ABNORMAL HAEMOGLOBINS IN MALTA: THE SIGNIFICANCE OF TWO FOETAL AND AN ADULT VARIANT

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The study of human haemoglobin variants has a special place in haemoglobin research for several reasons. Certain amino acid substitutions and deletions in haemoglobin A have contributed significantly to our understanding of the structure and function of the haemoglobin molecule as a cooperative tetramer and further progress in this area can be expected. Foetal haemoglobin variants have shed light on the complicated genetics of the γ-chain of foetal haemoglobin.

The discovery of haemoglobin F (Malta) by Cauchi *et al.* (1969) and the high incidence of this haemoglobin in Maltese newborns gave us the opportunity to initiate a systematic study of a foetal haemoglobin variant. During the course of our screening of cord blood samples we have found a new foetal variant, haemoglobin F-Malta-II. We have also found that some families with Hb-F-Malta-II have a new adult haemoglobin variant, which we have called "Haemoglobin St. Luke's". We have redesignated Hb F(Malta) as Hb-F-Malta-I. The purpose of this article is to give briefly the relevance of Hb-F-Malta-I and Hb-F-Malta-II to current concepts of the genetics of the γ-chain of foetal haemoglobin (Huisman *et al.*, 1972), and to give a brief description of Hb St. Luke's (Bannister *et al.*, 1972).

Foetal haemoglobin variants and the genetics of the γ-chain of foetal haemoglobin

Foetal haemoglobin has two α- and two γ-chains. The γ-chain of human HbF has 146 amino acid residues. Cleavage of the chain at methionyl residues by the action of cyanogen bromide yields three peptides one of which accounts for residues 134-146. Analysis of this peptide called

γCB-3 shows that γ-chains are heterogenous with respect to position 136, which is occupied by two amino acids — glycine and alanine. There are therefore two γ-chains: one with glycine in position 136 and one with alanine in this position. We designate these the Gγ- and the Aγ-chains. The ratio of Gγ- to Aγ-chains at birth is of the order of 2:1 The α-chains of rabbit, horse, goat and mouse haemoglobin are also known to be chemically heterogenous in that certain positions of the polypeptide chains are occupied by more than one amino acid residue. This phenomenon can be explained by invoking the presence of alleles of a single structural gene, by ambiguous translation of genetic material, or by the existence of more than one structural gene. Various considerations support the idea of multiple non-allelic structural human γ-genes (Huisman and Schroeder, 1971). This idea provides a satisfactory mechanistic interpretation of several observations on the γ-chain of human foetal haemoglobin. The original proposal of three Gγ- and one Aγ-genes by Schroeder *et al.* (1968) has been modified to account for recent observations on foetal haemoglobin variants.

It is found that haemoglobin F variants are either mutants of the Gγ- or the Aγ-chain. We consider here four variants which have permitted the formulation of a hypothesis of four non-allelic structural genes for the γ-chain: Hb-F-Malta-I, Hb-F-Malta-II, Hb-F-Jamaica and a Negro Hb-F_x. Hb-F-Malta-I has arginine instead of histidine in position 117 of the γ-chain (Cauchi *et al.*, 1969). We find the incidence of this variant in the Maltese population to be of the order of 2%.Hb-F-Malta-II has a much lower incidence. We have detected six cases of this variant over a period of one year. Paucity of material has so far prevented the elucidation of the amino acid substitution in Hb-F-Malta-II which only contributes a very small fraction to the total foetal haemoglobin when it is present. Hb-F-Jamaica has glutamic acid instead of lysine in position 61 of the γ-chain (Ahern *et al.*, 1970) and we may mention here Hb-F-Hull which has lysine instead of glutamic acid in position 121 (Sacker *et al.*, 1967) and is similar to Hb-F-Jamaica with respect to position 136 and the expression of the γ-chain. Hb-F_x is a Negro variant found in Georgia with a hitherto unknown amino acid substitution.

Hb-F-Malta-I and Hb-F_x are Gγ-variants, while Hb-F-Jamaica and Hb-F-Malta-II are Aγ-variants as shown by analysis of the cyanogen bromide peptide γCB-3 as well as the tryptic peptide γT-15 (residues 133-144) in the case of Hb-F-Malta-I (Cauchi *et al.*, 1969) and Hb-F-Jamaica (Ahern *et al.*, 1970). The normal haemoglobin F in the carriers of these variants shows the same Gγ- to Aγ- ratio as the haemoglobin F of normal newborns.

Haemoglobin F variants also manifest themselves in different, characteristic percentages of the total foetal haemoglobin. We find the percentages of Hb-F-Malta-I, Hb-F_x, Hb-F-Jamaica and Hb-F-Malta-II to be on the average 22.5, 13.5, 12.5 and 5.5, respectively, in heterozygotes. These percentages correspond approximately to 4/18, 2/18, 2/18 and 1/18, respectively, of the total foetal haemoglobin. We have assumed as a working hypothesis that the production of these variants in the heterozygote reflects not only the relative activity of the mutated γ-chain structural gene but also that of the allelic normal γ-chain gene, and accordingly, we have proposed the existence of four non-allelic structural genes for the γ-chain with a relative expression of 4:2:2:1 (Huisman *et al.*, 1972). We designate these presumptive genes $G\gamma_m$, $G\gamma_l$, $A\gamma_m$ and $A\gamma_l$, respectively, the symbols G and A referring to the type of γ-chain produced and the symbols m and l indicating the gene with more or lesser activity in each case.

Haemoglobin-F-Malta-I is a $G\gamma_m$-variant, Hb-F_x is a $G\gamma_l$-variant, Hb-F-Jamaica is a $A\gamma_m$-variant, and Hb-F-Malta-II is a $A\gamma_l$-variant.

The hypothesis of four γ-chain genes, $^{G}_{m}\gamma$, $^{G}_{l}\gamma$, $^{A}_{m}\gamma$, and $^{A}_{l}\gamma$, with an activity ratio of 4:2:2:1 requires a Gγ- to Aγ-chain ratio of 2:1. This ratio is observed at birth. However, the ratio changes to 2:3 by the fifth month of life and is maintained at this value in the minute amounts of foetal haemoglobin in adult life. It appears that the mechanism which produces the switch from γ-chain to β (and δ)-chain production after birth affects the γ-chain genes differentially. Preferential suppression of the $^{G}_{m}\gamma$-gene might explain the switch from the 2:1 ratio of Gγ- to Aγ-chains at birth to the 2:3 ratio of the adult. We are currently investigating the disappearance rates of Hb-F-Malta-I and Hb-F-Malta-II and the normal haemoglobin F in heterozygous carriers after birth as a way of testing this idea.

Haemoglobin St. Luke's

The haemoglobin molecule is built of two dissimilar sub-units, the α- and β-chains in Hb A which consists of two α- and two β-chains. Haemoglobin A dissociates into dimers containing one α and one β-chain each. The cleavage is believed to occur at the so-called $\alpha_1\beta_2(\alpha_2\beta_1)$ contact which involves 19 amino acids. Oxygenation of haemoglobin results in reorientation of the sub-units. The $\alpha_1\beta_2(\alpha_2\beta_1)$ contacts undergo greater shift than the $\alpha_1\beta_1(\alpha_2\beta_2)$ and assume a new steric orientation. Amino acid substitution in the region of $\alpha_1\beta_2(\alpha_2\beta_1)$ contact may be expected to affect the dissociation and oxygenation properties of haemoglobin. The amino acid substitution in Hb St. Luke's occurs in this region. The haemoglobin is an α-chain variant and in position 95 of the α-chain proline has been replaced by arginine. A comparable situation exists in Hb-G Georgia and Hb-Rampa.. These variants have leucine and serine, respectively, in position 95 of the α-chain (Smith *et al.*, 1972). Like them Hb St. Luke's shows a greatly increased dissociation into dimers in the oxygenated state which seems to be associated with increased affinity for oxygen. The oxygenation properties need further characterization. We have been impeded in this by methaemoglobin formation *in vitro*.

Hb St. Luke's constitutes 10% or less of the total haemoglobin in the cases we have encountered so far. The subjects are healthy and completely asymptomatic. *Table I* gives haematological data on these subjects. We have found Hb St. Luke's in three out of six families with Hb-F-Malta-II. The meaning of the occurrence of both haemoglobin variants in these families is not clear at the present time.

Table I — Haematological data on carriers of the Hb St. Luke's heterozygosity.

Family	*Subject*	*Hb*	*PCV*	*Reticulocytes*	*Hb A*	*Hb A_2*	*Hb St. Luke's*	*Hb A_2 St.Luke's*
		g/100ml	%	%	%	%	%	%
M	*	15.3	47	1.6	87.6	1.6	10.3	0.5
B	P.B.**	16.2	50	1.6	89.4	2.9	7.4	0.3
C	T.C.***	13.9	45	1.3	89.8	2.1	7.9	0.2
G	J.G.****	15.9	46	1.0	90.6	1.9	7.2	0.3

* Average data for four carriers, two males and two females, one being the father of an Hb-F-Malta-II baby.

** Father of newborn baby with Hb St. Luke's heterozygosity detected as 4% Hb-F-St. Luke's.

*** Mother of Hb-F-Malta II baby.

**** Father of Hb-F-Malta II baby.

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