ABNORMALITIES OF HAEMOGLOBIN A_2

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Summary

1. **Hereditary abnormalities of the A_2 fraction of human haemoglobin may be: (a) structural, in which a genetically determined change in the amino acid composition and sequence of either the alpha or the delta polypetide chains, results in the production of a haemoglobin A_2 variant and (b) quantitative, in which changes in concentration in the haemoglobin A_2 fraction occur as a result of activation or repression of the genes for either the alpha or the delta chains or as a result of the replacement of the normal genes for either of these chains by structurally mutant genes. Because the beta and delta chains of human haemoglobins are linked, a number of conditions also involving the beta chain genes (delta-beta hybrid chain haemoglobins, hereditary persistence of foetal haemoglobin, the beta thalassaemias) produce changes in haemoglobin A_2 concentration.**
2. **Several non-hereditary states have been shown to produce quantitative changes in the haemoglobin A_2 fraction.**

At least four types of haemoglobin molecule are known to occur at various stages of development in normal human subjects. These are: haemoglobin A_1 (the major adult haemoglobin, accounting for some 98% of the total haemoglobin after the first year of extra-uterine life), haemoglobin A_2 (the minor adult haemoglobin, accounting for some 2-3% of the total haemoglobin within a few months after birth), haemoglobin F (the major foetal haemoglobin, produced in the foetus from about the tenth week of intra-uterine life till birth and of which traces only occur after the neonatal period) and haemoglobin Gower-2 (the embryonal or 'primitive' haemoglobin produced up to the twelfth week of gestation). All these haemoglobin types are similar in that they have a common tetrameric structure comprising two different pairs of polypeptide chains per molecule, of which one pair is common to all forms (the alpha chain) and the second pair is characteristic of each form (and called beta chains in A_2, gamma chains in F and epsilon chains in Gower-2). In addition to the above four haemoglobins, small amounts of haemoglobins containing only one type of chain also occur under normal conditions, thus, Gower-1 (containing four epsilon chains per molecule) occurs together with Gower-2 during embryonal development, while haemoglobin Bart's (containing four gamma chains per molecule) occurs in traces in many normal infants at birth.

Abnormalities of haemoglobin A_2 which affect the structure of this haemoglobin fraction arise on a hereditary basis, whereas quantitative abnormalities affecting this fraction may be either hereditary or non-hereditary. A brief summary of the available knowledge on these abnormalities will be presented here.

Normal haemoglobin A_2

Kunkel and Wallenius (1955) discovered that electro-phoresis of haemoglobin solutions from normal persons on starch grains in an alkaline medium, reveals the existence of a second haemoglobin band (A_2) constituting 2-3% of the total pigment, in addition to the major haemoglobin band (A_1). To these workers also is due the discovery that the haemoglobin A_2 concentration is almost doubled in patients with thalassaemia minor. At birth, the A_2 fraction is

present in trace amounts (0.2 - 0.3%) (Horton *et al.*, 1962), but increases as the concentration of haemoglobin F decreases, so that adult levels are reached within the first year of life.

Detailed studies of purified haemoglobin A_2 (by the techniques of peptide map analysis, amino acid composition and sequence determination) have shown that each molecule contains a pair of alpha polypeptide chains and a pair of chains similar to, but not identical with, the beta chains of haemoglobin A_1. These specific chains (delta chains) differ from beta chains in 10 amino acid residues out of the 146 that constitute each of these two kinds of chains (Schroeder and Jones, 1965). Because of this marked similarity between normal delta and beta chains, Ingram (1963) has proposed that the delta chain gene has evolved from the beta chain gene and this suggestion has been supported by the finding of a haemoglobin A_2-like component only in the blood of primates. Haemoglobin A_2 has a higher affinity for oxygen than haemoglobin A (Meyering *et al.*, 1960; Huisman, 1963), and the finding of the same property in haemoglobin variants containing abnormal delta chains (e.g., haemoglobin A'_2 and haemoglobin Lepore) (Huisman, 1963) indicate that this property is related to the structure of the delta chains. Evidence has been obtained that the genetic loci responsible for synthesis of the beta and delta chains are closely linked (Boyer *et al.*, 1963).

A change in the structure (i.e. information content) of the genes for the alpha or delta chain will result in structural variants of haemoglobin A_2. In the heterozygous state for such a mutant gene, the amount of normal haemoglobin A_2 produced will be approximately half that found in normal persons, while in the homozygous state for such a mutant gene, haemoglobin A_2 will be absent. Thus, production of a structural variant of haemoglobin A_2 will automatically decrease the concentration of structurally normal A^2 synthesised. In the absence of such structural mutant genes, the production of alpha or delta chains may be increased or decreased by activation or repression respectively of these genes and this may have a hereditary or a non-hereditary basis.

The most popular technique used in the study of abnormalities of the haemoglobin A_2 fraction is electrophoresis, making use of starch grain slabs or starch gels, filter paper or cellulose acetate as a supporting medium and either densitometric scanning, or elution and spectro-photometry for quantitative determinations.

Structural variation in haemoglobin A_2

(a) Alpha chain variants

Since alpha chains occur in the four normal human haemoglobins, production of a variant of the alpha chain will give rise not only to a variant of haemoglobin A_1 but also to variants of haemoglobin A_2 (beyond the neonatal period), of haemoglobin F (at birth) and of haemoglobin Gower-2 (during the embryonal period) so that in the individual heterozygous for an alpha chain variant both normal and abnormal haemoglobins A_1, A_2, F and Gower-2 will be produced. In an individual homozygous for an alpha chain variant, only abnormal haemoglobins A_1, A_2, F and Gower-2 will be produced. No such instance has yet been reported, presumably because the production of abnormal haemoglobins in the embryo has lethal results. In most alpha chain variants that have been described, decreased concentrations of haemoglobin A_2 have been found.

(b) Delta chain variants

Four variants of the delta chain which contain single amino acid substitutions have been discovered. These are:

(1) haemoglobin A'_2 (or B_2), characterized as δ 16 gly $\rightarrow$ arg (Ball *et al.*, 1966; Jones *et al.*, 1967);

(2) haemoglobin A_2 Sphakia, characterized as δ 2 his $\rightarrow$ arg (Jones *et al.*, 1966);

(3) haemoglobin A_2 Flatbush, characterized as δ 22 ala $\rightarrow$ glu (Jones *et al.*, 1967), and

(4) haemoglobin A_2 Babinga, characterized as δ 136 gly $\rightarrow$ asp (De Jong and Bernini, 1968).

In addition, at least two haemoglobin variants which appear to be hybrids of beta and delta chains (the Lepore haemoglobins) are known. No variants in which a deletion

of genetic material from the delta chain gene has occurred have so far been described.

(i) **Haemoglobin A'_2**

This is by far the commonest of the delta chain variants. The heterozygous state for this variant has been found in 2% of an American negro population (Huisman, 1969), and has only been reported sporadically from Italy (Silvestroni *et al.*, 1963), Turkey (Aksoy and Erdem, 1966), Germany (Betke, 1966), and Venezuela (Arends, 1963). A variant that is believed to be A'_2 has recently been found in 1.2% of some 6600 North American Indian subjects in the province of Alberta (Canada) (Vella, 1969), though not in the same ethnic group in the province of Saskatchewan (Vella and Guzak, 1968). Ten instances of the same variant in subjects of English, Scottish or Eastern European origin have also been found during a study of some 30,000 Canadians from the province of Manitoba (Vella, 1969), though none was found in a similar study in Saskatchewan (Vella, 1967).

In the heterozygous state, haemoglobin A'_2 occurs in the presence of approximately half the normal amount of A_2, while in the homozygous state, there is complete absence of haemoglobin A_2 (Ceppellini, 1959; Huisman *et al.*, 1961). This variant has also been reported in association with β-thalassaemia when both the A_2 and A'_2 fractions were increased in amount) (Ceppellini, 1959; Huisman *et al.*, 1961), with haemoglobin Flatbush (Huisman and Lee, 1965), with haemoglobin S (Huisman, 1969) and with haemoglobin C (Huisman, 1969).

(ii) **Haemoglobin A_2 Sphakia**

This variant was discovered in one family during the course of screening of a small population in Sphakia, an isolated mountainous region in Southern Crete. It was only found in the heterozygous state and was associated with a reduced level of haemoglobin A_2.

(iii) **Haemoglobin A_2 Flatbush**

This variant was discovered by Ranney *et al.*, (1963) during the investigation of a Puerto Rican family with sickle cell anaemia. The same haemoglobin has also been found in two unrelated negro families (Huisman and Lee, 1965). The homozygous state has not yet been reported, though the variant has been found in association with haemoglobin A'_2 (Huisman and Lee, 1965), and with haemoglobin S (Ranney *et al.*, 1963).

(iv) **Haemoglobin A_2 Babinga**

A detailed report on this variant is not yet available.

(c) **Delta-beta hybrid chain variants**

Not all the known haemoglobin variants are characterised by single amino acid substitutions. In the Lepore haemoglobins, Baglioni (1962) showed that the alpha chains were normal, but the non-alpha chains had the N-terminal sequence of the delta chain, the length of the delta-beta hybrid being the same as that of the beta and delta chains. The most likely explanation for the origin of these hybrid variants is a non-homologous crossing-over between parts of the delta and beta chain genes, resulting in the formation of unequal genetic products of which one is a delta-beta hybrid gene which codes for a Lepore haemoglobin (Baglioni, 1962). The known proximity of the structural genes for the beta and delta chains supports this explanation.

Haemoglobin Lepore (or Lepore Boston) was first described by Gerald and Diamond (1958) in association with a clinical picture of thalassaemia in a negro family. A number of haemoglobin variants with properties similar to those of Lepore Boston, and associated with a clinical picture of thalassaemia, have since been described (Lepore Hollandia — Barnabas and Muller, 1962; Pylos — Fessas *et al.*, 1962; Lepore Cyprus — Beaven *et al.*, 1964; Lepore Bronx — Ranney and Jacobs, 1964; Lepore Washington and Lepore Augusta — Labie *et al.*, 1966). There seem to be at least two types of haemoglobin Lepore differing from each other in the proportions of beta and delta chain residues that occur in the non-alpha chains. In the first type, the delta chain sequence contributes the first 87 or so amino acid residues (as occurs in the Boston, Augusta, Washington, Bronx variants and probably also in Cyprus and Pylos), while in the second group the delta chain contributes on-

ly the first 22 or so amino acid residues (as occurs in the Hollandia variant), the rest of the chain being contributed by the beta chain sequence.

The level of haemoglobin A_2 is usually decreased in heterozygotes for a Lepore variant, while the A_2 fraction is absent in homozygotes for a Lepore variant. Haemoglobin Lepore variants have been reported in combination with β-thalassaemia (Pearson *et al.*, 1959), with haemoglobin S (Stomatoyannopoulos and Fessas, 1962), and with haemoglobin C (Ranney and Jacobs, 1964).

Quantitative changes in haemoglobin A_2 levels

Besides the reduction in the level of haemoglobin A_2 which arises as a result of the production of structural variants of eiher alpha or delta chains, several genetically determined conditions may either increase or decrease the level of haemoglobin A_2 without being associated with structural changes in either of the polypeptide chains of this haemoglobin. Of primary importance are the thalassaemias.

The term "thalassaemia" refers to a number of haematologic phenotypes, each of which is caused by a genetically determined inhibition of synthesis of one of the polypeptide chains of the physiologic haemoglobins that occur in man. The inhibition of synthesis of a polypeptide chain may vary in expression from complete to partial. Since five different polypeptide chains enter into the composition of the human haemoglobins, in theory, at least five types of thalassaemia can be distinguished on the basis of which chain is primarily affected. A severe degree of inhibition of synthesis of either alpha or gamma or epsilon chains, arising on the basis of homozygosity for the particular thelassaemia gene, because of the interference with production of embryonal and foetal haemoglobins would be lethal, produce death in utero and be difficult to detect. Three of the five possible forms of thalassaemia are well documented (alpha–, beta– and delta-thalassaemia). There is very good evidence for the existence of several varieties of beta thalassaemia and of at least two of alpha thalassaemia (Weatherall, 1965; Weatherall, 1967).

(a) Alpha thalassaemia

This type of thalassaemia may be very difficult to detect especially in the heterozygous state and in individuals beyond the neonatal period. In the immediate post-natal period, infants with this trait produce abnormal amounts of haemoglobin Bart's with, very often, no other distinct haematological changes. The haemoglobin Bart's usually disappears completely within six months, but traces of it and of haemoglobin H may occur in adults, but the haemoglobin A_2 levels are usually normal.

In haemoglobin H disease (also sometimes called "alpha thalassaemia intermedia"), a more severe inhibition of alpha chain synthesis leads to a disbalance between the relative amounts of alpha and non-alpha chains produced, the resulting excess of non-alpha chains leading to the production of tetramers which contain only one type of chain (only beta chains in haemoglobin H and only gamma chains in haemoglobin Bart's). Small amounts of a haemoglobin containing only delta chains have been isolated in this disease (Dance and Huehns, 1962). Because the haemoglobin A_2 level is almost invariably low in this disease, whereas in alpha thalassaemia heterozygotes it is usually within normal limits, it has been suggested that two different alpha thalassaemia genes may be required to produce haemoglobin H disease, one causing almost total suppression of alpha chain synthesis, the other being 'silent' or not expressed appreciably (Huehns, 1965).

In homozygous alpha thalassaemia, the predominant haemoglobin at birth is haemoglobin Bart's, and haemoglobin A_2 is absent. The condition is incompatible with life and has only been found in still-born infants with the clinical picture of erythroblastosis foetalis (Lie-Injo, 1962).

(b) Beta thalassaemia

Since the first observation of Kunkel and Wallenius (1955) that the haemoglobin A_2 fraction was raised in thalassaemia minor to two to three times the level in normal subjects, this finding has become the main diagnostic feature of the beta thalassaemia trait. The mechanism of the increase in the haemoglobin A_2 fraction in the presence of a

beta chain gene which is functioning subnormally is not yet clear. Since individuals doubly heterozygous for beta thalassaemia and for haemoglobin A'_2 show increased levels of both the normal and the abnormal haemoglobin A_2 fractions, the beta thalassaemia gene results in increased activity of both the delta chain loci. The amount of haemoglobin A_2 in homozygous beta thalassaemia (Cooley's anaemia, thalassaemia major) is very variable and an elevated level is not always found. Several varieties of beta thalassaemia have been described on the basis of the haemoglobin pattern in presumed heterozygotes, as follows:

(i) **Classical beta thalassaemia ("A_2 thalassaemia").** This is the commonest variety and is characterized by high levels of haemoglobin A_2 (3.5 – 7.0%), normal or near normal levels of haemoglobin F (1.5 – 6.5%) and the characteristic erythrocyte morphology of thalassaemia.

(ii) **Beta thalassaemia with raised haemoglobins A_2 and F.** This variety is similar to (i) but the haemoglobin F level tends to be higher (5 – 15%).

(iii) **Beta thalassaemia with isolated raised haemoglobin A_2.** In this variety only the haemoglobin A_2 level is raised, the haemoglobin F concentration and erythrocyte morphology being normal.

(iv) **Beta thalassaemia with normal A_2 and raised haemoglobin F ("δ -β thalassaemia", "F thalassaemia").** In this variety, the haemoglobin F level is raised (8 – 36%), the erythrocyte morphology is abnormal, but the haemoglobin A_2 is within normal limits.

(v) **Beta thalassaemia with normal haemoglobins A_2 and F.** In this variety normal haemoglobins A_2 and F levels occur in the presence of an abnormal erythrocyte morphology and the finding of a thalassaemia-like state in a close relative.

(c) Delta thalassaemia

Fessas and Stomatoyannopoulous (1962) reported on a Greek patient whose haemoglobin was completely deficient in A_2 fraction. A similar finding has been reported in a white American patient (Thompson *et al.*, 1965 a). No other abnormality of haemoglobin pattern was found in either of these patients and it was concluded that synthesis of delta chains in them was completely inactive or inhibited. Absence or marked deficiency of haemoglobin A_2 is considered to arise from homozygosity for a delta thalassaemia gene.

Fraser *et al.*, (1964) have found approximately half the normal concentrations of haemoglobin A_2 in several Greek families and consider these subjects to be heterozygous for a delta thalassaemia gene. Reports are also available of a delta thalasssaemia gene in association with hereditary persistence of haemoglobin F (Thompson *et al.*, 1965 b), with sickle cell anaemia and sickle cell trait (Thompson *et al.*, 1966 a), with haemoglobin D trait (Thompson *et al.*, 1966 b), as well as with beta thalassaemia trait (Thompson *et al.*, 1966 b, Fraser *et al.*, 1964).

(d) Delta-beta hybrid chain thalassaemias

The Lepore haemoglobins appear to be structural hybrids of delta and beta polypeptide chains and are associated with a clinical and haemotological picture of thalassaemia. They are often considered as thalassaemia phenotypes (Motulsky, 1964) and have been referred to above.

(e) Hereditary persistence of haemoglobin F

This condition is characterized by persistence of high levels of haemoglobin F into adult life. Though high levels of haemoglobin F are usually associated with thalassaemia, and in particular with homozygous beta thalassaemia, hereditary persistence of haemoglobin F can be clearly distinguished from the thalassaemia syndromes. The anomaly appears to arise from failure or inactivity of the beta and delta chain genes, with resulting production of gamma chains and persistence of haemoglobin F into adulthood. The haemoglobin F is homogeneuolsy distributed throughout the erythrocytes. In the hetreozygous form the anomaly is not associated with any clinical or haematological abnormalities, though the haemoglobin A_2 levels are decreased. In the homozygous form the haemoglobin A_2 fraction is completely absent (Conloy *et al.*, 1962).

(f) Haemoglobin Zurich

This is an interesting inherited haemoglobinopathy, in which increased amounts of

haemoglobin A_2 occur. The mechanism by which the A_2 level is raised is however probably different from that operating in the case of beta thalassaemia genes.

Haemoglobin Zurich was discovered in two related patients who developed severe haemolytic crises after oral therapy with sulfonamides (Frick *et al.*, 1962). The variant was found in 15 members, over four generations of this family. During the haemolytic episode most of the erythrocytes contained inclusion bodies, but these were absent outside the haemolytic episodes. Intra-erythrocytic inclusion bodies can be produced by a variety of oxidizing agents in erythrocytes containing haemoglobin Zurich.

A second family with this haemoglobinopathy has been reported (Rieder *et al.*, 1965), in which the haemoglobin variant was associated with raised concentrations of haemoglobin A_2. The authors have explained the abnormal levels of A_2 as arising from the preferential loss of the unstable haemoglobin Zurich after synthesis.

(g) Non-hereditary conditions associated with abnormal haemoglobin A_2 levels

Decreased amounts of haemoglobin A_2 have been reported in erythroleukaemia (Aksoy and Erdem, 1967) and in severe and long-standing iron deficiency anaemia (Chernoff, 1964).

Increased amounts of haemoglobin A_2 have been reported in pernicious anaemia in relapse (Josephson *et al.*, 1958), hereditary spherocytosis (Harmeling and Moquin, 1967), acute and chronic *Plasmodium vivax* malaria (Arends, 1967), in later stages of pregnancy (Okcuoglu, *et al.*, 1963; Okcuoglu, 1965) and in recipients of foetal haemopoietic tissue (Bridges *et al.*, 1961).

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