eco RI, Hind III, Hpa I and Bam HI, and the probes γ IVS and pRK28. These analyses gave identical band sizes to those of the DNA from patient B.A. (Table II). These results identify an identical deletion of 34 kbp in all 20 subjects whose DNA was analysed and presumably also in the 11 additional affected relatives who are listed in Table I.

Subject I.L. of Table I is unusual in his non-deletion chromosome—he has two γ genes (and no γ gene). The relevant evidence is presented in Fig 3. Pst I digestion of DNA from I.L. gave two abnormal-sized fragments after hybridizing to the γ IVS probe. The 1.9 kbp fragment originates from the chromosome with the deletion (Table II), while the 3.7 kbp fragment is due to the loss of the Pst I restriction site in the third exon of the 3' γ gene. This situation is characteristic of the $\gamma\gamma$ -chromosomes described by Powers *et al* (1984).

The number of α -globin genes present was determined in 25 subjects. Fourteen had the full complement of four α genes ($\alpha\alpha/\alpha\alpha$), while 11 had three α genes ($-\alpha/\alpha\alpha$) corresponding to the α -thal-2 heterozygosity. The results for the individual subjects are listed in Table I.

DISCUSSION

Our study of the DNA of 20 Black individuals previously classified as either $\gamma(\delta\beta)^{\circ}$ -thal or $\gamma(\beta)^{\circ}$ -HPFH heterozygotes shows that all 20 (and presumably the 11 relatives with a similar phenotype) had the same genetic defect—a deletion of 34 kbp of DNA. The 5' end of the deletion is located within 62 bp of DNA between the Hpa I site in the IVS II and the Sst I site in the third exon of the γ globin gene, while the 3' end is estimated to be within 200 bp of the

Fig 3. Autoradiogram from genomic mapping of DNA from a normal control person (C) and from an affected individual (P) who is subject I.L. listed in Table III. See text for further details.

Kpn I site, 13 kbp 3' to the β globin gene. This large deletion eliminates part of the $^A\gamma$ -, and all of the $\psi\beta$ -, the δ - and the β -globin genes and in heterozygotes leads to a deficiency of the corresponding non- α globin genes. In addition, there is an increase in the synthesis of G_γ chains.

Since the deletion is shared by all these individuals and because the heterozygotes for this condition have haematological features compatible with thalassaemia rather than HPFH, we propose that this 34 kbp deletion be described as $(^A\gamma\delta\beta)^\circ$ -thal. The haematological parameters of this abnormality are heterogeneous but, in most cases, deviate little from normal. These slight deviations may account for previous ambiguities in classification of such persons. Four other deletions resulting in an $(^A\gamma\delta\beta)^\circ$ -thal condition have been described in Turkey, Malaysia, China and India (Fritsch *et al*, 1979; Orkin *et al*, 1979; Jones *et al*, 1981a, b; Nakatsuji *et al*, 1984; Trent *et al*, 1984). The molecular defects of these conditions (reviewed by Trent *et al*, 1984) are different from the one described here. For instance, the 5' end of the deletion in the Turkish and Malaysian patients was rather close to the 3' end of the G_γ gene and thus involved the entire $^A\gamma$ gene. The 5' end of the Chinese deletion is within the $^A\gamma$ gene, like the 5' end of the deletion described here, but the Chinese deletion is much larger

Table III. Haematological and haemoglobin composition data in HPFH and $\delta\beta$ -thalassaemia heterozygotes*

	$G_\gamma^{A\gamma}$ -HPFH (n = 54)	$G_\gamma^{A\gamma}$ - $\delta\beta$ (n = 62)	G_γ - $\delta\beta$ (n = 27)	($A\gamma\delta\beta$) $^\circ$ -Thal (n = 24)
Hb (g/dl)	12.9 $\pm$ 1.6	12.2 $\pm$ 1.0	12.5 $\pm$ 1.5	12.6 $\pm$ 1.5
MCV (fl)	80.1 $\pm$ 7.3	76.7 $\pm$ 6.4	76.0 $\pm$ 5.3	74.3 $\pm$ 7.3
MCH (pg)	26.6 $\pm$ 2.7	23.4 $\pm$ 2.9	24.0 $\pm$ 1.7	24.1 $\pm$ 2.7
Hb F (%)	26.3 $\pm$ 4.6	10.5 $\pm$ 3.3	12.2 $\pm$ 3.1	11.2 $\pm$ 3.3
Hb A ₂ (%)	1.9 $\pm$ 0.4	2.3 $\pm$ 0.5	2.2 $\pm$ 0.5	2.3 $\pm$ 0.4
G_γ in Hb F (%)	41 $\pm$ 5	41 $\pm$ 9	101 $\pm$ 3	93.2 $\pm$ 4
Hb A (pg/cell)	19 $\pm$ 1.7	20.4 $\pm$ 2.6	20.5 $\pm$ 1.4	20.8 $\pm$ 1.9
Hb F (pg/cell)	7.1 $\pm$ 1.6	2.4 $\pm$ 0.8	2.9 $\pm$ 0.8	2.8 $\pm$ 1.0
Hb A ₂ (pg/cell)	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1
G_γ (pg/cell)	1.45	0.49	1.45	1.4

* Data are from Wood *et al* (1979) except for those of the last column which are averages and SD of the individual values listed in Table I. The G_γ - $\delta\beta$ condition is presently listed as ($A\gamma\delta\beta$) $^\circ$ -thal.

in the 3' direction (Vanin *et al*, 1983; Dixie Mager, personal communication). The Indian ($A\gamma\delta\beta$) $^\circ$ -thal patients have a molecular defect involving a complex rearrangement which includes deletions of DNA as well as inversion of the DNA between the $A\gamma$ and δ genes (Jones *et al*, 1981b; Nakatsuji *et al*, 1984). Despite the apparent differences in the deletion of DNA causing these thalassaemic conditions, the haematological observations among heterozygotes are remarkably similar. Wood *et al* (1979) summarized such data for G_γ^{+} -($\delta\beta$) $^\circ$ -thal [= ($A\gamma\delta\beta$) $^\circ$ -thal] heterozygotes, and these results are again listed in Table III, together with the average values for our ($A\gamma\delta\beta$) $^\circ$ -thalassaemic heterozygotes. Although the group of $G_\gamma(\delta\beta)$ $^\circ$ -thalassaemic heterozygotes referred to by Wood *et al* (1979) may well be a mixture of disorders with different molecular defects, the similarities between these data and those for our 24 ($A\gamma\delta\beta$) $^\circ$ -thal heterozygotes are most striking.

The presence of a β^S determinant on the opposite chromosome of some patients in our present study does not greatly affect the haematological parameters, although these compound heterozygotes suffer from a mild form of sickle cell disease. Their MCV (average 80.7 fl) and MCH (average 26.9 pg) values are somewhat higher than seen in the simple heterozygotes (Table I). Most striking is the increased level of Hb F (average 19.2%) which is about double that of simple ($A\gamma\delta\beta$) $^\circ$ heterozygotes but contains the same level of G_γ chain (average 93.1%). This increase is probably due to a Hb F red cell survival which is longer than that of cells containing mainly Hb S.

The data of Fig 4 show the distribution of MCV, MCH, %Hb F and % G_γ values among the ($A\gamma\delta\beta$) $^\circ$ -thalassaemia heterozygotes with or without a Hb S or an α -thalassaemia-2 heterozygosity. These data suggest that an α -thalassaemia-2 heterozygosity ($-\alpha/\alpha\alpha$) has no consistent or significant effect on the four parameters shown, as well as the additional data given in Table I.

Fig 4. A comparison of some haematological and haemoglobin composition data, listed in Table I. AF refers to the ($\gamma\delta\beta$) $^\circ$ -thalassaemia heterozygote; SF to the Hb S-($\gamma\delta\beta$) $^\circ$ -thalassaemia condition.

Subject I.L., heterozygous for the ($\gamma\delta\beta$) $^\circ$ -thal chromosome and a variant chromosome having two G_γ globin genes (and no A_γ globin gene), is of some special interest. As a consequence of this double heterozygosity, his fetal Hb does not contain any A_γ chains. About 3% of the Black population is heterozygous for the $G_\gamma G_\gamma$ chromosomal variant and the normal $G_\gamma A_\gamma$ chromosome (Gilman & Huisman, 1984), and the detection of a similar chromosome among the 20 Black persons tested is, therefore, not surprising.

We report the characterization of a fifth type of chromosomal alteration in the β -globin gene cluster responsible for ($\gamma\delta\beta$) $^\circ$ -thalassaemia. In spite of differences in the molecular nature of the lesion, these all show similar clinical conditions. The molecular tools of DNA analysis are therefore necessary in order to properly differentiate the various $\delta\beta$ thalassaemic disorders present in the human population.

ACKNOWLEDGMENTS

The authors are indebted to H. F. Harris and B. L. Abraham for their help in the family studies, to Dr Russell E. Kaufman for providing plasmids pRK20 and pRK28, and to Dr John G. Gilman for discussions. These investigations were supported by NIH GM 20069 and AM 20120, by USPHS Research Grants HLB-05168 and HLB-15158, and by designated research funds of the Veterans Administration. This is paper no. 2722 from the Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, and contribution no. 0816 from the Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, U.S.A.

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