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A Second Type of Hereditary Persistence of Foetal Haemoglobin in India

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Summary. Six individuals in four Indian families have 25–30% foetal haemoglobin in which the two types of γ chains (the $^{G}\gamma$ chain with glycine in position 136 and the $^{A}\gamma$ chain with alanine in that position) are present in a ratio of 70:30. It is suggested that these heterozygotes form a distinct subgroup of the $Hb_{G\gamma}$ $Hb_{A\gamma}$ class of the hereditary persistence of foetal haemoglobin. In three relatives this HPFH condition occurs together with β-thalassaemia. It is concluded that the β-thalassaemia is of the type in which the ratio of $^{G}\gamma:^{A}\gamma$ chains is 3:1 as in the newborn.

In 'hereditary persistence of foetal haemoglobin' (HPFH), the heterozygote is characterized by a familial persistence of 5–40% foetal haemoglobin (Hb-F) in adult life, by the absence of haematological abnormalities, by low normal or decreased amounts of Hb-A_2, and by a more or less uniform cellular distribution of Hb-F. Although this condition has most frequently been observed in Negroes, it has also been detected in Greeks and other Caucasians, in Thais, and in Asiatic Indians (Huisman *et al*, 1969, 1970b; Huisman, 1972; Sukumaran *et al*, 1972). Chemical analyses of the Hb-F of over 100 cases of HPFH have shown a marked heterogeneity at the molecular level. Most cases have an Hb-F which, like that of the normal newborn and the normal adult, contains two types of γ chains. These $^{G}\gamma$ and $^{A}\gamma$ chains are distinguished by the amino acid residue in position 136; it is glycyl in the $^{G}\gamma$ chain and alanyl in the $^{A}\gamma$ chain (Huisman *et al*, 1969, 1970b; Schroeder *et al*, 1968, 1971). This type of HPFH has been termed the $Hb_{G\gamma}$ $Hb_{A\gamma}$ class because both types of γ chains are produced. In addition, two other classes have been recognized. Subjects in the $Hb_{A\gamma}$ class, which has been found in Greeks (Huisman *et al*, 1970b) and in one additional Caucasian family (Huisman *et al*, 1970a), produce Hb-F with $^{A}\gamma$ chains only. Subjects in the other, the $Hb_{G\gamma}$ class, come from the Negro race (Huisman *et al*, 1969; Sukumaran *et al*, 1972; and unpublished data) and from Indian families from the area of Bombay (Sukumaran *et al*, 1972); their Hb-F has $^{G}\gamma$ chains only.

The $Hb_{G\gamma}$ $Hb_{A\gamma}$ class of HPFH has much heterogeneity of expression. The majority of heterozygotes produce about 25% Hb-F with a $^{G}\gamma:^{A}\gamma$ ratio of about 40:60. In a few Negro

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TABLE I. Haematological and biochemical data on members of four families with the HPFH or the HPFH–β-thalassaemia condition

Case	*Age*	*Relation*	*Condition*	*Hb* (*g*/100 *ml*)	*PCV* (%)	*RBC* ($10^6/\mu l$)	*MCV* (*fl*)	*MCH* (*pg*)	*MCHC* (%)	*Osmotic fragility*	*Hb-F distribution*	*Hb-A_2* (%)	% *Hb-F**		*Peptide γCB-3*	
													A.D.	*Ile*	*Gly*	*Ala*
FAMILY C																
B.P.C.	29	Mother	HPFH	13.6	43	4.81	89	28	32	Normal	Even	1.6	24	30	0.73	2.38
R.P.C.	7	Daughter	HPFH	14.5	44	4.74	93	31	33	Normal	Even	2.2	25	28	0.68	2.34
FAMILY D																
N.H.D.	38	Father	HPFH	14.8	45	5.47	82	27	33	Normal	Even	1.8	17	23	0.72	2.36
K.H.D.	52	Brother§	HPFH	14.5	47	4.75	99	30	31	Normal	Even	1.5	24	27	0.71	2.33
V.N.D.	31	Mother	β-Thal.	11.6	37	5.66	66	21	31	Decreased	—	4.9	1.0	1.9	—	—
P.N.D.‡	7	Son	HPFH–β-thal.	5.5	21	3.73	56	15	26	Decreased	Even	2.5	52	84	0.75	2.27
FAMILY T.R.†																
Raj.R.‡	8	Brother	HPFH–β-thal.	6.7	25	3.51	72	19	26	Decreased	Even	3.2	63	87	0.70	2.33
Ram.R.	5	Brother	HPFH	12.2	36	4.06	89	30	34	Normal	Even	1.5	21	22	0.66	2.36
FAMILY R																
Ca.G.	6	Niece	HPFH	13.0	44	4.81	92	27	30	Normal	Even	2.0	24	29	0.72	2.33
Cl.R.‡	23	Uncle	HPFH–β-thal.	9.0	30	4.69	61	19	30	Decreased	Even	3.3	63	84	0.70	2.32

* F_{AD} = %Hb-F determined by alkali denaturation (Betke *et al*, 1959). F_{IIe} = Hb-F determined by a chemical procedure (Schroeder *et al*, 1970b).
† Father has been identified as a HPFH heterozygote and the mother as a β-thalassaemia heterozygote.
‡ DEAE-Sephadex chromatography showed the presence of small amounts of Hb-A namely 4.4% (P.N.D.), 4.3% (Raj.R.) and 4.6% (Cl.R.)
§ K.H.D. is the brother of N.H.D.

families, the amount of Hb-F is similar, but the $^{G}\gamma:^{A}\gamma$ ratio is significantly higher, namely, about 60:40. On the other hand, heterozygotes of two Negro families with about 17% Hb-F show a decreased ratio of about 20:80 (for a detailed discussion of these subclasses, see Huisman, 1972; Sukumaran *et al*, 1972).

Here we describe an additional subgroup of the $Hb_{G\gamma}$ $Hb_{A\gamma}$ class of HPFH in four Indian families. This is the second type of HPFH in India; the first, which belongs to the $Hb_{G\gamma}$ class, has been discussed earlier (Sukumaran *et al*, 1972).

MATERIALS AND METHODS

Blood samples from several members of the four families to be discussed here have been studied repeatedly at the Cancer Research Institute in Bombay. Haematological evaluation used standard methods (Wintrobe, 1962). The distribution of Hb-F in the cells was evaluated by the method of Kleihauer (1966).

Samples were shipped airmail, special delivery, from Bombay to Augusta, Georgia, as washed red cells with added streptomycin. Red-cell haemolysates were analysed by starch-gel electrophoresis (Efremov *et al*, 1969) and by DEAE-Sephadex chromatography (Huisman & Dozy, 1965; Dozy *et al*, 1968). The Hb-F content was determined by an alkali denaturation procedure (Betke *et al*, 1959; results are presented as % $F_{A\cdot D.}$) and by a procedure which used chromatography and amino acid analysis (Schroeder *et al*, 1968; results as % F_{IIe}). The ratio of the $^{G}\gamma:^{A}\gamma$ chains was determined by amino acid analysis of the γCB-3 peptide (residues 134–146 inclusive of the γ chain) which was isolated from globin F after treatment with cyanogen bromide (Schroeder *et al*, 1968). The glycine content of this peptide, which may or may not be a mixture, can vary from zero to one residue. The ratio of the $^{G}\gamma:^{A}\gamma$ chains can be calculated from the glycine content; thus, if glycine is 0.50, the ratio is 50:50, or if glycine is 0.75, the ratio is 75:25.

RESULTS

Haematological and haemoglobin data on 10 members of the four families are presented in Table I.

DISCUSSION

That the anomaly is hereditary is clear from the relationships within these families. For all six heterozygotes for HPFH, the haematological data are within the normal range, and the amount of Hb-F (% F_{IIe}) which varies from 22 to 30% (with a mean of 26.5%) is comparable to that in nearly all Negro HPFH heterozygotes of the $Hb_{G\gamma}$ $Hb_{A\gamma}$ class. The equal distribution of Hb-F within the red cells is considered to be specific for the HPFH anomaly. The levels of Hb-A_2 (1.5–2.2%) are slightly below the range of 2.2–3.0% that normal adults show with our methods.

Of special interest is the fact that, in the γCB-3 peptides from the Hb-F of these six heterozygotes, the mean value for glycine is 0.70 with a range of 0.66–0.73. The $^{G}\gamma:^{A}\gamma$ ratio, thus,

is 70:30: it appears to be distinct from the 20:80, 40:60 or 60:40 ratios that were noted above and to constitute a new subgroup.

It is, indeed, pertinent to question whether the analytical accuracy and precision of the method is sufficient to distinguish between a 70:30 and a 60:40 ratio. The accuracy and precision of the method has been discussed briefly by Huisman *et al* (1969) and in more detail by Schroeder & Huisman (1970). It was concluded that the precision and accuracy is of the order of ± 0.05 residues of glycine. Many additional data since these reports verify this conclusion. If one were to compare two isolated and unrelated cases which had $^{G}\gamma:^{A}\gamma$ ratios of 60:40 and 70:30, there would be a reasonable doubt as to whether they were, in fact, different. However, when groups of related individuals with the same condition show such a difference, it is reasonable to conclude that ratios of 70:30 and 60:40 indeed represent subclasses.

This separate subgroup of the $Hb_{G\gamma}$ $Hb_{A\gamma}$ class among Indian HPFH heterozygotes has a $^{G}\gamma:^{A}\gamma$ ratio which is identical with that in more than 100 newborns (Schroeder *et al*, 1972). The most common $Hb_{G\gamma}$ $Hb_{A\gamma}$ class with a ratio of 40:60 has the same ratio as in Hb-F of the adult (Schroeder *et al*, 1971; Schroeder & Huisman, 1970). An explanation of the differences in these ratios may be forthcoming when uncertainties about the number of Hb_{γ} structural loci per chromosome are resolved (Huisman *et al*, 1972). At present, the differences cannot be satisfactorily explained. However, it appears that the mechanism which is responsible for the change in the $^{G}\gamma:^{A}\gamma$ ratio after birth from 70:30 to 40:60 (Schroeder *et al*, 1971), whatever this mechanism may be, is not active (at least on the chromosome with the HPFH determinant) in these Indian $Hb_{G\gamma}$ $Hb_{A\gamma}$ heterozygotes.

The $Hb_{G\gamma}$ $Hb_{A\gamma}$ heterozygotes of these four Indian families fit all the criteria that define the HPFH condition. In contrast, individuals with the $Hb_{G\gamma}$ type may have some characteristics of F-thalassaemia as previously discussed in detail (Sukumaran *et al*, 1972). Th cause of these differences is, at present, as undetermined as that of the variability of the $^{G}\gamma:^{A}\gamma$ ratio within the $Hb_{G\gamma}$ $Hb_{A\gamma}$ type.

Three children who have both HPFH and β-thalassaemia (Table I) are rather severely anaemic and their cells are hypochromic and microcytic. Their Hb-A_2 levels (mean 3.0%) are distinctly higher than those of the simple HPFH heterozygotes (mean 1.8%) and the amount of Hb-F is increased about threefold. In HPFH–β-thalassaemia cases among Negroes (Huisman *et al*, 1971) and Greeks (Huisman *et al*, 1970b), Hb-A_2 and Hb-F are likewise increased although among the Greeks the increase in Hb-F is less. The Negro cases show microcytosis but not anaemia or hypochromia, and the Greek cases are only mildly anaemic.

The mean value of 0.72 for glycine in the Hb-F of these three patients is essentially the same as that of the HPFH heterozygotes. Previous analyses of Hb-F of comparable Greek and Negro cases (Huisman *et al*, 1970b, 1971) have led to the conclusion that the excess amount of γ chain is synthesized by the chromosome which carries the β-thalassaemia determinant. If this be true also for the Indian HPFH–β-thalassaemia patients, one may conclude that the ratio of $^{G}\gamma:^{A}\gamma$ chains in the Hb-F from the β-thalassaemia chromosome is the same as that from the chromosome with the HPFH determinant. On the basis of the $^{G}\gamma:^{A}\gamma$ ratio of the Hb-F, β-thalassaemia may be classified into two groups (Schroeder *et al*, 1970a); in one, the $^{G}\gamma:^{A}\gamma$ ratio is 70:30 as in the newborn, and in the other it is about 40:60 as in the adult. Our data indicate that the β-thalassaemia in these Indian families belongs to the first group.

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REFERENCES

BETKE, K., MARTI, H. & SCHLICHT, I. (1959) Estimation of small percentages of foetal haemoglobin. *Nature*, **184,** 1877.

DOZY, A.M., KLEIHAUER, E.F. & HUISMAN, T.H.J. (1968) Studies on the heterogeneity of hemoglobin. XIII. Chromatography of various human and animal hemoglobin types on DEAE-Sephadex. *Journal of Chromatography*, **32,** 723.

EFREMOV, G.D., HUISMAN, T.H.J., SMITH, L.L., WILSON, J.B., KITCHENS, J.L., WRIGHTSTONE, R.N. & ADAMS, H.R. (1969) Hemoglobin Richmond a human hemoglobin which forms asymmetric hybrids with other hemoglobins. *Journal of Biological Chemistry*, **244,** 6105.

HUISMAN, T.H.J. (1972) Normal and abnormal hemoglobins. *Advances in Clinical Chemistry*, **15,** 150.

HUISMAN, T.H.J. & DOZY, A.M. (1965) Studies on the heterogeneity of hemoglobin. IX. The use of tris (hydroxymethyl)aminomethane-HCl buffers in the anion-exchange chromatography of hemoglobins. *Journal of Chromatography*, **19,** 160.

HUISMAN, T.H.J., SCHROEDER, W.A., ADAMS, H.R., SHELTON, J.R., SHELTON, J.B. & APELL, G. (1970a) A possible subclass of the hereditary persistence of fetal hemoglobin. *Blood*, **36,** 1.

HUISMAN, T.H.J., SCHROEDER, W.A., BANNISTER, W.H. & GRECH, J.L. (1972) Evidence of four nonallelic structural genes of the γ chain of human fetal hemoglobin. *Biochemical Genetics*, **7,** 131.

HUISMAN, T.H.J., SCHROEDER, W.A., CHARACHE, S., BETHLENFALVAY, N.C., BOUVER, N., SHELTON, J.R., SHELTON, J.B. & APELL, G. (1971) Hereditary persistence of fetal hemoglobin: heterogeneity of fetal hemoglobin in homozygotes and in conjunction with β-thalassemia. *New England Journal of Medicine*, **285,** 711.

HUISMAN, T.H.J., SCHROEDER, W.A., DOZY, A.M., SHELTON, J.R., SHELTON, J.B., BOYD, E.M. & APELL, G. (1969) Evidence for multiple structural genes for the gamma-chain of human fetal hemoglobin in hereditary persistence of fetal hemoglobin. *Annals of the New York Academy of Sciences*, **165,** 320.

HUISMAN, T.H.J., SCHROEDER, W.A., STAMATOYANNOPOULOS, G., BOUVER, N., SHELTON, J.R., SHELTON, J.B. & APELL, G. (1970b) Nature of fetal hemoglobin in the Greek type of hereditary persistence of fetal hemoglobin with and without concurrent β-thalassemia. *Journal of Clinical Investigation*, **49,** 1035.

KLEIHAUER, E. (1966) *Fetales Hämoglobin und Fetale Erythrozyten*. Ferdinand Enke, Stuttgart.

SCHROEDER, W.A. & HUISMAN, T.H.J. (1970) Investigations of molecular variation in human fetal hemoglobin in the infant and in certain hematological conditions in the adult. *Protides of the Biological Fluids, 17th Colloquium* 1969, p 249. Pergamon Press, Oxford.

SCHROEDER, W.A., HUISMAN, T.H.J., BROWN, A.K., UY, R., BOUVER, N.G., LERCH, P.O., SHELTON, J.R., SHELTON, J.B. & APELL, G. (1971) Postnatal changes in the chemical heterogeneity of human fetal hemoglobin. *Pediatric Research*, **5,** 493.

SCHROEDER, W.A., HUISMAN, T.H.J., SHELTON, J.R., SHELTON, J.B., APELL, G. & BOUVER, N. (1970a) Heterogeneity of fetal hemoglobin in β-thalassemia in the Negro. *American Journal of Human Genetics*, **22,** 505.

SCHROEDER, W.A., HUISMAN, T.H.J., SHELTON, J.R., SHELTON, J.B., KLEIHAUER, E.F., DOZY, A.M. & ROBBERSON, B. (1968) Evidence for multiple structural genes for the γ chain of human fetal hemoglobin. *Proceedings of the National Academy of Sciences of the United States of America*, **60,** 537.

SCHROEDER, W.A., HUISMAN, T.H.J., SHELTON, J.R. & WILSON, J.B. (1970b) An improved method for the quantitative determination of human fetal hemoglobin. *Analytical Biochemistry*, **35,** 235.

SCHROEDER, W.A., SHELTON, J.R., SHELTON, J.B., APELL, G., HUISMAN, T.H.J. & BOUVER, N. (1972) Worldwide occurrence of nonallelic genes for the γ chain of human foetal haemoglobin in newborns. *Nature*, **240,** 273.

SUKUMARAN, P.K., HUISMAN, T.H.J., SCHROEDER, W.A., MCCURDY, P.R., FREEHAFER, J.T., BOUVER, N., SHELTON, J.R., SHELTON, J.B. & APELL, G. (1972) A homozygote for the $Hb_{G\gamma}$ type of foetal haemoglobin in India: a study of two Indian and four Negro families. *British Journal of Haematology*, **23,** 403.

WINTROBE, M.M. (1962) *Clinical Hematology*, 5th edn. Lea & Febiger, Philadelphia.