$G\gamma\beta^+$ type of hereditary persistence of fetal haemoglobin in association with Hb C

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SUMMARY This report describes a Negro family with the $G\gamma\beta^+$ type of hereditary persistence of fetal haemoglobin. Family members with levels of haemoglobin F of 17 to 23% had normal red cell indices, balanced globin chain synthesis, and a pancellular distribution of the fetal haemoglobin, showing that these subjects have a form of HPFH. The production of Hb A and C in addition to the large amount of Hb F in one family member showed that there was an active β^A gene in cis to the HPFH determinant, while structural analysis of the Hb F revealed the presence of only $G\gamma$ chains. The criteria for the diagnosis of $G\gamma\beta^+$ HPFH, and the relevance of such conditions to the control of globin gene expression, are discussed.

Hereditary persistence of fetal haemoglobin (HPFH) is a condition in which increased levels of fetal haemoglobin (Hb F, $\alpha_2\gamma_2$) are present in persons who are clinically normal, and who have minimal or no haematological abnormalities (Weatherall and Clegg, 1975). It is a heterogeneous group of disorders which can be divided into pancellular and heterocellular types according to whether the Hb F is present in all, or only a proportion, of the red blood cells (Boyer *et al.*, 1977). The condition can be further subdivided, depending on the amount and structure of the Hb F. The γ chains of Hb F have either glycine ($G\gamma$) or alanine ($A\gamma$) at position 136 (Schroeder *et al.*, 1968). The $G\gamma$ and $A\gamma$ chains are the product of distinct genetic loci which are believed to be linked to the β and δ chain genes in the order $G\gamma-A\gamma-\delta-\beta$ (Huisman *et al.*, 1972; Weatherall and Clegg, 1975) on chromosome 11 (Deisseroth *et al.*, 1978).

In the most common pancellular HPFH (Negro HPFH), both $G\gamma$ and $A\gamma$ genes are expressed in the absence of β and δ chain production in cis to the HPFH determinant (Conley *et al.*, 1963; Ringelhan *et al.*, 1970; Huisman *et al.*, 1974). Recently it has been shown that this condition results from a deletion involving parts of the δ and β chain genes (Kan *et al.*, 1975; Forget *et al.*, 1976; Ottolenghi *et al.*, 1976; Ramirez *et al.*, 1976).

Other HPFH cases have been reported in which only $G\gamma$ chains were present (Sukumaran *et al.*, 1972; Huisman *et al.*, 1975a, b; Altay *et al.*, 1976, 1977). Included among these are two Negro families in

which 15% Hb F production was associated with both Hb A and S, indicating the production of β^A chains in cis to the HPFH determinant (Huisman *et al.*, 1975b; Friedman and Schwartz, 1976). This condition has been called $G\gamma\beta^+$ HPFH. In this report we describe a further Negro family with $G\gamma\beta^+$ HPFH, in this case associated with Hb C.

Methods

HAEMATOLOGICAL STUDIES AND HAEMOGLOBIN ANALYSIS

Peripheral blood samples were collected into heparin or EDTA. Haemoglobin and red cell indices were measured with a Coulter Counter S. Fresh blood smears were made from each family member. Haemoglobin electrophoresis was carried out in starch gel with a tris-EDTA-borate buffer system, pH 8.6 (Weatherall and Clegg, 1972). The proportion of Hb A₂ (or Hb C and A₂) was measured by eluting from cellulose acetate strips (Weatherall and Clegg, 1972).

The Hb F levels were measured by alkali denaturation (Betke *et al.*, 1959) as modified by Pembrey *et al.* (1972). The intracellular distribution of Hb F was examined by both the acid elution technique (Kleihauer *et al.*, 1957) and by immunofluorescence (Wood *et al.*, 1975). The haemoglobin composition of the red cells from two family members (GT and JaT) were also examined after the cells had been fractionated after centrifugation. The packed cells obtained from 30 ml peripheral blood were centrifuged at 64 000 g for 60 min at 15°C. After

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Fig. 1 Pedigree of family. Arrow indicates proband.

gene. The two sibs with HPFH have 17.7 and 17.4% Hb F, respectively, and low levels of Hb A₂; both are haematologically normal. Two sibs with Hb C trait have approximately 35% Hb C and target cells were seen in the peripheral blood. In the 5-year-old child (MG) of one of these, the presence of abnormal red cell indices, together with anisopoikilocytosis, was noted. He also had a lower than average level of Hb C (28%) and a raised level of Hb F (approximately 4%) unevenly distributed in his red cells. Unfortunately, the child's father was not available for study.

In those patients with the HPFH gene, both acid elution smears and examination of blood films by the immunofluorescence technique showed that Hb F was present in virtually all of the cells. With the acid elution technique there was marked variability in the staining intensity from cell to cell (Fig. 3).

The results of the differential centrifugation of the red cells from the HPFH heterozygote (JaT) and the HPFH/Hb C compound heterozygote (GT) are

shown in Table 1. The decreasing MCV and increasing MCHC from top to bottom of the spin cells indicate that a separation of the cells according to their density was achieved. No significant differences in the proportions of the haemoglobin present in these cells were observed between various fractions.

GLOBIN CHAIN SYNTHESIS

The biosynthesis data show that in both the single heterozygote for HPFH and the compound heterozygote for HPFH and Hb C the α /non- α ratio was balanced (Table 3). It was not possible to separate the γ peak from the pre- β peak, or the β^C peak from the pre- α peak by CMC chromatography (Fig. 4). Nevertheless, the proportion of β^C chain synthesis in GT approximated to the amount of Hb C present in the peripheral blood. Similarly, the proportion of γ chain synthesis was similar to the Hb F level in the peripheral blood. The specific activity of the γ chain was less than that of the β^A chain on both of these

Table 2 Summary of haematological data

Subject	RBC ($\times 10^{12}/l$)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)	H	A	P	Po	T	A* (%)	A ₂ (%)	C (%)	F (%)	F cells (%)	Gly	Δ la
GT	5.45	14.7	44.3	81	26.5	33.5	—	—	—	—	+	34.80 30.10	— —	44.2 46.9	21.0 23.0	100	1.00	2.00
VT	4.78	12.7	38.7	82	26.1	31.5	—	—	—	—	—	96.98	2.35	—	0.67	—	—	—
VG	5.00	13.4	39.3	78	27.0	33.8	—	—	—	—	+	64.20	—	35.0	0.80	7.5 $\pm$ 0.1	—	—
MT	4.27	12.6	35.7	84	29.0	34.8	—	—	—	—	—	80.50 79.20	1.80 1.60	— —	17.7 19.2	100	1.30	2.00
JaT	4.12	13.0	39.3	95	31.6	32.7	—	—	—	—	—	80.40 77.08	2.20 2.32	— —	17.4 20.6	100	0.99	2.00
JocT	4.84	12.8	35.8	73	26.6	35.4	—	—	—	—	+	62.40 62.15	— —	34.9 36.0	0.90 1.85	12.0 $\pm$ 1.8	—	—
MG	5.90	12.1	37.6	63	20.8	32.1	+	+	+	±	+	67.90 66.00	— —	28.2 29.5	3.9 4.5	23.6 $\pm$ 0.2	—	—

*By subtraction. H, hypochromia; A, anisocytosis; P, poikilocytosis; Po, polychromasia; T, target cells.

subjects ($\gamma/\beta^A = 0.71$ and 0.77). In VT the $\alpha/\text{non-}\alpha$ ratio was 0.82 . In the absence of further biosynthetic and genetic data the significance of this finding is uncertain.

COMPOSITION OF Hb F

Analysis of the $\gamma\text{CB } 3$ from the γ chain of MT gave values for glycine and alanine of 1.00 and 2.00 residues, respectively. This established that the Hb F was solely of the $G\gamma$ type. Analysis of peptide $\gamma 9$, isolated from a two dimensional peptide map of a

tryptic digest of γ chains, showed that position $\gamma 75$ was occupied by isoleucine.

Discussion

The term 'hereditary persistence of fetal haemoglobin' (HPFH) is given to a group of conditions characterised by persistent fetal haemoglobin synthesis beyond the neonatal period in the absence of any major haematological abnormalities. In the common form found in Negroes, there is abundant

Fig. 2 Starch gel electrophoresis of haemolysates prepared from (i) GT, (ii) VG, (iii) MT, (iv) JaT, (v) JocT, (vi) MG.

Fig. 3 Peripheral blood smears from (a) MT and (b) JaT, stained by the acid elution technique, showing that all cells are stained but there is variability in intensity of staining from cell to cell.

Table 3 Summary of biosynthetic data

	Total counts/min/0.5 ml							Specific activities (cpm/OD ²⁸⁰)			
	γ	β ^A	β ^C	α	α/non-α	γ/non-α	β ^C /non-α	α/β ^A	α/β ^C	γ/β ^A	γ/β ^C
Father (GT)	1203	1799	4689	8614	1.12	0.156	0.609	1.36	1.20	0.71	0.63
Mother (VT)	—	1001	—	827	0.82	—	—	0.83	—	—	—
Daughter (JaT)	539	1847	—	2739	1.15	0.225	—	0.99	—	0.77	—

Fig. 4 Chromatographic separation of ³H leucine labelled globin chains from (top) VT, (middle) JaT, and (bottom) GT.

evidence to show the absence of β and δ chain synthesis directed by the chromosome carrying the HPFH gene (Wheeler and Krevans, 1961; Conley *et al.*, 1963). Two families have been reported previously, however, in which an HPFH condition

has been found in association with β^A and β^S chains (Huisman *et al.*, 1975b; Friedman and Schwartz, 1976). The Hb F in these subjects contained only α chains and this, together with the low level of Hb A, suggested that the condition arose from a deletion of

the $A\gamma$ and δ genes from the $G\gamma A\gamma \delta\beta$ globin gene complex. Hence, this form of HPFH has been called the $G\gamma\beta^+$ type (Friedman and Schwartz, 1976).

The present studies indicate that the family described here also carry a $G\gamma\beta^+$ type of HPFH. This is shown by the presence in GT of both Hb A and C, together with the high level of Hb F, in the absence of any haematological abnormalities. Clearly there must be β chain synthesis in cis to the HPFH gene. Among his offspring, those with Hb C had normal adult levels of Hb F, while those with high levels of Hb F had a low-normal level of Hb A₂, with the remainder of their haemoglobin being Hb A. Thus, the β^A gene is in cis to the HPFH gene.

Unlike the two previous families in which the $G\gamma\beta^+$ type of HPFH was found together with Hb S, in the present case it was found in association with Hb C. However, there was little if any difference between the Hb C/ $G\gamma\beta^+$ HPFH and the Hb S/ $G\gamma\beta^+$ HPFH compound heterozygotes.

The distribution of Hb F in the $G\gamma\beta^+$ HPFH heterozygotes and the Hb C/ $G\gamma\beta^+$ HPFH compound heterozygote was pancellular by both the acid elution and immunofluorescence techniques. However, by acid elution there appeared to be more variable staining from cell to cell than is seen in $G\gamma A\gamma$ Negro HPFH. This may reflect the slightly lower Hb F level (15 to 20%) in $G\gamma\beta^+$ HPFH than in the more common $G\gamma A\gamma$ HPFH (20 to 30%), which also shows some heterogeneity in the amount of Hb F per cell.

The α /non- α synthesis ratios in the $G\gamma\beta^+$ HPFH heterozygotes were close to unity. However, in both subjects studied, the specific activity of the γ chain was significantly lower than that of the β chain. This could be explained on the basis of differential survival of the cells with most Hb F, as has been shown to occur in β - and $\delta\beta$ -thalassaemia (Gabuzda *et al.*, 1963; Loukopoulos and Fessas, 1965; Wood *et al.*, 1977). However, the Hb F was evenly distributed throughout all red cell fractions obtained by differential centrifugation. This method separates cells by virtue of their relative densities, which usually reflect cell age (Borun *et al.*, 1957; Rigas and Koler, 1961), and hence suggests that there is little differential survival of red cells containing variable amounts of Hb F. A possible alternative explanation for the lower γ specific activity is that γ chain mRNA may disappear from reticulocytes at a slightly faster rate than β mRNA (Ringelmann *et al.*, 1977). If this were so, γ chain synthesis *in vitro* might not be truly representative of *in vivo* synthesis. This hypothesis could be tested by performing simultaneous bone marrow and peripheral blood incubations from a patient such as GT.

Deletions of various parts of the $G\gamma A\gamma \delta\beta$ complex

have been advanced as the most likely explanation of the pancellular HPFH conditions, and in some cases of $G\gamma A\gamma$ HPFH deletions involving the δ and β genes have been detected, both by cDNA hybridisation (Kan *et al.*, 1975; Forget *et al.*, 1976; Ottolenghi *et al.*, 1976; Ramirez *et al.*, 1976), and by restriction endonuclease analysis of genomic DNA (Mears *et al.*, 1978). A similar deletion involving the δ and β loci is also found in some cases of $\delta\beta$ -thalassaemia (Ottolenghi *et al.*, 1976; Ramirez *et al.*, 1976). In the case of $G\gamma\beta^+$ HPFH, such a deletion would presumably involve the $A\gamma$ and δ genes, the loss of this material allowing the continued expression of the β locus, but removing the normal repression of the $G\gamma$ gene in adult life. Whether the reduced output of the β gene results directly from the deletion, or whether the reduction results from the apparently reciprocal relationship between γ and β chain production (Weatherall *et al.*, 1979) remains to be determined.

In addition to $G\gamma\beta^+$ HPFH, increased levels of Hb F containing only $G\gamma$ chain are also found in Hb Kenya (Huisman *et al.*, 1972; Smith *et al.*, 1973; Nute *et al.*, 1976), $G\gamma \delta\beta$ -thalassaemia (Mann *et al.*, 1972; Huisman *et al.*, 1975a; Wood *et al.*, 1977), $G\gamma\beta^0$ HPFH (Altay *et al.*, 1977), and the Atlanta type of heterocellular HPFH (Altay *et al.*, 1976). These cases have been reviewed recently by Altay *et al.* (1977). There is considerable difficulty involved in the differential diagnosis of HPFH and $\delta\beta$ -thalassaemia, particularly those involving the $G\gamma$ types (Mann *et al.*, 1972; Sukumaran *et al.*, 1972; Huisman *et al.*, 1975a,b; Altay *et al.*, 1977; Wood *et al.*, 1977). The usual criteria of haematology (normal red cell indices in HPFH versus low MCH and MCV in $\delta\beta$ -thalassaemia), globin chain synthesis (balanced versus unbalanced), and Hb F distribution (pancellular versus heterocellular) all show considerable overlap in the two conditions and may not all be consistent. The recent report of balanced globin chain synthesis in some $\delta\beta$ -thalassaemia heterozygotes with abnormal haematology and heterocellular Hb F distribution (Kinney *et al.*, 1978) exemplifies this point. In addition, the existence of HPFH cases with very low proportions of $A\gamma$ chains makes very careful structural analysis of the Hb F essential (Sukumaran *et al.*, 1972; Boyer *et al.*, 1977). The diagnosis of $G\gamma\beta^0$ HPFH is further complicated by the necessity of having an abnormal β chain locus in trans in order to show the absence of β^A synthesis in cis to the HPFH gene, that is, to differentiate it from $G\gamma\beta^+$ HPFH. By the strict criteria of normal red cell indices, balanced chain synthesis, pancellular Hb F distribution, solely $G\gamma$ chains, and proof of the absence of cis β^A chain production, only two possible $G\gamma\beta^0$ families have been described. These are family J of Sukumaran *et al.* (1972) and Huisman *et al.*

(1975a) (though the critical family member for the exclusion of $\gamma\gamma\beta^+$ HPFH is omitted from the pedigree in the second publication) and family M of Altay *et al.* (1977) where the remote possibility of a $\gamma\gamma\beta^+$ HPFH could not be excluded.

The importance of these probable deletion conditions lies in the information they provide as to the control of gene expression and, in particular, the control of fetal and adult haemoglobins during human development. Only by thorough characterisation of the haematological manifestations of these variants will it be possible to interpret the results of molecular analysis obtained by restriction endonuclease mapping and DNA sequencing. It is hoped that in this way some understanding will be gained as to the variable degree of γ chain compensation in HPFH and $\delta\beta$ -thalassaemia. $\gamma\gamma\beta^+$ HPFH would appear to be a particularly suitable candidate for such analysis.

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Note added in proof

Since this paper was submitted for publication, a further case of $\text{G}\gamma\beta^+$ HPFH has been reported in association with Hb S and α -thalassaemia (Tatsis (1978), *Blood*, **52**, (Suppl.) 119). In addition we have become aware of two early reports of HPFH in association with Hb S and A, and C and A, which seem very likely to be further examples of this condition (McCormick and Humphreys (1960), *Blood*, **16**, 1736–1744; Schneider *et al.* (1960), *New England Journal of Medicine*, **265**, 1278–1283).