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Haemoglobinopathies and their occurrence in South East Asia *

by

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Abstract

A great number of hereditary abnormalities in the rate of haemoglobin synthesis and in structure of haemoglobins are known at the moment. Most of these abnormalities occur only in a few families and are not very important for the health of a population. Some forms occur, however, rather frequently. In Eastern Asia both alfa and beta thalassaemia cases are rather common. The alfa thalassaemia gene seems to occur in a rather high frequency in people from Chinese extraction. The beta thalassaemia gene, too, is not rare in the population of many etnic groups in South-East Asia.

The symptoms of homozygocity, heterozygocity and double-heterozygocity for the different thalassaemia genes are described. In most Asian countries genetic abnormalities, causing abnormal haemoglobins, are not as common as they are for instance in many African groups. Forms of Lepore haemoglobin, Hb E, Hb Thai, Hb O, Hb S, have been found in East Asian populations, Hb Thai being rather frequent in Thailand, Hb E in Ceylon.

The different clinical pictures of heterozygocity, homozygocity and double-heterozygocity for these genetic abnormalities are described.

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* Invited Paper at the Third National Indonesian Paediatric Congress, Surabaya, July 1-6, 1974.

Hereditary diseases encounter growing interest. Since the paediatrician is the first person who will examine the developing child for this type of affections, it is of great importance that he keeps pace with the different diagnoses of the hereditary diseases to which the group of haemoglobinopathies belongs.

Haemoglobinopathies can be divided into a number of affections, which are caused by a structural anomaly of the haemoglobin molecule and hereditary errors in the rate of synthesis of one or more of the polypeptide chains which form together the normal haemoglobin molecule.

Structure

Haemoglobin consists of two pairs of identical polypeptide chains; each chain carries one haeme group. One pair of chains, the alfa chains, differ in amino acid sequence and in length from the other pair, the beta chains. The alfa chain contains 141 and the beta 146 amino acid residues. Although there are marked differences in the amino acid sequences (there is an agreement at 61 places) the folding of the alfa and beta chains and their tertiary structures are rather similar. The haeme group is situated between loops of the polypeptide chain. The iron atom of the haeme is attached to a histidine group of the polypeptide chain by a covalent bound. In the alfa chain it is the 87th and in the beta chain the 92nd amino acid residue from the N-terminal end of the chain. Moreover, the iron atom of the haeme group is especially close to the histidyl group numbered 58 of the alfa chain and numbered 67 of the beta chain.

The amino acids in the polypeptide chain do not form a straight chain, but form alfa helices. The alfa helices are stabilized by hydrogen bounds between the amino acids laying above each other in adjacent turns of the helix. About 70 percent of the amino acid residues of the chains of globin are in the various helical parts. Between the eight helical parts are amino acid residues which are not arranged in a helix; in these areas the polypeptide chain is twisted in fixed patterns. It is of interest that prolyl residues are often found in these areas of the chain. The four iron atoms of the haeme group are relatively far apart. The forces that keep the two chain pairs together are relatively weak. The dimensions of the haemoglobin molecule are 64 by 55 and 50 A, the molecular weight being about 65,500. In oxygenated haemoglobin the distance between the beta chains is less than in the reduced form in which H_2O has replaced the oxygen.

Normal haemoglobin variants

In addition to haemoglobin A_1 (or alfa$_2$ beta$_2$) normal blood contains a small amount of another fraction, haemoglobin A_2, the percentage being 2 to 3%. It consists of one pair of alfa chains and one pair of chains which differ from the beta chain in amino acid sequence at ten positions. This chain is called delta chain (Hb A_2 = alfa$_2$ delta$_2$). Hb A_2 can be separated from Hb A_1 by electrophoretic and chromatographic methods. The specific function of this second haemoglobin fraction, if any, is unknown. The same applies to foetal haemoglobin which forms the main haemoglobin fraction in the blood of the newborn. It consists of one pair of alfa chains and one pair of so-called gamma chains. The latter chain differs from the normal beta chain at 39 places (Hb F = alfa$_2$ gamma$_2$). The gamma chain contains four isoleucyl residues which are found in neither the alfa nor in the beta chain. It is remarkable that in spite of these differences in amino acid sequences between the beta and gamma chains the functional differences are small. The oxygen affinity of foetal blood is somewhat higher than that of adult blood; this higher affinity for oxygen, however, disappears when the red cell haemolysates are thoroughly dialyzed. In most mammals the oxygen affinity of foetal haemoglobin is higher than that of the adult form.

In the early stages of development of the human embryo special embryonic haemoglobins, Hb Gower (ε_4) Hb Gower$_2$ ($\alpha_2 \varepsilon_2$) and Hb Portland I have been detected. These haemoglobins already appear early during the gestational period, and will not be discussed here.

After birth the percentage of foetal haemoglobin drops sharply in the first months, thereafter slower. In children of about one year old foetal haemoglobin is present from one to five percent. At that time the distribution of foetal haemoglobin over the red cell is very unequal. Some red cells contain 10 to 20 percent of foetal haemoglobin, most of them however contain none. In the blood of normal children older than three foetal haemoglobin is usually less than one percent.

The 2, 3 glycerophosphate that is present in the red cell reduces the oxygen affinity of normal haemoglobin and to a far less extent that of

foetal haemoglobin. Therefore the oxygen affinity of the foetal erythrocyte is higher than that of the adult haemoglobin.

A great number of hereditary abnormalities in the amino acid sequence of one of the chains of the haemoglobin, resulting in the occurrence of an abnormal haemoglobin, is known. Hb S $\alpha_2 \beta_2$ 6 glu-val. Hb C $\alpha_2 \beta_2$ 6 glu-lys and Hb E $\alpha_2 \beta_2$ 26 glysis are most frequent, the latter occurring frequently in certain populations of East Asia. I hall come back tions of East Asia. I shall come back carriers of this abnormal haemoglobin.

Besides abnormalities in the beta chain there are also found abnormalities in the alfa chain. Carriers of the alfa chain abnormalities, however, are rare compared with those of haemoglobins S, C and E.

The following datas will show a survey of a number of variants.

Besides substitution of one amino acid by another, there are also variants in which one or some amino acids are missing and variants in which one of the chains is considerably longer, f.i. Hb Thai = Hb Constant Spring. In some forms the haemoglobin is less stable and less soluble. Sometimes, when the amino acid substitution is in direct spatial relation to the haeme group, methaemoglobin is formed. In other variants, especially in sickle cell haemoglobins, there is an increasing tendency to crystallize out needle-shaped in its desoxygenated form.

A specific structural gene controls the amino acid sequence of each chain, while an operator gene controls the synthesis quantitatively. The synthesis takes place in the protoplasma of the still nucleated erythrocyte on the ribosomal agregates. The genetical information is stored in the chromosomal DNA. This information is carried to the ribosomes by the ribosomal RNA. A mutation of the gene regulating the structure of the polypeptide chains causes a change in the coding system of the specific messenger RNA.

After birth the rates of synthesis of the alfa and beta chains are by far the highest, there is a limited, but constant, production of delta chains and a decreasing synthesis of gamma chains. During intra-uterine life, till the 36th week of pregnancy, mainly alfa and gamma chains are

synthesized. The change-over of the synthesis of foetal to adult haemoglobin is caused by stimulation of the synthesis of beta chains and depression of that of gamma chains.

A number of diseases is known which are due to a poor or totally absent synthesis of one of the chains.

There are the beta thalassaemia in which there is an interference in the synthesis of the beta chains, the beta-delta thalassaemia and the alfa thalassaemia. In the beta thalassaemia Hb A_1 will not, or only in small amounts, be formed. In the beta-delta thalassaemia the synthesis of both Hb A_1 and A_2 is disturbed. In various forms of alfa-thalassaemia the synthesis of foetal haemoglobin is also disturbed. Generally, the heterozygous carrier will be only slightly affected since he has a normal gene still at his disposal. The presence of a normal gene makes the synthesis of normal haemoglobin still possible. The heterozygous carrier of beta thalassaemia is characterized by an elevation of the Hb A_2 concentration, whereas the heterozigosity for beta-delta thalassaemia causes an increase of the foetal haemoglobin production.

Homozygosity for beta thalassaemia will considerably lower or completely interfere with the synthesis of haemoglobin A_1. To survive synthesis of foetal haemoglobin is the only solution. The majority of these patients present a severe anaemia. This is also seen in beta-delta thalassaemia.

Homozygosity for the alfa-thalassaemia gene is incompatible with extra-uterine life, because any synthesis of haemoglobin A and F is completely or almost impossible. Only haemoglobin Barts (gamma$_4$), haemoglobin H (beta$_4$) and Hb Portland I can be synthesized during intra-uterine life.

There is a third form of haemoglobinopathies in which a chain is synthesized that shows partly the structure of a beta chain and partly that of a delta chain. It is assumed that here a crossing-over of the beta and delta genes has taken place.

This haemoglobin, whose rate of synthesis is only slightly higher than that of Hb A_2, is called haemoglobin Lepore. The clinical picture of the homozygous carrier of haemoglobin Lepore does not differ from that of a moderately severe thalassaemia.

When one of the parents is heterozygous for one form of thalassaemia and the other, for example, for a structural anomaly in the beta chain, it is possible that children from such marriage are doubly heterozygous for two haemoglobinopa-

thies. We know patients who are carriers both of haemoglobins S and C, or of haemoglobins E and S.

As for the clinical pictures I will restrict myself to those forms that are frequently found in East Asia. This concerns mainly carriers of the haemoglobin E gene and of the various forms of thalassaemia. There are, however, observed some other forms. We should take into account the occurrence of carriers of Hb Thai, which combined with heterozygosity for an alfa thalassaemia leads to a form of the Hb H disease. Moreover Hb O (Indonesia) and Hb Lepore (Hb Hollandia) are also known to occur. The occurrence of the latter form is probably limited to East Indonesia. The clinical picture of homozygous carrier is that of beta-thalassaemia. As I mentioned already, the clinical pictures of heterozygosity for beta and beta-delta thalassaemia are not severe. The haematological picture can clinically hardly be differentiated from the slight iron deficiency anaemia. The striking difference lies in the fact that the serum iron content is not found to be decreased, but rather increased. In the blood either an increased Hb A_2 concentration, or also after the age of one year, a moderately increased foetal haemoglobin content are found. It is important to distinguish this form from iron deficiency anaemia since iron administration will lead to iron storage and deterioration of the patient's condition.

Individuals who are homozygous for beta and beta-delta thalassaemia show a severe anaemia, whilst their erythrocytes are normally too small, showing an abnormal form with occasional target cells. Especially when the usually much enlarged spleen has been removed, a reticulocytosis is found. Normoblasts are often found in the peripheral blood. The main part of the haemoglobin is foetal haemoglobin. The bone marrow is hyperactive, which causes on the one side a typical X-ray picture, especially that of the carpal bones which may show hypertrophy of the medullary portion of the bone, and on the other side an excessive haemoproduction which changes the patient's complexion and colours the urine dark brown by its metabolic products. Since the synthesis of foetal haemoglobin is unimpaired, the child is normal at birth. The anaemia appears only at the end of the first year of life. At birth small amounts of Hb Barts gamma$_4$ are detectable in the blood.

Double heterozygosity for beta thalassaemia and haemoglobin E occurs rather frequently in East Asia. The clinical picture is that of a tha-

lassaemia intermedia. Haemoglobin E and F are the main haemoglobin components in the blood. The number of erythrocytes is considerably increased, while the spleen is much enlarged. Treatment of thalassaemia is still unsatisfactory. A diet rich in folic acid and vitamin B_{12} is of importance since in spite of the actually small haemoglobin production, the hyperactive bone marrow needs a good supply of these vitamins, a lack of which may cause aplastic crises. Moreover, prevention of infections is important while in many cases the administration of androgenic hormones lessens the seriousness of the anaemia to a certain extent. Spleen extirpation is not recommendable, except in cases where the size of the spleen is causing troubles.

The homozygous and heterozygous carriers of Hb E show few abnormalities. They are healthy or only slightly anaemic. The great number of target cells in the blood smear is striking.

Much more severe is the clinical picture of alfathalassaemia whose gene frequency seems to be high, especially in the population of South China and Thailand. Homozygosity

for the alfathalassaemia gene is incompatible with extra-uterine life. Infants (often prematurely) born with this abnormality die soon after birth, showing the clinical and haematological picture of hydrops foetalis.

The majority of heterozygous carriers is relatively symptomless. Individuals who are doubly heterozygous for alfathalassaemia and another anomaly like Hb Thai, may show the haemoglobin H disease. It is often assumed, that there are different alfathalassaemia genes which may explain the differences in seriousness of the affections. Patients suffering from Hb H disease might also be heterozygous for two forms of alfa thalassaemia. The Hb H disease is a form of haemolytical anaemia in which the blood-smear shows that a part of the haemoglobin in the erythrocyte is partly insoluble and is coagulated. The seriousness of this form of haemolytical anaemia can vary. Spleen extirpation, however, is not advisable.

The clinical picture of the haemoglobin Lepore is that of a somewhat lighter thalassaemia. Patients with methaemoglobinaemia on the base of one of the forms of haemoglobin M have as far as I know not yet been

described in Indonesia. We may assume, however, that by intensive investigation the occurrence of sporadic cases of other haemoglobinopathies may be demonstrated. For the pattern of diseases in Indonesia, the knowledge of the occurrence of different forms of thalassaemia and haemoglobin E is of special importance.