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The Interaction of α-Thalassaemia and Haemoglobin G Philadelphia

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SUMMARY. An American Negro woman was found to have HbH disease in association with HbG Philadelphia (α68-asn→lys). Starch gel electrophoresis failed to reveal the presence of any HbA or HbA_2 and studies of globin chain synthesis indicated absence of α^A production. The α^G/β synthesis ratio was 0.63. The woman's son and her two half-sibs had α-thalassaemia trait with no HbH and α/β synthesis ratios of 0.84, 0.84 and 0.76. The data indicate that there is no functioning α^A gene linked to the α^G gene. The absence of α^A synthesis by the propositus also indicates that the α-thalassaemia gene trans to the α^G gene completely suppresses α chain production, the first evidence for such a gene in Negroes.

The α-thalassaemias are a group of disorders of varying clinical severity characterized by different degrees of decreased synthesis of the α polypeptide chain of human haemoglobin (Wasi *et al*, 1974). The most severe form of the disease, seen primarily in Asians, results in still-birth with hydrops fetalis and a haemoglobin composition almost exclusively Hb Bart's, a γ^4 tetramer (Luan Eng *et al*, 1962; Hunt & Lehmann, 1959). A milder disorder, HbH disease, is characterized by slight to moderate haemolytic anaemia and the presence of 5–30% of HbH, a $\beta 4$ tetramer (Rigas *et al*, 1956; Jones & Schroeder, 1963). α-Thalassaemia trait is usually associated with no clinical manifestations except for erythrocyte hypochromia (Wasi *et al*, 1974).

The genetic bases for these syndromes have not been conclusively determined. In one scheme that has been proposed, the Hb Bart's–hydrops fetalis disorder results from homozygosity for a gene (α-$thal_1$) which causes complete suppression of α globin synthesis (Pootrakul *et al*, 1967). HbH disease would be due to heterozygosity for α-$thal_1$ and a gene (α-$thal_2$) which directs a partially depressed level of α chain production (Wasi *et al*, 1964). Simple heterozygosity for α-$thal_1$ would result in α-thalassaemia trait. There is evidence that in some human populations (Hollán *et al*, 1972), but not all (Abramson *et al*, 1970), α globin synthesis is directed by two linked structural loci. Accordingly, it has been postulated that classic α-thalassaemia trait, HbH disease, and the hydrops fetalis syndrome, respectively, result from the presence of a total of two, three or four equivalent thalassaemia genes at the 4α structural sites on the chromosome pair (Lehmann, 1970).

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Although the frequent occurrence of small amounts of Hb Bart's in the neonatal period suggests a high incidence of the gene for α-thalassaemia in Negroes (Weatherall, 1963; Folayan Esan, 1970), HbH disease is considered rare in that race and the Hb Bart's–hydrops fetalis syndrome has never been reported. As a result it has been postulated that the α-thalassaemia genes in Negroes differ from those in Asians and do not totally suppress α chain synthesis (Stamatoyannoupoulos, 1972). A haematological study and an investigation of haemoglobin synthesis in an American Negro woman with HbG Philadelphia (α68 asn→lys)–HbH disease, and in members of her family, has provided additional information on the genetics of α-thalassaemia.

METHODS

Haematological methods. Haematological studies were carried out using standard methods (Cartwright, 1968).

Haemoglobin analysis. Haemoglobin electrophoresis on starch gel at pH 8.5 and pH 7.0 was performed as described by Weatherall & Clegg (1972). The level of HbF was determined by the method of Betke *et al* (1959) and the level of HbA_2 by the method of Bernini (1969). HbH was quantitated by photometric scanning of cellulose acetate electrophoretic strips (Helena Laboratories, Beaumont, Texas). The separation of globin chains on carboxymethylcellulose columns in 8 M urea and fingerprint analysis were performed as previously described (Clegg *et al*, 1966).

Haemoglobin synthesis. Globin chain biosynthesis was studied by *in vitro* incubation of peripheral blood for 30 min in the presence of [^{3}H]leucine (Rieder, 1971). Globin was prepared immediately from the entire haemolysate including membranes. After separation of the chains by carboxymethylcellulose chromatography (Clegg *et al*, 1966), incorporated radioactivity was measured by liquid scintillation counting.

RESULTS

The propositus (II-4, Table I) is 28 years old and healthy but has known of mild anaemia and abnormal haemoglobin for several years. Her peripheral blood film is typical of HbH disease with marked anisocytosis, poikilocytosis, microcytosis and hypochromia (Table I). Incubation of her erythrocytes with new methylene blue resulted in the formation of multiple inclusions (HbH bodies) in almost all cells (Rigas *et al*, 1956; Cartwright, 1968). Starch gel electrophoresis at pH 8.6 (Weatherall & Clegg, 1972) revealed haemoglobin bands in the position of HbH, HbG Philadelphia (Weatherall *et al*, 1962) and $HbG_2(\alpha_2{}^G\delta_2)$ (Fig 1). No HbA or HbA_2 was evident. The level of HbH was 3.5–7.1% when measured by cellulose acetate electrophoresis. Starch gel electrophoresis at pH 7.0 (Weatherall & Clegg, 1972) and fingerprint and amino acid analyses confirmed the presence of HbH and HbG Philadelphia (Baglioni & Ingram, 1961).

The son of the propositus (III-1, Table I) is mildly anaemic (PCV 0.352) with hypochromic, microcytic red blood cells (MCH 18.8 pg, MCV 63 fl). The peripheral blood film was characteristic of thalassaemia trait. A rare cell with inclusion bodies was noted after incubation with new methylene blue. Starch gel electrophoresis at pH 8.6 revealed only HbA and HbA_2. The level of HbA_2 was normal (2.7%) when measured by DEAE-cellulose chromatography

TABLE I. Haematological findings in family members

Subject	*PCV*	*Hb* (*g/dl*)	*RBC* (10^{12}/*l.*)	*MCV* (*fl*)	*MCH* (*pg*)	*MCHC* (*g/dl*)	*HbH* (%)	*HbA*$_2$ (%)	*HbF* (%)	*Synthesis ratio* (*αcpm/βcpm*)
II-4 (propositus)	0.375	10.6	6.18	60.7	17.3	28.3	3.5–8.1	1.4*	4.5†	0.63
III-1 (son)	0.352	10.6	5.6	63.0	18.8	29.9	—	1.9	0.2	0.84
II-1 (half-sister)	0.412	12.7	6.16	67	20.6	30.8	—	1.4	1.3	0.82
II-2 (half-brother)	0.385	11.9	6.0	66	20.4	30.7	—	1.3	0.5	0.76
I-1 (mother)	0.444	12.8	6.1	72	21.0	28.8	—	2.3	0.5	—

* $HbG_2(\alpha_2{}^G\delta_2)$.
† Presumably HbG/F $(\alpha_2{}^G\gamma_2)$.

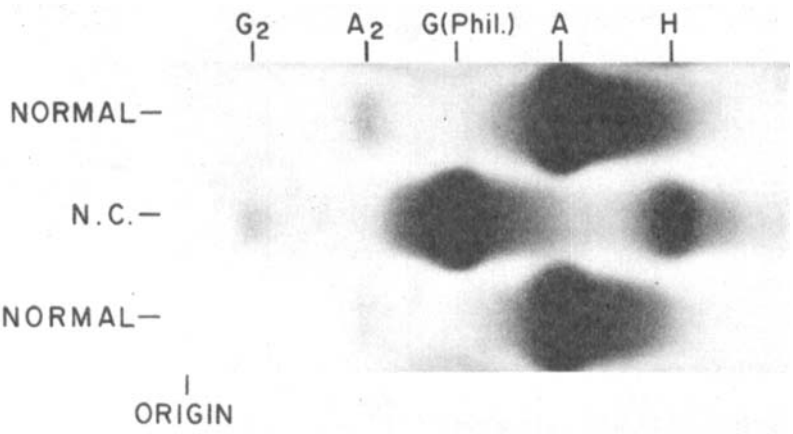

FIG 1. Starch gel electrophoresis in Tris–EDTA–borate buffer, pH 8.6, of haemolysates from normal individual (normal) and propositus (N.C.) with HbG Philadelphia–HbH disease, stained with Amido Black. The anode is towards the right.

(Bernini, 1969). No HbH was demonstrated by haemoglobin electrophoresis on starch gel at pH 7.0. Haematological examination of the mother (I-1, Table I), a half-sister and a half-brother (II-1, II-2, Table I) of the propositus gave results similar to those found in the son. All these subjects had evidence of α-thalassaemia trait with very slight anaemia, moderate anisocytosis, poikilocytosis, microcytosis and hypochromia with normal levels of HbA_2 and HbF (Table I).

Haemoglobin synthesis was studied *in vitro* in the peripheral blood of the propositus and her relatives. Fig 2 shows the column chromatographic separation of radioactive β and α^G globin from the blood of the propositus. There was deficient synthesis of α^G globin relative

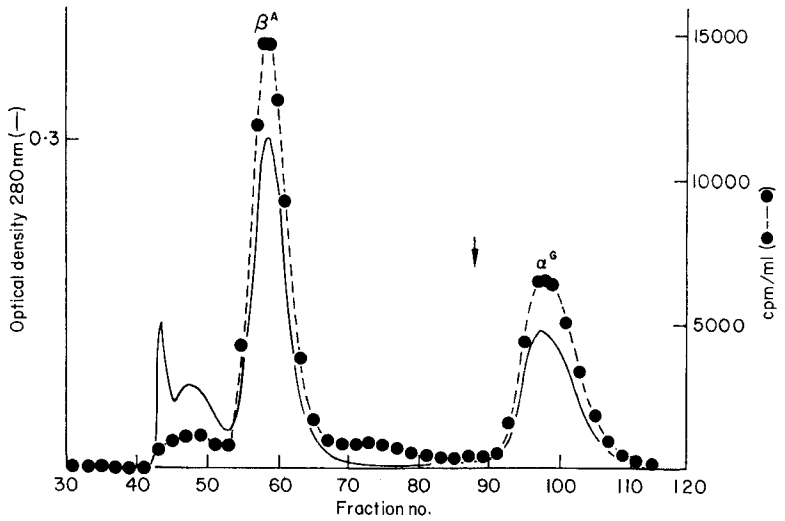

FIG 2. Carboxymethyl cellulose chromatography of radioactive globin prepared from the blood of the subject with HbG Philadelphia–HbH disease. The arrow indicates the position expected for α^A. The α^G/β synthesis ratio is 0.61.

to β globin; in two experiments the α^G/β synthesis ratios were 0.66 and 0.63 (Table I). These results are similar to the α/β synthesis ratios previously reported in studies of haemoglobin synthesis in Negroes with HbH disease (Schwartz & Atwater, 1972) but are somewhat higher than reported in other racial groups (Kan *et al*, 1968). There was no evidence of any α^A production by the erythrocytes of the propositus (Fig 2).

Globin chain synthesis was also unbalanced in the blood of the son (III-1). The α/β synthesis ratio was 0.84 (Table I). Similar results were obtained when blood specimens from the half-sister (α/β = 0.82) and half-brother (α/β = 0.76) of the propositus were incubated (Table I). The results are similar to the synthetic ratios reported in α-thalassaemia trait.

DISCUSSION

The haematological and biosynthetic studies indicate that this propositus with HbH disease is heterozygous for α-thalassaemia and the structural mutant, α^G Philadelphia. It seems certain that the son inherited α-thalassaemia trait from his mother and that she received it from her mother. The other two relatives of the propositus also appear to be carriers of α-thalassaemia trait. The failure to demonstrate HbA, or any synthesis of α^A, in the blood of the propositus indicates that the α-thalassaemia gene situated trans to α^G, is of the type that results in complete suppression of α globin production. This is the first demonstration of such a gene in the Negro race. In addition, the absence of HbA indicates that there is also no functioning α^A gene linked on the chromosome bearing the α^G Philadelphia gene in the propositus. Because some heterozygotes for HbG Philadelphia (Rucknagel & Dublin, 1974) possess more abnormal haemoglobin (40%) in their erythrocytes than is usual for an α chain mutant (20–25%) it has been suggested that the α^G Philadelphia gene is located on a chromosome that contains only one Hb_α locus or is frequently linked to an α-thalassaemia gene (French & Lehmann, 1971). Either hypothesis would explain why the propositus manifested HbH disease while her son who inherited the opposite chromosome and its α-thalassaemia gene had only α-thalassaemia trait. The biosynthetic studies also are suggestive of greater α/β imbalance in the mother than the son.

The haemoglobinopathy manifested by the propositus is thus similar to the syndrome in Asian subjects with HbH disease due to heterozygosity for HbQ and α-thalassaemia (Luan Eng *et al*, 1966). No HbA is found in that disorder either. Apparently α^Q is also linked to an α-thalassaemia gene. In contrast, a Negro woman heterozygous for α-thalassaemia and the α mutant HbI, did not have HbH and demonstrated 30% HbA (Atwater *et al*, 1960). Her children inherited α-thalassaemia trait, exhibiting 10% Hb Bart's in the neonatal period (Atwater *et al*, 1960). Possibly there is a functioning α^A gene linked to α^I.

Studies of DNA–DNA hybridization suggest that in the hydrops fetalis–Hb Bart's syndrome there is deletion of α globin genetic material (Ottolenghi *et al*, 1974; Taylor *et al*, 1974). The simplest explanation for the present observations would be that normally two α genes are linked per chromosome. HbH disease occurs when an individual inherits one chromosome with both α genes deleted and one chromosome with one gene deleted. Recently published DNA hybridization data support this possibility (Kan *et al*, 1975). Both the α^Q and α^G Philadelphia genes seem to occur on chromosomes without a second functioning α globin gene. Whether a single chromosome (Rucknagel & Dublin, 1974) is synonymous

with classic α-thalassaemia remains to be seen. In any event when the chromosomes bearing these mutant α genes are paired with a chromosome having both α genes deleted, HbG Philadelphia–HbH disease or HbQ–HbH disease results.

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