VARIANTS OF HAEMOGLOBIN F AND OBSERVATIONS ON HAEMOGLOBIN F (MALTA)

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Introduction

The major haemoglobin component found in the blood of humans at birth is foetal haemoglobin, haemoglobin F. In common with most other human haemoglobins it has a tetrameric structure, each molecule being made up of two different pairs of polypeptide chains. In the case of haemoglobin F these are the α-chains and the γ-chains, and haemoglobin F thus has the molecular formula $\alpha_2 \gamma_2$. Whereas the α-chains are common to the major adult haemoglobin component, haemoglobin A, to the minor adult haemoglobin component, haemoglobin A_2, and to the embryonic haemoglobin Gower-II, the γ-chains are unique to haemoglobin F. At birth haemoglobin F accounts for 60-80% of the haemoglobin present in the blood. The other haemoglobins present at birth are haemoglobin A ($\alpha_2 \beta_2$) which accounts for 20-40% of the haemoglobin and a very small amount of haemoglobin A_2 ($\alpha_2 \delta_2$), less than 0.5%. As a child matures, the level of haemoglobin F in the blood decreases until, by the age of 3-6 months ,it is less than 5%. The majority of the rest of the haemoglobin is then haemoglobin A, but there is also an increased amount of haemoglobin A_2 (2-3%).

Because haemoglobin F consists of two types of polypeptide chains, two classes of haemoglobin F variants are possible, those possessing abnormal α-chains and those with abnormal γ-chains.

Alpha chain variants

Because the α-chains are common to both haemoglobins A and F, any child inheriting an α-chain mutation will have four main haemoglobin components present at birth, haemoglobin A ($\alpha_2\ \beta_2$), haemoglobin F ($\alpha_2\ \gamma_2$) and the corresponding variants, haemoglobins A^x ($\alpha_2{}^x\ \beta_2$) and F^x ($\alpha_2{}^x\ \gamma_2$). This has been observed, for example, in the case of an infant with the α-chain variant, haemoglobin G Philadelphia (Minnich *et al.*, 1962, and Weatherall and Baglioni, 1962). Both the foetal haemoglobins disappear as the baby matures and in the adult blood there will be present haemoglobin A, haemoglobin A^x, haemoglobin A_2, and the α-variant of haemoglobin $A_2{}^x$. Observations such as this have made it appear likely that a single gene controls α-chain synthesis both in foetal and in adult life.

Gamma chain variants

Relatively few γ-chain variants have been described so far and in those that have been found, the amino acid substitution appears to have little effect on the function of the haemoglobin molecule. Because there are so few γ-chain variants known it is possible to discuss each one separately. It should be noted, however, that not all of them have been fully characterized so that it is possible that two haemoglobins discussed under separate headings are in fact the same variant. Their electrophoretic mobilities have in general been expressed relative to haemoglobin A and its two common variants haemoglobin S and haemoglobin C. The order of increasing anodic mobility of these haemoglobins at alkaline pH is C,S,A. Normal foetal haemoglobin, haemoglobin F, has a slightly lower mobility than haemoglobin A.

(i) *Haemoglobin F-Texas I*

This variant was observed during a survey of cord blood carried out in Britain (Jenkins *et al.*, 1967). In electrophoresis at alkaline pH a haemoglobin component with a slower mobility than haemoglobin C was found in a healthy, full-term female child of West Indian parentage. The variant was absent in the parents and had the typical foetal haemoglobin properties of resistance to denaturation by alkali and the presence of tryptophan "notch" in the absorption spectrum at 289-290 nm. The amount of this haemoglobin diminished in the first few months of life and peptide patterns of tryptic digests showed that the substitution was lysine for glutamic acid in position 5 of the γ-chain. Only one case of this variant was found.

(ii) *Haemoglobin F-Texas II*

This variant, which may be the same as haemoglobin F-Texas I, was found in the United States of America in the cord blood of five Negro infants (three of them siblings) and one Caucasian infant (Schneider *et al.*, 1964; Schneider and Jones, 1965). All the babies were born at term and appeared healthy, the abnormal haemoglobin making up 12% or less of the total haemoglobin. The total foetal haemoglobin values as determined by alkali denaturation were in the range 68-82%. At six months the variant was barely detectable in the infants. In the initial reports haemoglobin F-Texas II was not observed in the parents but later (Schneider *et al.*, 1966) it was found that a trace component with the electrophoretic mobility of haemoglobin F-Texas II was in fact present in the blood of the father of the three sibs. In electrophoresis in an alkaline buffer, haemoglobin F-Texas II had a lower anodic mobility than haemoglobin C. It behaved in the same way as normal haemoglobin F in immunodiffusion but hybridisation studies did indicate that an alteration in structure had occurred in the γ-chain. This alteration did not affect the characteristic tryptophan fine structure band in the spectrum at 289-290 nm. Peptide analyses by column chromatography of tryptic hydrolysates of the variant were carried out by Schneider and Jones (1965), and the results indicated that one of the glutamic acid residues at positions 5 and 6 of the γ-chain had been substituted by lysine.

(iii) *Haemoglobin F-Roma*

One case of this variant has been observed in a healthy, female child born to parents in Rome (Silvestroni and Bianco, 1963). This haemoglobin which had an electrophoretic mobility at alkaline pH greater than that of haemoglobin F was resistant to denaturation by alkali. However, the tryptophan absorption at 289-290 nm was apparently very slightly increased as compared with that of haemoglobin F. Not enough material was available to work out the substitution but it was differentiated from haemoglobin Bart's (γ_4) which has a similar electrophoretic mobility at alkaline pH, and hybridisation experiments showed that the change had taken place in the γ-chains. Silvestroni and Bianco (1963) were also able to measure the relative percentages of haemoglobin F Roma and the total foetal haemoglobin (determined by alkali denaturation) as the child matures *(Table 1)*.

TABLE I

Relative rates of disappearance of haemoglobin F-Roma and total foetal haemoglobin (from Silvestroni and Bianco 1963)

	F-Roma (per cent of total Hb)	Total foetal Hb (per cent of total Hb)	F-Roma (per cent of total foetal Hb)
newborn	17.0	79.0	21.5
45 days	12.0	37.0	32.5
3 months	5.82	18.0	32.3
5 months	0	3.5	-

(iv) *Haemoglobin F-Warren (F-Houston)*

This variant was first found in the cord blood of a healthy Negro newborn by Huisman *et al.* (1965) who called it haemoglobin F-Warren. Electrophoretically, its mobility was between that of haemoglobins S and C at an alkaline pH. An apparently identical haemoglobin was reported by Schneider *et al.* (1966) who gave it the name of haemoglobin F-Houston. In both cases the haemoglobin amounted to 13-15% of the total haemoglobin at birth and had declined to low levels by four months. In their one infant Huisman *et al.* (1965) were able to measure the relative rates of disappearance of haemoglobins F-Warren and F and found that as the child matured the level of F-Warren fell more slowly than that of F *(Table II)*. No clinical or haematological abnormalities were apparent due to the presence of this variant which was antigenically and spectrally indistinguishable from haemoglobin F. Hybridisation showed that it was a γ-chain mutant. It is interesting that both

TABLE II

Relative rates of disappearance of haemoglobin F-Warren and total foetal haemoglobin (from Huisman et al., 1965)

	F-Warren (per cent of total Hb)	Total foetal Hb (per cent of total Hb)	F. Warren (per cent of total foetal Hb)
newborn	13.2	94	14.1
6 weeks	13.7	78	17.6
10 weeks	10.0	48	20.8
16 weeks	4.9	16	30.6

groups of workers reported relatives, in the case of Huisman *et al.* (1965), a brother and sister, in the case of Schneider *et al.* (1966), the father, with very small amounts of a band with electrophoretic mobility similar to the foetal haemoglobin variant. A change of glutamic acid to alanine has been suggested for this variant by Schneider *et al.* (1966), but purely on the basis of the relative amino acid compositions of haemoglobins F and F-Warren.

(v) *Haemoglobin F-Hull*

Three cases of this variant were found in two unrelated families in Kingston-upon-Hull, England (Sacker *et al.*, 1967). In one family an otherwise normal, healthy baby had 14% of the variant at birth. A second child born to the same parents had 9% at birth but was born prematurely and died. The third case, like the first, was a healthy baby with 7% of the variant. This

variant was not observed again during a survey of 12,000 cord bloods in Britain (Sacker *et al.*, 1967). It was not found in the parents and the amounts in the children declined during the first four months of life. Spectrally it showed a typical tryptophan "notch" at 289-290 nm. On paper electrophoresis at pH 8.9, its anodic mobility was less than that of haemoglobin C, suggesting again the change of an acidic to a basic amino acid. This was confirmed by the peptide maps of tryptic digests of the haemoglobin which showed that glutamic acid at position 121 of the γ-chain had been altered to lysine.

(vi) *Haemoglobin F-Alexandra*

This variant has not been well characterised so it may be the same as one of the others reported. One case was found by Fessas *et al.* (1959) in Greece, and an apparently similar component was reported by Vella *et al.* (1959) in Singapore. The variant of Fessas *et al.* (1959) had an electrophoretic mobility at alkaline pH between haemoglobins S and C but had the same mobility as haemoglobins S at pH 6.7. At birth the variant amounted to 18.3% of the total haemoglobin compared with a total alkali-resistant haemoglobin level of 60%. Over a period of fifteen weeks the amount of haemoglobin F-Alexandra declined from 18.3 to 2.2% and the total foetal haemoglobin from 60% to 6% (F-Alexandra was thus 30.5% of the total foetal haemoglobin at birth and 36.6% at the age of fifteen weeks). No clinical or haematological abnormality was observed in the child and the variant was absent in the mother. It showed typical foetal haemoglobin properties, the rate of alkali denaturation being the same as normal haemoglobin F and the ultraviolet spectrum showing a marked tryptophan "notch". The variant of Vella *et al.*, (1959) was found in a Chinese baby and amounted to 15% of the haemoglobin at birth. It had the same electrophoretic mobility and the same spectrum as the component of Fessas *et al.* (1959). Again both parents were normal.

(vii) *Haemoglobin F-Malta*

This is the most recent variant discovered and is of special interest to Malta as it is only here that it has been reported (Cauchi *et al.*, 1969). Haemoglobin F-Malta is unique among the foetal variants in that it has a very high incidence, being present at birth in the blood of one Maltese child in every fifty. Electrophoretically, it migrates more slowly than haemoglobin F but more rapidly than haemoglobin S at an alkaline pH and has the same mobility as haemoglobin F at pH 7.0. Haemoglobin F-Malta is also readily separated from normal haemoglobin F on isoelectric focussing in a pH gradient (Brown and Grech, unpublished observations), and with this technique has an isoelectric point at pH 7.44 as compared with 7.22 for normal haemoglobin F. These observations are in qualitative agreement with the amino acid substitution which has been shown to be a change of histidine to arginine at position 117 of the γ-chain (Cauchi *et al.*, 1969). The ultraviolet spectrum of haemoglobin F Malta is identical in the 289-290 nm region with that of haemoglobin F and the rates of denaturation of the two haemoglobins by alkali are very similar (Brown and Grech, unpublished observations).

The variant disappears as the baby matures, in common with other foetal haemoglobin variants, and has not been observed in any parents. Infants born with haemoglobin F-Malta are apparently healthy and have no other haematological anomalies.

Cauchi *et al.* (1969), in a quantitative examination of twelve cases reported that at birth the proportion of the abnormal component ranged from 14.8–22.5% of the total haemoglobin or 20.3–27.4% of the total foetal haemoglobin. To date we have found 47 cases of haemoglobin F-Malta and have quantitated the levels of total foetal haemoglobin, as measured by the alkali denaturation method of Jonxis and Visser (1956), and the amounts of haemoglobin F-Malta, as measured after cellulose acetate electrophoresis by the method of Marengo-Rowe, (1965). Our

values for haemoglobin F-Malta at birth range from 17.7–27.9% of the total haemoglobin or 27.6–37.1% of the total foetal haemoglobin. Thus our values are substantially higher than those of Cauchi *et al.* (1969).

We are studying the relative rates of disappearance of the haemoglobins F-Malta and F as the infants mature. Because of the high incidence of haemoglobin F-Malta it is possible to carry out a much more comprehensive study on the relative rates of disappearance than it is in the case of other foetal variants. Our results to date are shown in *Table III*. The post-natal samples were all obtained from different infants at varying periods after birth. For convenience the results have been grouped into ten day periods as shown in *Table III*. It seems quite clear that the amounts of haemoglobin F-Malta and haemoglobin F are declining at the same relative rate, at least in the first 3 months of life.

We believe observations such as these to be important. It has very recently become apparent that the genetics of γ-chain formation are much more complicated than was formerly thought. Thus Schroeder *et al.* (1968) have shown that at birth, normal human foetal haemoglobin consists of two components which cannot be separated by electrophoresis or chromatography but which differ in having either glycine or alanine in position 136 of the γ-chain. Quantitatively they found that the foetal haemoglobin of newly born infants had three times as many γ-chains with glycine at position 136 as with alanine at position 136. From this and other evidence it now appears likely that there are multiple non-allelic structural genes for the human γ-chain. Schroeder *et al.* (1968) drew up a genetic model in which there were four γ-chain loci on the relevant chromosome, three directing synthesis of γ-chains with glycine at position 136 and one directing synthesis of γ-chains with alanine at position 136. According to this model, γ-chain variants should have either alanine or glycin at position 136 of the aberrant γ-chain and they should amount to about 12.5% of the total foetal haemoglobin. Schroeder *et al.* (1968) in further cases of newborns with haemoglobin F-Texas II and haemoglobin

TABLE III

Relative rates of disappearance of haemoglobin F-Malta and haemoglobin F.

Days after Birth	No. of Observations	F-Malta (per cent of total Hb)	Total foetal Hb (per cent of total Hb)	F-Malta (per cent of total foetal Hb)
0	46	22.9 ± 2.7 (S.D.) (range 17.7 - 27.9)	70.2 ± 9.0 (S.D.) (range 51.5 - 89.5)	32.8 ± 2.3 (S.D.) (27.6 - 37.1)
1-10	-	-	-	-
11-20	3	18.6	56.3	33.7
21-30	1	15.4	42.5	36.2
31-40	2	18.1	51.8	34.9
41-50	4	14.0	46.9	29.6
51-60	2	11.5	33.8	34.0
61-70	2	11.3	40.2	29.7
71-80	1	6.3	19.5	32.3
101-110	1	2.4	9.2	26.1

F-Warren showed that the model predicted correctly the amounts of haemoglobin F-Texas II, which has only alanine in position 136, (12.2% of total foetal haemoglobin in one case) and haemoglobin F-Warren, which has only glycine at position 136, (13.5% and 12.8% of the total foetal haemoglobin in two cases). However, it is inadequate for explaining the quantitative relationships between haemoglobin F-Malta (glycine in position 136 of the γ-chain) and haemoglobin F and also between haemoglobin F-Roma and haemoglobin F (see *Table I* and *Table III*).

The situation has been further complicated by the observations of Schroeder and Huisman (1970) on the haemoglobin F of infants studied from birth to the age of several months. They have found that the ratio of γ-chains with glycine at position 136 to γ-chains with alanine at position 136 changes from 3 : 1 at birth to 2 : 3 after 150 days. The latter ratio was very close to the values obtained for the glycine: alanine ratios in haemoglobin F isolated from normal adults. However, the preliminary observations on the relative rates of decline of haemoglobin F-Warren and haemoglobin F *(Table II)* and of haemoglobin F-Malta and haemoglobin F *(Table III)* are not in accord with this change in ratio. Both variants have glycine at position 136 of the γ-chain and so might be expected to decline more rapidly than haemoglobin F. This does not happen. It is interesting too to mention that Schroeder and Huisman (1970) have found the condition of hereditary persistence of foetal haemoglobin to be very heterogeneous at the molecular level in that they could classify patients with this condition into groups whose haemoglobin F γ-chains contained either only glycine or only alanine, or both, at position 136. This grouping did not depend on the level of haemoglobin F in the blood. It is thus not possible at this stage to draw up a comprehensive genetic model to explain the inheritance and the control of formation of γ-chains, but these observations of Schroeder and Huisman (1970) do increase the importance of the study of foetal haemoglobin variants and their rates of disappearance for the fuller understanding of γ-chain genetics.

A note of caution must, however, enter into any discussion of the genetics of foetal haemoglobin variants. The relative levels of haemoglobin F and haemoglobin F variants in blood not only depend on their relative rates of formation but also of course on their relative rates of destruction. We have here in fact a system where the rate of removal of the foetal haemoglobins from the blood is exceeding their rate of synthesis. Moreover, haemoglobin F and haemoglobin F variants need not necessarily be either formed or destroyed at the same rate as each other.

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