Observations on the high foetal haemoglobin gene and its interaction with the thalassaemia gene

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SYNOPSIS A family of mixed Indian-Portuguese ancestry is reported in which there is a hereditary persistence of foetal haemoglobin and β -chain thalassaemia.

The proband, a 17-year-old boy, was found to have a mild haemolytic anaemia characterized by slight splenomegaly, microcytosis, numerous target cells, decreased osmotic fragility, a very high level of foetal haemoglobin (75%), and normal haemoglobin A_2 level.

Examination of 12 other members of the family showed the following: Three individuals (father, sister, and nephew) had high levels of foetal haemoglobin (25%) but without other clinical or haematological abnormalities. Two individuals (mother and sister) had the features of thalassaemia trait with increased haemoglobin A_2 and normal levels of foetal haemoglobin.

The condition in the proband appears to be the result of heterozygosity for a gene which is responsible for the hereditary persistence of foetal haemoglobin (high F gene) combined with heterozygosity for a β -thalassaemia gene and provides further evidence for allelism of these genes.

The possible genetic basis for the high F state and β -chain thalassaemia is discussed.

Fifty to eighty per cent of the haemoglobin of newborn infants consists of foetal haemoglobin. This is gradually replaced by adult haemoglobin which constitutes the major fraction after the age of 3 months. By the end of the second year of life normal children possess less than 1% of alkali-resistant haemoglobin (White and Beaven, 1959).

It now appears that foetal haemoglobin may persist in some families in the absence of any clinical or other haematological abnormalities (Edington and Lehmann, 1955; Jacob and Raper, 1958; Went and MacIver, 1958; Herman and Conley, 1960; Kraus, Koch, and Burckett, 1961; Thompson, Mitchener, and Huisman, 1961). However, it remains uncertain whether this is the consequence of a primary defect in the genetic mechanism responsible for the switchover from foetal haemoglobin to the production of adult haemoglobin or whether it represents a compensatory response to some undetectable abnormality in the synthesis of adult haemoglobin. For convenience, the term 'high foetal' or 'F gene' (Went and MacIver, 1958) will be used in referring to this condition. The quoted studies suggest allelism of the F gene to the S and C haemoglobin

genes. Family II of Kraus *et al.* (1961) and the patients reported by Wheeler and Krevans (1961) suggest allelism also with the gene of classical thalassaemia; the possibility of an interaction of this kind was suggested by Oleson, Oleson, Livingstone, Cohen, Zuelzer, Robinson, and Neel (1959) in their study of atypical thalassaemia cases in Liberia.

In this paper we report the hereditary persistence of foetal haemoglobin in three generations of a family of mixed Indian and Portuguese descent; as far as we know this appears to be the first account of this condition in a non-Negro family. One of the members has a mild haemolytic anaemia which is believed to result from the simultaneous presence of a thalassaemia gene derived from his mother with an F haemoglobin gene derived from his father, and this lends further support for the possibility of allelism between the F gene and the classical thalassaemia gene.

MATERIALS AND METHODS

Haemoglobin, packed cell volume, red cell count, and red cell osmotic fragility were determined by standard methods (Dacie, 1956). Haemolysates were prepared and foetal haemoglobin (Singer, one-minute method) was determined as described by Lehmann and Ager (1961).

ELECTROPHORESIS OF HAEMOGLOBIN (a) On cellulose acetate paper (Oxoid) at room temperature using veronal buffer, pH 8.6, ionic strength 0.05 (duration of run approximately four hours); the strips were examined in the unstained state.

(b) On starch gel with a tris-borate discontinuous buffer system (Poulik, 1957); hydrolysed starch (Connaught Laboratories, Toronto) was used for making the gel.

(c) On a starch block at 4°C. for the determination of haemoglobin A₂, essentially as described by Kunkel, Ceppellini, Müller-Eberhard, and Wolf (1957); the Dutch potato starch (Mulder) supplied by Griffin and George, London, was found to be satisfactory for this purpose. The maize starch supplied by British Drug Houses, Poole, Dorset, was equally satisfactory.

Red cells containing foetal haemoglobin were demonstrated in peripheral blood films by the acid elution method of Kleihauer, Braun, and Betke (1957) as modified by Zipursky, Hull, White, and Israels (1959).

FAMILY STUDIES

THE PROPOSITUS This boy (Fig. 1, II.9) aged 17 years, an apprentice decorator, attended St. Mary's Hospital with the complaint of intermittent attacks of pain in the left hypochondrium which occurred, apparently spontaneously, at intervals of a few weeks. The pain was stabbing in character, remained localized to the left hypochondrium, and was sometimes associated with nausea. Apart from the pain he felt well. On physical examination he was of normal development for a boy of his age. The only abnormal physical finding was an enlarged spleen which was palpable about two finger-breadths below the left costal margin on inspiration; it was firm and somewhat tender.

The blood count was as follows: Haemoglobin 11.6 g./100 ml., red cells 5.8 m./c.mm., P.C.V. 36%, M.C.H. 20 µg., M.C.V. 66 c.µ., reticulocytes 5% (Table I). The film showed anisocytosis and numerous target cells. The leucocyte and platelet counts were within normal limits. Serum bilirubin was 0.5 mg./100 ml. and serum iron 102 µg./100 ml. Electrophoresis of the haemoglobin on cellulose-acetate paper showed a single component with a mobility between that of haemoglobin A and haemoglobin S. The pattern suggested the presence of an increased amount of foetal haemoglobin; this was confirmed by the alkali denaturation test which showed 75% alkali-resistant haemoglobin.

FIG. 1. The family tree. The shaded symbols indicate those individuals with high foetal haemoglobin, the degree of shading representing approximately the amount of foetal haemoglobin. n.t. = not tested T = thalassaemia trait.

The haematological examination was repeated several times during the succeeding six months and showed a persistent mild anaemia with reticulocytosis, numerous target cells, increased foetal haemoglobin, and a normal serum iron concentration; the red cell osmotic fragility was markedly decreased (Fig. 2). The proportion of A₂ haemoglobin was within normal limits (Table I). Nucleated red cells were not seen in the peripheral blood films. At no time did he appear jaundiced. There was no evidence of blood loss.

Since the blood picture suggested a haemoglobinopathy, the other members of the family were examined.

THE FAMILY Thirteen members were available for study (Fig. 1). All were born in India, and came to England nine years ago. They look typically Indian. Both parents have Indian and Portuguese ancestry, the Portuguese element having been introduced from Goa a few generations earlier.

The parents were clinically well but had some abnormal blood findings (Table I). The father (I.1) had 23% alkali-resistant haemoglobin but no other abnormal haematological findings; the red cells appeared normocytic and normochromic and their osmotic fragility was within normal limits (Fig. 2). The film of the mother's

TABLE I
HAEMATOLOGICAL FINDINGS IN AFFECTED MEMBERS OF THE FAMILY

Pedigree	Sex	Age (yr.)	Hb (g./100 ml.)	P.C.V. (%)	Red Cells (m./c. mm.)	M.C.H.C. (%)	M.C.H. (µµg.)	M.C.V. (c.µ.)	Reticu-locytes (%)	Foetal Hb (%)	A ₂ Hb (%)	Target Cells	Osmotic Fragility	Serum Iron (µg./100 ml.)
I.1	M	59	15.5	48	5.4	32	28	94	1.5	25	1.3	Nil	Normal	76
I.2	F	54	13.6	44	5.7	31	24	77	2.0	<1	4.5	Some	Decreased	150
II.4	F	29	11.9	37	4.1	32	28	90	1.2	28	1.5	Nil	Normal	82
II.6	F	26	8.8	28	3.0	31	29	93	2.5	<1	4.5	Nil	Not done	Not done
II.9	M	17	11.2	36	5.7	31	20	63	5.8	75	2.2	Many	Decreased	102
III.3	M	12	11.8	35	4.1	33	29	85	2.0	25	1.6	Nil	Normal	100

FIG. 2. Red cell osmotic fragility curves from I.1, I.2, II.9, and III.3. The area enclosed by the interrupted lines is the normal range.

(I.2) blood showed an increased number of target cells; the M.C.H.C. was only slightly reduced, but the M.C.H. was low; the osmotic fragility was decreased (Fig. 2); the serum iron and bilirubin concentrations were normal; the haemoglobin A₂ fraction was increased to 4.5% (Table I). The A₂ component was easily visible after electrophoresis of the mother's haemolysate on cellulose acetate paper.

An older sister (II.4) and one of her sons (III.3) were found to have increased amounts of foetal haemoglobin (25%) but their blood pictures appeared to be otherwise normal (Table I); the osmotic fragility of the blood of III.3 was within normal limits. Another sister (II.6) who was about 36 weeks pregnant at the time of examination had a moderate anaemia. Some target cells were present. Foetal haemoglobin was within normal limits. However, since she showed a haemoglobin A₂ value of 4.5% (Table I) which is definitely raised, she was presumed to have the thalassaemia trait. The blood films of the remaining members of the family appeared normal and their haematological values were within normal limits. Two brothers of the propositus were in India and were not studied. They were said to be well.

STARCH GEL AND STARCH BLOCK ELECTROPHORESIS PATTERNS The haemoglobin patterns, on starch gel, of the parents (I.1 and I.2), the propositus (II.9), his sister II.4, and his nephew (III.3) were as follows:—

The major haemoglobin band of the propositus moved somewhat more slowly than that of the other members of the family and appeared to have a similar mobility to that of cord blood but unlike cord blood it showed distinct A₂ and protein X components. Starch block electrophoresis of the haemolysates from II.9 and III.3 showed a distinct separation between the haemoglobin A and F components, the latter constituting in the case of II.9 the major component (Fig. 3).

CELLULAR DISTRIBUTION OF FOETAL HAEMOGLOBIN Examination of the treated films showed that all the red cells contained some foetal haemoglobin. In the case of the propositus there appeared to be a fairly uniform distribution (Fig. 4b); his sister (II.4) and his nephew (III.3) showed a similar distribution. The father's cells, however, varied in their foetal haemoglobin content; all appeared positive but there was a gradation from cells which stained relatively palely to those which were deeply stained (Fig. 4c). The red cells of the mother reacted like normal cells but many showed small inclusion bodies whose nature is unknown (Fig. 4d).

INTERPRETATION OF THE HAEMATOLOGICAL FINDINGS

THE PROPOSITUS The clinical and haematological findings are suggestive of thalassaemia minor but the

FIG. 3. Starch block electrophoretic patterns of haemolysates from III.3 and II.9 showing the separation of haemoglobins A, F, and A₂.
O = origin.

very high percentage of foetal haemoglobin appears to exclude this diagnosis. Thus, Beaven, Ellis, and White (1961), in a study of blood samples from 131 cases of thalassaemia minor, found that the percentage of foetal haemoglobin never exceeded 8 and was within normal limits in two-thirds of the cases. Very high levels of foetal haemoglobin are found in thalassaemia major but the clinical and haematological findings considered together make it most unlikely that the *propositus* has thalassaemia major.

THE PARENTS Apart from the large amount of foetal haemoglobin, the father is haematologically normal. This picture conforms with those cases reported as having a hereditary persistence of foetal haemoglobin. The mother has the haematological features of classical thalassaemia trait.

The most likely interpretation of the findings in the *propositus* is that he has acquired a thalassaemia gene from his mother and a 'high foetal' haemoglobin gene from his father. He is therefore carrying two abnormal genes which have interacted to produce a mild haemolytic anaemia.

The other two members (II.4 and III.3) with high foetal haemoglobins presumably possess a 'high foetal' gene and a normal gene.

DISCUSSION

The hereditary persistence of foetal haemoglobin has been described only in Negro families, and this report is the first account of this condition in a non-

Negro family, and the third report of the apparent interaction with the gene of classical thalassaemia.

The persistent alkali-resistant haemoglobin appears to be identical with the foetal haemoglobin of cord blood. Bradley, Brawner, and Conley (1961) found it indistinguishable from foetal haemoglobin by electrophoresis in various buffers and by elution from chromatography columns. Like foetal haemoglobin it could be separated from adult haemoglobin by electrophoresis on agar in a citrate buffer at pH 6.0 where it showed the same mobility as the major component from cord blood. The kinetics of its denaturation by alkali and its absorption curve in the ultra-violet spectrum were similar to those of foetal haemoglobin. The amino-acid sequence of the globin moiety appears to be similar to that of foetal haemoglobin (Thompson *et al.*, 1961).

A further similarity with foetal haemoglobin is in the acid elution test; the red cells from the 'high foetal' individuals were found to behave like those from normal cord blood. It would, therefore, seem that all the red cells contain foetal haemoglobin in greater or lesser degree. This makes it seem unlikely that two different clones are involved, the one producing cells with only adult haemoglobin and the other producing cells with only foetal haemoglobin. The *propositus* has therefore inherited two genes, each of which presumably impairs or suppresses the formation of haemoglobin A and allows its replacement with foetal haemoglobin. It is interesting that he has approximately three times as much foetal haemoglobin as the AF individuals, a finding similar to that reported by Kraus *et al.* (1961) and Wheeler and Krevans (1961).

That the basis for thalassaemia may be a structurally abnormal but electrophoretically normal haemoglobin synthesized at a subnormal rate was suggested by Pauling (1955) and Itano (1957). Ingram and Stretton (1959) then proposed that thalassaemia is a mutation either of an α or of a β haemoglobin gene; they suggested that the gene of classical thalassaemia which characteristically is associated with a raised A₂ value was at the Hb β^A locus. On this hypothesis the mother of the *propositus* has a β -chain thalassaemia. Direct demonstration of a molecular alteration involving an amino-acid substitution is still awaited. It is possible, however, that the various thalassaemic genes only suppress the synthesis of α or β chains without leading to the formation of an abnormal haemoglobin. This block may operate at different points in the synthetic pathway depending on the particular gene involved and may secondarily affect the synthesis of γ chains (foetal haemoglobin) accordingly. It is generally assumed that there is a reduced rate of synthesis of

FIG. 4a

FIG. 4b

FIG. 4c

FIG. 4d

FIG. 4. *Appearance of red cells after acid elution (phase-contrast microscopy). Cells containing foetal haemoglobin stand out darkly. Normal adult cells appear as ghosts. (a) Mixture of cord and adult blood, (b) I.1.9, (c) I.1.1, (d) I.1.2.*

adult haemoglobin in classical thalassaemia but it remains to be established whether this is due primarily to an abnormality of haem synthesis or an abnormality of globin synthesis, or of both, or whether a failure in one determines the abnormal production of the other.

On the basis of the hypothesis and haemoglobin symbols proposed by Ingram and Stretton (1959) we may write the genotype of the affected members of our family as follows: The father $\text{Hb}_{\alpha}^{\text{A}}/\text{Hb}_{\alpha}^{\text{A}}$, $\text{Hb}_{\beta}^{\text{A}}/\text{Hb}_{\beta}^{\text{x}}$; the mother $\text{Hb}_{\alpha}^{\text{A}}/\text{Hb}_{\alpha}^{\text{A}}$, $\text{Hb}_{\beta}^{\text{A}}/\text{Hb}_{\beta}^{\text{Th}}$; the propositus, $\text{Hb}_{\alpha}^{\text{A}}/\text{Hb}_{\alpha}^{\text{A}}$, $\text{Hb}_{\beta}^{\text{x}}/\text{Hb}_{\beta}^{\text{Th}}$, where x is the gene responsible for the persistence of foetal haemoglobin and Th the gene for classical thalassaemia. The genotypes of the other two members (II.4 and III.3) with just the 'high F' gene would be the same as that of the father. If these genotypes are correct it follows that the haemoglobin A of the propositus is abnormal since both genes for the $\text{Hb}_{\beta}^{\text{A}}$ locus are abnormal. The fact that his haemoglobin A component appears normal electrophoretically is still consistent with this interpretation since the molecular abnormality may be electrophoretically 'silent'. A detailed study of the amino-acid sequence of his haemoglobin A might give further helpful information.

The basic assumption of these interpretations is that the 'high F' gene and the thalassaemia gene are allelic but we have no conclusive proof of this since the propositus has not yet produced any children. A further assumption is that the persistence of foetal haemoglobin is a consequence of a genetic defect in the production of the β chains of A haemoglobin and not due to a primary activity of a specific 'high F' structural gene; this abnormality in some way prevents the switchover from γ chain (of F haemoglobin) production to normal β chain production. Wheeler and Krevans (1961) have reported that a child apparently homozygous for the high F gene possessed no detectable A or A_2 haemoglobin and they consider that this absence of both β and δ chains favours the concept of the high F gene as resulting from mutation at a controller locus. The findings in this child have led Neel (1961) to a similar concept. The terms 'controller', 'regulator', or 'operator' gene were suggested by Jacob and Monod (1961) and would appear to be similar to the 'tap' genes of Freese (1958). Ingram and Stretton (1959) considered the hypothesis of 'tap' gene mutations as an alternative to the 'substitution' hypothesis but felt the latter to be more tenable. As yet, there is no reported evidence for the substitution hypothesis despite what

must be, according to Neel (1961), a brisk search in several laboratories, and he feels that this lends indirect support to the 'tap' hypothesis. Motulsky (1961) has put forward somewhat similar views. He postulates that the high haemoglobin F condition represents a mutation of a switching gene which exerts reciprocal control over the formation of γ and β chains. During foetal life this gene normally suppresses β chain formation. After birth, the identical gene suppresses γ chain formation but allows β chain production. A mutation of the switching gene (high F gene) causes failure of the normal switchover from γ to β chains. He considered that the normal to low haemoglobin A_2 levels in the high F syndrome and the low haemoglobin A_2 levels in normal neonatal bloods suggests that the switching gene also controls the δ locus. This mutant switching gene and the β -thalassaemia gene are presumably linked or allelic to the normal β chain locus.

If the regulator or switchover gene concept is the correct one, then there is no need to postulate a β chain mutation as the basis of the high F syndrome. The genetic apparatus for producing normal β chains must then be presumed to be present but functionally suppressed by the mutant regulator gene and no abnormal haemoglobin would be expected. However, assuming the validity of this hypothesis, it is not clear why the interaction of the mutant switchover gene and β -thalassaemia gene should result in an even greater production of F haemoglobin since the heterozygous state of the latter, unlike the heterozygous state of the former, is usually not associated with increased levels of F haemoglobin. But, on the other hand, homozygous β thalassaemia (thalassaemia major) is usually associated with a greatly increased production of F haemoglobin which suggests a close relationship amongst these genes.

While the individuals with the 'high F' trait (I.1 and III.3) appear to have a normal red cell osmotic fragility, and the mother of the propositus has a moderately reduced osmotic fragility, the propositus has a marked reduction in osmotic fragility. It would seem that though the 'high F' gene by itself does not produce any abnormality in red cell osmotic fragility, its association with a β -thalassaemia gene enhances the osmotic resistance found with the thalassaemia gene alone. The explanation of these findings is unknown.

A further finding for which no adequate explanation can be given is the normal haemoglobin A_2 value in the propositus. Having acquired the gene of classical thalassaemia, which is characteristically associated with a high A_2 value, he might be expected to have had a raised A_2 component. However, many

patients with thalassaemia major (homozygous β -gene thalassaemia) also show normal A_2 values. Kraus *et al.* (1961) also observed a normal A_2 value in their high F-thalassaemia patient. They suggest that the A_2 value should be related only to the amount of A haemoglobin. If this is done then the A_2 percentage is markedly raised in these individuals.

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