



HEREDITARY DISEASES PROGRAMME
DIVISION OF NONCOMMUNICABLE DISEASES

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REPORT OF THE VTH ANNUAL MEETING OF THE WHO WORKING GROUP
ON THE FEASIBILITY STUDY ON
HEREDITARY DISEASE COMMUNITY CONTROL PROGRAMMES
(HEREDITARY ANAEMIAS: ALPHA THALASSAEMIA)*

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1. INTRODUCTION

Inherited disorders of haemoglobin, including the thalassaemias and sickle cell disease, constitute a public health problem in many parts of the world^(1,2). The β thalassaemias were the first topic addressed by the WHO Working Group on Community Control of Hereditary Anaemias, because of their frequency and severity, our rapidly increasing knowledge of their molecular basis, and the increasing possibilities for treatment and prevention⁽³⁾. Understanding of the α thalassaemias has now reached a similar level, so the 5th meeting of the Working Group, held in Crete in October 1987 in association with the 2nd Mediterranean Conference on Thalassaemia, was dedicated to a review of the epidemiology, molecular basis, clinical features, health burden and possibilities for control of the α thalassaemias.

Globally, though α thalassaemia is extremely common, it causes serious pathology only in parts of South-East Asia and the Eastern Mediterranean Region, and among groups originating from those areas. Here the presence of severe α -thalassaemia genes causes HbH disease, which can be chronically debilitating, and α thalassaemia hydrops fetalis, which is lethal for the fetus and life-threatening for the mother. Where these disorders constitute a public health problem, it is desirable to develop strategies for diagnosing and informing α thalassaemia carriers, providing optimal management for patients with HbH disease, and making prenatal diagnosis available, particularly for α thalassaemia hydrops fetalis. Since the severe α thalassaemias occur only in areas where β haemoglobinopathies are also common, such programmes should be only one component of a comprehensive haemoglobinopathy control programme.

2. THE ALPHA THALASSAEMIAS

The summary below, and the fuller molecular description in Annex 1, draws heavily on a working paper provided for the meeting by Dr D. Higgs. Detailed references may be found in a published review by the same author⁽⁴⁾.

2.1 The normal α globin genes

Table 1 summarizes the composition and developmental sequence of the normal human haemoglobins. All contain four polypeptide chains, two coded by the α globin gene cluster, which is located at the tip of the short arm of chromosome 16, and two coded by the β globin gene cluster on chromosome 11. The α globin gene cluster contains three functional genes (Figure 1): one ζ gene and two α genes, α_2 and α_1 . Like the genes of the β -globin cluster, they are switched on and off sequentially during embryonic development. The single ζ gene functions in the early embryo to produce Hb Gower 1 ($\zeta_2\epsilon_2$) and Hb Portland ($\zeta_2\gamma_2$), but at five to six weeks of embryonic life, the ζ gene is switched off while both α globin genes are switched on. They first produce Hb Gower 2 ($\alpha_2\epsilon_2$), then haemoglobin F ($\alpha_2\gamma_2$), and finally haemoglobin A ($\alpha_2\beta_2$). Though the sequences of the two α genes and their gene products are identical, the α_2 locus is responsible for about 75% of α globin production⁽⁵⁾.

2.2 The alpha thalassaemias

Alpha thalassaemia means reduced production of α globin chains. It may be caused by deletion of one or both of the two α genes on a chromosome, or by point mutations that interfere with the normal functioning of one or both genes. Some mutations directly reduce α globin output, others alter the coding sequence to produce α chains that are abnormal in both structure and quantity. The known groups of α thalassaemia mutations are summarised in Table 2.

Despite their molecular complexity, for practical purposes the α thalassaemias fall into two broad groups indicated in Table 3. Group 1 includes severe mutations that cause disease in the homozygous state or when combined with a Group 2 mutation. Group 2 mutations are mild in both the heterozygous and the homozygous state, and cause clinical problems only when combined with a Group 1 mutation. For clinical and genetic counselling purposes, accurate diagnosis is essential for Group 1 mutations, but is really important for Group 2 mutations only when Group 1 mutations are also present in a population or family.

The pathology caused by the severe α thalassaemias in the homozygous or compound heterozygous state is due both to a reduced total haemoglobin content in the red cell, and to unbalanced synthesis of globin chains. The deficiency of α chains leaves unpaired non- α chains which aggregate into soluble tetramers, γ^4 (= Hb Bart's) in the fetus and newborn, and β^4 (= Hb H) in adults. Since these abnormal tetramers bind oxygen tightly and do not release it to the tissues, the oxygen-carrying capacity of the red cells is reduced in proportion to the concentration of Hb H and Hb Barts. Unpaired non- α chains also precipitate to form inclusions that can lead to haemolysis and a shortened red cell life-span, with compensatory marrow hypertrophy. Inclusions are usually removed in the spleen, so a high level of Hb H or Hb Barts often leads to hypersplenism. The clinical consequences of the cellular pathology are discussed in section 6.

2.3 Classification

Up to the late 1970s, the α -thalassaemias could be defined only in terms of their effect on the blood picture, and the way in which they interact with each other clinically to produce mild α thalassaemias, Hb H disease or Hb Bart's hydrops fetalis. The more severe two-gene deletions were called α thalassaemia 1, the milder one-gene deletions were called α thalassaemia 2. As the α thalassaemias are now better understood, the more scientific classification used in Table 2 has been proposed.

Alpha thalassaemias in which there is no normal α globin production are described as α^0 thalassaemias, and those in which α globin output is reduced are described as α^+ thalassaemias (bringing the classification of α thalassaemias in line with that of the β thalassaemias). Both the α^0 and α^+ thalassaemias are further sub-divided into deletional and non-deletional types, and information on the precise mutation involved can be included when it is available.

The normal α -globin haplotype (= the pattern of genes present on a single chromosome) is written $\alpha\alpha$, representing the $\alpha 2$ and $\alpha 1$ genes respectively. A normal individual has the genotype $\alpha\alpha/\alpha\alpha$.

A deletion involving one ($-\alpha$) or both ($--$) α genes may be further defined by the size of the deletion (when this is known) written as a superscript; thus $-\alpha^{3.7}$ indicates a 3.7 kb deletion. Where the size of a deletion has not yet been established, a superscript describing the geographical or individual origin of the deletion is used; thus $--^{MED}$ describes the deletion of both α -genes, first identified in individuals of Mediterranean origin.

Alpha thalassaemias where both genes are intact (i.e. non-deletional α thalassaemias) are indicated by $\alpha\alpha^T$. When the precise molecular defect is known (as in Hb Constant Spring) $\alpha\alpha^T$ can be replaced by the more informative $\alpha^{CS}\alpha$, the superscript being attached to the $\alpha 2$ or the $\alpha 1$ gene as appropriate.

This system provides an useful shorthand for accurately describing various α thalassaemia gene interactions. For example, the genotype $--^{SEA}/\alpha^{CS}\alpha$ denotes Hb H disease due to an interaction of the Hb Constant Spring mutation with the common south-east Asian α^0 defect.

2.4 Molecular basis

The commonest forms of α thalassaemia involve deletion of one α globin gene from a chromosome. There are two common types of 1-gene deletion: deletion of 4.2 kilobases (kb) of DNA including the $\alpha 2$ gene, leaving a functioning $\alpha 1$ gene (leftward deletion), or deletion of 3.7 kb of DNA, usually including most of the $\alpha 1$ gene, and leaving a functioning, hybrid but predominantly $\alpha 2$ gene (rightward deletion). Despite the difference in the normal level of activity of the two α genes, the single gene remaining after both types of deletion produces about half the normal amount of α globin. Therefore these mutations have similar effects, and α globin synthesis is decreased by about a quarter in heterozygotes, and by about a half in homozygotes. They are the mildest common α thalassaemia mutations⁽⁶⁾.

Deletion of both α globin genes leave no α chain production from that chromosome. In heterozygotes α globin production is reduced by half, with effects on the red cell resembling those of β thalassaemia trait. In homozygotes α chain production is abolished altogether, and this leads to hydrops fetalis. Unlike the one-gene deletions and non-deletional mutations (see below), two-gene deletions may allow some de-repression of the ζ gene⁽⁷⁾. This permits homozygous fetuses to survive throughout most of intrauterine life by making some Hb Portland (which, unlike Hb Barts, can carry oxygen effectively). Similarly, traces of ζ globin may be detected in the red cells of heterozygotes. Twelve types of deletion involving both α genes are known to date (see Annex Figure 3). Some of the less common ones delete the ζ gene as well as both α genes. In the homozygous state these mutations cause early spontaneous abortion rather than hydrops fetalis, since they permit no haemoglobin formation of any kind in the embryo. The commonest two-gene deletions are the South East Asian and the Mediterranean types, which leave the ζ gene intact.

About 18 kinds of non-deletional α thalassaemia are known to date (see Annex 1). Most affect the dominant $\alpha 2$ gene and have more severe effects than the 1-gene deletions described above, because they reduce total α globin production from the affected chromosome by about 75%. They fall roughly into four groups:

- (a) Non-deletional mutations that reduce α chain production by the affected gene.
- (b) Non-deletional mutations that reduce α globin production by both α genes, e.g. the Saudi Arabian form of non-deletional α thalassaemia allows only about 12% of the normal amount of α globin production from the affected chromosome⁽⁸⁾. These are particularly severe.
- (c) Chain termination mutations that produce an abnormally elongated α chain that is synthesised at a reduced rate - e.g. Hb Constant Spring.
- (d) Structural mutations that cause a very unstable α chain.

Correlation of DNA genotype with blood picture shows that in heterozygotes each form of α thalassaemia has a characteristic effect on the red cell indices (Table 4)^(4,9). In general, single α gene deletions ($-\alpha$) produce the mildest changes, non-deletion mutations affecting the dominant α gene cause more marked changes, and deletional mutations involving both α genes such as the Mediterranean or South-East Asian forms cause the most marked effects. Therefore the interactions of two α thalassaemia genes in homozygotes or compound heterozygotes can produce a continuous spectrum of conditions ranging from asymptomatic homozygotes for the common deletional form of α^+ thalassaemia to individuals with Hb H disease and fetuses with α thalassaemia hydrops fetalis (Table 5).

3. GENETIC RISKS

Disease due to α thalassaemia, and the risk of having children with an α thalassaemia syndrome is associated only with severe (e.g., α^0 and α^T Saudi) forms of α thalassaemia. The genetic implications of the common severe deletional α thalassaemia genes are clear, but with non-deletional forms of α thalassaemia it can still be difficult to be sure of the mutation involved, or of its precise clinical implications.

Matings between two carriers of mild α^+ thalassaemia ($-\alpha/\alpha\alpha$) have no genetic risk. There is a one in four chance that each offspring will have homozygous α^+ thalassaemia ($-\alpha/-\alpha$), which causes microcytosis resembling that of α^0 thalassaemia trait and is asymptomatic. Carriers of the common mild α^+ thalassaemia have a genetic risk only when severe α^0 thalassaemia is also present in the population.

Matings between an α^0 and an α^+ thalassaemia carrier have a 25% or 50% risk of HbH disease in the offspring, depending on whether the α^+ carrier is heterozygous or homozygous ($-\alpha/\alpha\alpha$ or $-\alpha/-\alpha$). If the α^+ gene involved is of a non-deletional type e.g., α^T SAUDI or α^{CS} , the resulting Hb H disease is usually more severe.

Matings between two carriers of α^0 thalassaemia ($--/\alpha\alpha$) have a 25% risk of hydrops fetalis in the offspring.

People with the common form of Hb H disease ($-\alpha/--$) are at particularly high genetic risk. If the partner has:

- (a) Hb H disease, there is a 25% risk of α^0 hydrops fetalis, and a 50% risk of Hb H disease in the offspring.
- (b) α^0 thalassaemia trait, there is a 25% risk of hydrops fetalis and a 25% risk of Hb H disease in the offspring.
- (c) α^+ thalassaemia trait, there is a 25% risk of Hb H disease in the offspring.

A draft counselling booklet for carriers of one of the above forms of α thalassaemia is presented in Annex 2.

Carriers of severe non-deletional α thalassaemias, such as the Saudi Arabian form risk having children with severe Hb H disease: some may be transfusion-dependent. However, more information is needed on the spectrum and natural history of the different interactions involving this gene before it will be possible to provide adequate genetic counselling.

4. DIAGNOSIS

4.1 Conventional methods

Methods for diagnosing the α -thalassaemias are listed in Table 6(10). Each method has its limitations, and even together they rarely allow accurate definition of the genotype. In the past, this was usually possible only when a family history of α thalassaemia hydrops fetalis or Hb H disease unambiguously revealed the types of α thalassaemia mutations present. In practice DNA studies are often needed to define the genotype accurately enough for genetic counselling.

Microcytosis is typical for the thalassaemias. However, there is a continuous gradation between normal red cell indices and those associated with α thalassaemia (by contrast with the situation in β thalassaemia). Different types of α thalassaemia reduce red cell size to different degrees, and a large proportion of populations where α thalassaemias are common have microcytosis, ranging from minimal changes associated with α^+ thalassaemia, to the severe microcytosis of Hb H disease.

The Hb A₂ level is usually normal or low in α thalassaemias. However, microcytosis with a normal level of Hb A₂ is also found in iron deficiency anaemia and in some β thalassaemias including "normal Hb A₂" β thalassaemia trait(1).

Serum iron estimation (or an alternative method of assessing iron deficiency) is an essential tool in the diagnosis of α thalassaemia. A normal serum iron permits the diagnosis of a thalassaemia on the basis of microcytosis (MCH <25-27pg) alone.

The observation of Hb H inclusions is diagnostic but tedious. Failure to find them does not exclude α thalassaemia, and they are often not seen in milder forms of α thalassaemia in any case.

Globin biosynthesis studies of the relative rates of synthesis of the α and β chains can distinguish between α and β thalassaemia, or indicate when the two are present together. However, the results are not diagnostic for particular forms of α thalassaemia.

A monoclonal anti- ζ antibody can be used to detect the traces of ζ chain that are present with the --SEA deletion, and this may lead to a specific test for population-screening in South East Asia. However, the method does not pick heterozygotes with the --MED mutation or severe α thalassaemia trait of the Saudi Arabian type, or that due to deletions

that include the ζ gene. The technique still needs simplifying; and the test needs evaluation in the field.

Family studies are often critical for establishing a diagnosis, e.g. the distinction between $-\alpha/-\alpha$ and $--/\alpha\alpha$ thalassaemia traits.

The population concerned must also be taken into account in interpreting the haematological findings (see Section 5.2).

For population screening and genetic counselling, it is also necessary to have simple screening methods suitable for primary health care in developing countries. A 1-tube osmotic fragility test detects microcytosis almost as efficiently as an electronic red cell counter⁽¹¹⁾, and so constitutes a useful primary screen. It picks up a group including carriers of $--/\alpha\alpha$ and $-\alpha/-\alpha$, and people with HbH disease, along with β thalassaemia trait, many heterozygotes and homozygotes for Hb E and some other haematological abnormalities, to whom definitive laboratory diagnosis can then be offered. The ability of a one-tube fragility test to detect the severe Saudi Arabian form of non-deletional α thalassaemia, and the level of ζ chain production in this condition, require further investigation.

In populations where α thalassaemia is common, it is an important genetic determinant of the "normal" values for the red cell indices. Iron deficiency anaemia is also common in many of the relevant populations, especially in Asia and Africa, and it can be difficult to distinguish the contribution of these two factors to the abnormalities that are detected with routine haematological methods. Therefore the first step in setting up an anaemia or haemoglobinopathy screening programme is to define the red cell indices characteristic for the population, and the local prevalence of iron-deficiency anaemia.

The approximate prevalence of α thalassaemia in a population can be estimated by using an automated particle size analyser to establish the frequency distribution of the red cell indices (MCH or MCV) in adult males (who are least likely to have iron deficiency). Comparison with the MCH or MCV distribution among males in a population that is free from thalassaemias (e.g. a northern European population) as in Figure 2⁽¹²⁾, can give an approximate indication of the total prevalence of thalassaemias in the population under study. Hb A₂ estimation in those with MCH less than 27pg then discriminates between β thalassaemia trait and α thalassaemia trait or iron deficiency. Since the normal red cell indices are the same in males and females, comparing the frequency-distribution of the MCH or MCV in males and females gives useful guidance on the prevalence of iron-deficiency among females in the population. This approach also provides a simple and non-invasive method for monitoring the results of public health initiatives to control iron deficiency anaemia.

4.2 Problems in population screening

Screening for α thalassaemias is already (inevitably) routine in existing thalassaemia control programmes, and this experience has revealed some important problems both in populations where severe α thalassaemia mutations occur, and in those where only mild mutations are found.

In populations where severe α thalassaemias occur, it is essential to identify and warn carriers who are at genetic risk.

(a) The commonest and most important problem arises from the fact that, using classical diagnostic methods, it is usually impossible to distinguish homozygous α^+ thalassaemia ($-\alpha/-\alpha$) from α^0 thalassaemia trait ($--/\alpha\alpha$). This is a critically important, since the first carries only a minimal genetic risk, while the second poses a potential threat to the health of female carriers, as well as the risk of hydrops fetalis in their offspring. This problem is usually dealt with pragmatically by identifying such individuals as "possible carriers of α^0 thalassaemia trait", and testing their partner in due course. If the partner is haematologically completely normal, the couple can be told they are not at risk of having children with a severe α thalassaemia syndrome, with no further investigation

(though there is in fact a small risk of a child with Hb H disease, since some α^+ thalassaemia carriers are haematologically normal). But if the partner shows any red cell abnormality at all, a definitive diagnosis is necessary for both partners. This usually requires DNA methods, though family studies may prove helpful. The monoclonal anti- ζ antibody may also prove useful, since it detects traces of ζ globin in newborn and adult carriers of the south-east Asian type of α^0 thalassaemia, but not of homozygous α^+ thalassaemia⁽⁷⁾.

(b) Co-inheritance of α thalassaemia trait and β thalassaemia trait by the same person is common and causes important diagnostic problems. Since it reduces chain imbalance it moderates the pathology associated with both conditions.

(i) It leads to larger red cells than expected, and an Hb A₂ level at the lower end of the β -thalassaemia range, and so can cause the diagnosis of β thalassaemia trait to be missed.

(ii) The diagnosis of β thalassaemia trait does not necessarily exclude coincidental α^0 thalassaemia trait. If one of a couple has β thalassaemia trait and the other has α^0 thalassaemia trait, it is possible that they are at risk of having children with a severe α thalassaemia syndrome, so it is wise to investigate the β thalassaemia carrier further for α thalassaemia by globin biosynthesis or DNA studies¹.

(iii) Alpha thalassaemia trait in heterozygotes for the abnormal β haemoglobins HbS and HbE causes microcytosis and reduces the proportion of abnormal Hb present.

(c) In some Mediterranean populations, and possibly in some other parts of the world, the uncommon "normal Hb A₂" β thalassaemia trait presents the same haematological picture as α^0 thalassaemia trait.

Such problems have been encountered in screening programmes in the Mediterranean area and in North-West Europe, North and South America, and the Caribbean, where people of South East Asian or Mediterranean extraction have migrated. They will be particularly important when screening is started in South East Asia and the Middle East. DNA analysis allows a definitive diagnosis in nearly all cases, and so can resolve the above problems. Fortunately, DNA methods are rapidly becoming more suitable for use in less specialised laboratories.

In populations where severe α thalassaemia genes are not found, e.g., in sub-Saharan Africa and the Indian sub-continent, there is no indication for screening for α thalassaemia. However, the very high prevalence of both mild α^+ thalassaemia and iron deficiency anaemia increases the expense and decreases the efficiency of conventional methods of screening for β thalassaemia. Most screening programmes start with measuring the red cell indices and electrophoresis as a primary screen, and only samples with an MCH of less than 27 pg are investigated further for thalassaemia. In most European and Mediterranean populations the approach is very efficient, as from 5 to 20% of samples fall into this category, and a high proportion of these prove to have β thalassaemia trait (see Figure 2b). By contrast, in many Asian populations, especially when pregnant females are screened, around 50% of samples have an MCH of less than 27pg but only 6 to 12% of these finally prove to represent β thalassaemia trait (see Figure 2c and d). In populations such as that of Thailand where Hb E also contributes to the high frequency of microcytosis, the situation is even more complicated.

The importance of iron deficiency anaemia as a public health problem in many such populations means that haemoglobinopathy screening programmes in these areas must include

¹ Coincidental α thalassaemia can moderate the clinical picture of homozygous β^+ , but not usually of β^0 thalassaemia; the extent of moderation being proportional to the reduction of α globin synthesis.

appropriately cheap and simple methods for diagnosing iron deficiency, and should be conceived as part of a comprehensive anaemia prevention programme.

4.3 DNA diagnosis

Methods for DNA diagnosis^(14,15) continue to develop in terms of precision, simplicity and feasibility, and require decreasing time, equipment and radioactivity. Nevertheless, they should always be practised under the supervision of experts, because of possible pitfalls in interpreting the findings and the importance of other factors such as clinical observations and the family history, for genetic counselling.

The following methods are now used for diagnosing the α thalassaemias.

Southern blotting is used to identify deletional and some non-deletional forms. DNA is extracted from the sample under study and digested with a restriction enzyme (endonuclease) that cuts the DNA only at specific sequences in its coding or non-coding sections. The resulting fragments are separated according to size by electrophoresis, the shortest running fastest. The run is then exposed to a solution of a radioactive (P^{32} labelled) DNA probe complementary to the DNA sequence being sought - in the case of α thalassaemia, α gene probes, or both α and ζ gene probes, are used. The probe binds to the α (or ζ) gene, and the position of the band carrying the gene is recorded by autoradiographing and photographing the preparation. This method reveals the presence or absence of the gene, and the length of the fragment carrying it, which in turn indicates the distance apart of the two nearest restriction sites cut by that enzyme.

The restriction enzymes most often used to locate the genes of the α globin gene cluster are Bam HI and Bgl II. Table 7 shows the lengths (and therefore the relative mobility) of the characteristic fragments detected with α and ζ probes in normals and in deletional forms of α thalassaemia. After Bam HI digestion fragments run in characteristic positions, but one of the fragments produced by digestion with Bgl II includes the ζ gene together with the inter- ζ hypervariable region, and so may run in a slightly different position in different individuals. The two common 1-gene deletions produce characteristic changes in the position of the band detectable with an α probe, and so are easily identified. Deletion of both genes gives no visible band, but characteristically alters the mobility of the band carrying the ζ gene. Therefore, when probing for α^0 thalassaemia it is necessary to use both an α and a ζ gene probe, because in heterozygotes the α probe reveals only the normal α gene band, but the ζ probe reveals a band characteristic for α^0 thalassaemia. In homozygotes the ζ probe confirms that the absence of an α signal is not due to failure of DNA digestion.

Some non-deletional mutations (indicated in Annex Table 1) either abolish a restriction-enzyme site or create a new one, and so have a characteristic effect on the mobility of the α -containing fragment. These can be identified directly by digestion with the relevant enzyme followed by Southern blotting.

Other mutations can be detected using oligonucleotide probes⁽¹⁶⁾. These are short synthetic probes, usually about 19 base-pairs long, matched either with the normal or the mutated sequence that is being sought. Initially they were used on Southern blots to detect non-deletional thalassaemia mutations after restriction enzyme digestion. Genes containing such mutations run in the normal position, but bind only the matching oligonucleotide probe. A Southern blot is exposed to both the normal and the mutant probe either in parallel preparations, or in the same preparation sequentially. Homozygous normal DNA binds only the normal probe, homozygous mutant DNA binds only the specific mutant probe, and heterozygous DNA binds both probes.

These methods have been greatly simplified by the introduction of the polymerase chain reaction (PCR). This essentially simple method for selectively producing a large amount of the segment of DNA under study⁽¹¹⁾, requires precise knowledge of the sequence around this segment. Oligonucleotide probes complementary to sequences on either side of this segment are added to the DNA being examined, and anneal approximately, "bracketing" the DNA segment to be amplified. They are then used as primers for DNA synthesis. The enzyme DNA polymerase

and the appropriate nucleotides are added, and cyclical heating and cooling is applied to separate and reanneal the DNA sequences. This leads to doubling of the bracketed DNA segment in each cycle. Automated methods now exist for repeating the cycle as often as necessary. Twenty to 30 cycles, easily executed in a few hours, can amplify the target sequence to up to 10-30% of the entire DNA sample. The sequence can then be examined by traditional chemical methods. This approach has disadvantages, e.g., it is exquisitely sensitive to contamination, but has the following important advantages.

- (a) Amplification can be done without the labour-intensive DNA extraction and purification that has been necessary hitherto.
- (b) P^{32} labelled radioactive probes are unnecessary if the abnormality sought changes the position of the bands in a characteristic way. The amplified bands can be simply located using DNA stains, or, as DNA fluoresces after staining with ethidium bromide, they can be visualised with ultraviolet light and photographed.
- (c) Southern blotting is often unnecessary. The amplified DNA sample can be dot-blotted directly onto filter paper⁽¹⁸⁾. Radioactive oligonucleotide probes show whether the corresponding sequence is present or not, and can also indicate dosage of the gene.
- (d) Non-radioactive, (e.g., biotin-labelled) probes are becoming available⁽¹⁸⁾.
- (e) Amplified DNA can be run in a denaturing gel⁽¹⁹⁾ to simultaneously reveal the presence of a point mutation and to identify it. If a double-stranded DNA fragment differs by only one nucleotide from normal, it will denature (i.e., the complementary strands will separate) at a slightly different concentration of denaturing agents from normal. If double-stranded fragments e.g. of amplified normal α or β gene DNA, are electrophoresed in a gel containing a gradient of a denaturing agent, they will start to denature at a given position, and their migration is then effectively stopped. Fragments that differ by as little as one nucleotide pair denature at slightly different concentrations, and therefore migrate different distances in the gel; and each mutation may end at a different and characteristic position. Such methods hold out the possibility of very efficient DNA diagnosis even for abnormalities like the β thalassaemias, that have proved to be very heterogenous at the DNA level.
- (f) If the specific mutation present is not identified by the above methods, or is a new mutation, the amplified DNA can be directly sequenced to define the abnormality present.

These developments now make it realistic to start introducing DNA methods into developing countries where the haemoglobinopathies are such an important problem. A study of the forms of α thalassaemia present in a population is often a useful first step in introducing DNA methods, because the α thalassaemias are genetically rather simpler than the β thalassaemias.

5. GEOGRAPHICAL DISTRIBUTION

5.1 Defining the α thalassaemia mutations in a population

Investigation in the newborn by cord-blood electrophoresis, and measuring the percent of Hb Bart's present (see Table 5), is the classical approach for investigating the birth incidence of carriers of α -thalassaemia in a population. Hb Bart's (ζ^4) is more stable than Hb H (β^4), so the detectable excess of ζ chains in the fetus is greater than the excess of β chains in the adult. Thus mild α thalassaemias in which no Hb H is detectable in adults can often be identified by the presence of Hb Bart's in the newborn.

However, the level of Hb Bart's detected in the cord-blood depends on the mutation involved and the methods used, and the results can sometimes be confusing. A very high level (>80%) is unambiguous and indicates α thalassaemia hydrops fetalis (no functioning α genes). A level of 5-40% indicates HbH disease (effectively, one functioning α gene). A level of around 3-5% indicates 2 functioning α genes, i.e., either α^0 thalassaemia trait ($--/\alpha\alpha$) or homozygous α^+ thalassaemia ($-\alpha/-\alpha$). Lower levels of Hb

Bart's (>1%) indicate three functioning genes. Methods must be good to detect low levels reliably, especially as normal infants may have up to 0.5% of Hb Bart's. In addition, many newborns with $\alpha^{3.7}$ thalassaemia trait have no detectable Hb Bart's. Hence DNA-based studies practically always reveal a higher than previously suspected incidence of α^+ thalassaemia trait in a population.

In regions where severe α thalassaemia genes occur (α^0 or α^T Saudi etc), a convenient strategy for defining the types and relative frequencies of the α^0 and α^+ mutations present, is to study DNA from fetuses with hydrops fetalis, and from patients with Hb H disease. In these cases there are no normal chromosomes, and when deletional α^0 thalassaemia is present on one chromosome, the type of α thalassaemia gene present on the other chromosome should become clear. The results of one such study from China are summarized in Figure 3. This strategy has revealed the presence of non-deletional forms of severe α thalassaemia in Greece and Saudi Arabia, and the absence of non-deletional α thalassaemias (other than Hb Constant Spring) from Thailand. However, it does not give clear information on the prevalence of the mutations in the population.

In areas or populations where α thalassaemia hydrops fetalis or HbH disease do not occur, severe α thalassaemia genes may be assumed to be very rare or absent. In this case, most individuals with the typical blood picture of α^0 thalassaemia trait (marked microcytosis, normal or low Hb A₂ level, normal serum iron) will in fact have homozygous α^+ thalassaemia. An efficient strategy for defining the forms of α^+ thalassaemia present in these populations is therefore to analyse DNA from such individuals.

5.2 Distribution of α^+ and α^0 thalassaemia

Table 8 gives estimates for the probable global numbers of carriers of α thalassaemias.

The severe mutations determine the distribution of pathology due to α thalassaemia, but the background mild mutations affect the extent, frequency and severity of the pathology caused by the severe mutations. Migrations of populations carrying severe mutations, as from south-east Asia, are particularly significant in the spread of disease due to α thalassaemia.

Because of the limitations of the classical diagnostic methods mentioned above, it is difficult to be sure of the distribution and true frequency of the mild α^+ thalassaemia traits. It is however clear that they are extremely common in tropical areas, being carried by 30-50% of the population of sub-Saharan Africa, India and South-East Asia. In a few isolated areas the incidence of heterozygotes may be as high as 80%⁽²⁰⁾. However, relatively few studies so far are based on DNA analysis, so the distribution indicated in Figure 4 is necessarily impressionistic. The map is intended only to provide a basis for further investigation. Though α^+ thalassaemia is globally the commonest gene for a hereditary anaemia, outnumbering both the β haemoglobinopathy traits and G6PD deficiency, it causes pathology only when combined with a severe α thalassaemia trait, so in most areas it must be considered as a simple polymorphism. People of Indian or African origin with the haematological picture of α^0 thalassaemia trait, nearly always have homozygous α^+ thalassaemia and are not at risk of offspring with severe thalassaemia syndromes. This is important in genetic counselling.

The distribution of the severe forms of α thalassaemia is both more limited and better known. Alpha-zero thalassaemia is common in South-East Asia, as indicated in Figure 5. In the absence of definitive differentiation between homozygous α^+ and α^0 thalassaemia traits, the true frequency of α^0 thalassaemia can only be established by DNA analysis, or calculated from the birth incidence of fetuses with hydrops fetalis; but the latter approach requires good epidemiology and neonatal pathology. These approaches have shown that the incidence of α^0 thalassaemia trait in people originating from southern China (Guangdong) and resident in the USA, UK, Singapore or Hong Kong is 3-3.5%⁽²¹⁾. The incidence is similar in Thailand around Bangkok but higher in north-west Thailand⁽²²⁾, and reaches 6% in the Guangxi province of China⁽²³⁾. It is about 1.5% in Cyprus and parts of Greece⁽²⁴⁾. The Saudi Arabian form of non-deletional α thalassaemia⁽⁸⁾ is found in 1-3% of the population

of Eastern Saudi Arabia and Bahrain, and presumably occurs in other parts of the Middle East, but the limits of its range are unknown.

A given prevalence of α^0 thalassaemia trait creates most pathology in populations where α^+ thalassaemia is very common. For example, Table 9 shows that though the incidence of α^0 thalassaemia trait is similar in Singapore and Bangkok, the much higher local prevalence of α^+ thalassaemia trait in Bangkok leads to an five times higher prevalence of Hb H disease.

Consanguineous marriage, very common in many parts of the world, increases the chance that partners will both inherit the same mutation from a common ancestor, and so would increase the birth incidence of homozygotes with α^0 hydrops fetalis or α^+ Saudi Hb H disease for a given heterozygote frequency. However, it would have little effect on the frequency of $-\alpha/--$ Hb H disease, which is caused by interaction of two different genes.

Table 10 summarizes present knowledge about the incidence of α -thalassaemia in South-East Asia. Since we still have inadequate information for more than half the populations of the region, the estimated incidence of affected births is an absolute minimum. In this area at least 3,500 infants are born annually with α thalassaemia hydrops fetalis, so about 14,000 women a year must run the risk of obstetric complications due to this condition (see below). At least 13,000-16,000 infants are born annually with Hb H disease, and because of their long life-expectancy, HbH disease is the most prevalent major haemoglobinopathy in some areas. Allowing for a mean survival is about 50 years, there may be nearly a quarter of a million living individuals with Hb H disease in South-East Asia.

6. CLINICAL FEATURES AND HEALTH BURDEN

The clinical result of inheriting two α thalassaemia genes depends on the extent to which they suppress normal α globin synthesis.

6.1 Asymptomatic combinations

The homozygous state for the mild α thalassaemia deletions is usually asymptomatic, but examination of pure homozygotes for different mutations shows that the non-deletional mutations which reduce the expression of the dominant $\alpha 2$ gene have more marked effects than the α^+ deletions, which only reduce α chain synthesis by half.

Homozygotes ($-\alpha/-\alpha$) for the common deletional form of α^+ thalassaemia are asymptomatic, and clinically indistinguishable from individuals with α^0 thalassaemia trait ($--/\alpha\alpha$).

Homozygotes for the common chain-termination mutant Hb Constant Spring ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) show more noticeable effects. Most are anaemic with thalassaemic red cell changes, reticulocytosis, and often prominent basophilic stippling of the red cells. They may have mild jaundice and some hepatosplenomegaly, but in general they are clinically well.

A single Sardinian patient homozygous for the α^{Nco} non-deletional mutation has mild Hb H disease. This suggests that there is a threshold level of α chain synthesis below which the syndrome of Hb H disease occurs, and that the Constant Spring mutation permits marginally more, and the α^{Nco} mutation marginally less, than this critical amount of α chain synthesis.

Of course compound heterozygotes with one of the above mutations and one of another have an intermediate blood picture, allowing for a continuous gradation in the clinical picture of Hb H disease.

6.2 Hb H disease

Hb H disease is defined as an anaemia in which HbH is seen on electrophoresis. It most frequently results from the interaction of α^+ and α^0 thalassaemia, and is predominantly

found in south-east Asia (commonly --SEA/- $\alpha^{3.7}$) and the Mediterranean basin (commonly --MED/- $\alpha^{3.7}$) where both forms of α thalassaemia are common. It may also result from the interaction of non-deletion mutations affecting the predominant $\alpha 2$ globin gene ($\alpha^{Nco\alpha}$, α^T SAUDI or - α^T 3.7II).

The haematological features are variable. Haemoglobin levels ranging from 2.6 - 12.4 g/dl have been recorded, with reticulocytosis and typical thalassaemic red cell indices. The haemoglobin consists of Hb A with 2-40% of Hb H and sometimes Hb Barts. When peripheral blood is incubated with redox dyes, the Hb H level is reflected in the number of cells that contain typical Hb H inclusions.

The clinical picture of HbH disease ranges from mild anaemia to transfusion dependency (" α thalassaemia major"), but the commonest picture is a thalassaemia intermedia with hypochromic microcytic anaemia, jaundice and hepatosplenomegaly. Since the main mechanism of anaemia is haemolysis rather than ineffective erythropoiesis, only 35% of patients have marked bone-marrow expansion. Most patients with Hb H disease have an Hb level of 8-10 g/dl, jaundice and splenomegaly are mild and physical development is normal. In the steady state, most can function normally, but their life-long or intermittent jaundice is a major cause of anxiety, and often leads to a mis-diagnosis and inappropriate treatment⁽²⁵⁾. Correct diagnosis and information is therefore extremely important.

The commonest complications of Hb H disease are:

- (a) Development of hypersplenism, which exacerbates the chronic anaemia, and can make some patients transfusion-dependent. At present there are no statistics on the incidence, age at onset or causes of this complication, and no accepted criteria for its diagnosis or for splenectomy. Data from Thailand suggest that it is particularly common in Hb Constant Spring Hb H disease (--/ $\alpha^{CS\alpha}$), and that when the previous clinical status has been good and the spleen is very large (>6 cm), splenectomy may relieve most patients from transfusion-dependency⁽²⁶⁾. The decision to remove the spleen must be taken very carefully, as thrombotic complications may follow in some cases.
- (b) Acute anaemia due to haemolytic or aplastic episodes. Acute haemolytic crises are also particularly common in the --/ $\alpha^{CS\alpha}$ form of HbH disease.

Other complications include infection, leg ulcers, gallstones and relative folate deficiency, but iron overload of gastro-intestinal origin is uncommon.

The severity of Hb H disease appears to be directly related to the extent of suppression of α globin synthesis, which, of course, varies with different combinations of mutations. However, there have as yet been few systematic attempts to correlate genotype with phenotype. Table 11, from a recent study in Greece⁽²⁷⁾, illustrates the wide clinical range and shows that in general, patients with a non-deletion defect (affecting the predominant $\alpha 2$ gene) interacting with an α^0 thalassaemia determinant (--/ α^T or --/ $\alpha^{CS\alpha}$) have higher levels of Hb H, a lower haemoglobin and a more severe clinical picture than patients with the more common --/ α genotype. In both Greece and Thailand even cases with the same molecular basis (--MED/- $\alpha^{3.7}$, or --SEA/- $\alpha^{3.7}$) may have a different clinical course, indicating that other genetic and environmental factors play an important role in clinical variation, and three infants with severe Hb H disease associated with hydrops fetalis have been described. Obviously defining the α thalassaemia genotype is very important for genetic counselling, as couples at risk of the more severe syndromes (e.g., --/ α^T Saudi α) may wish for prenatal diagnosis.

The natural history of the more severe Saudi Arabian form of HbH disease has not been fully defined, but it appears that some affected infants may be born with anaemia and hypersplenism, and that some are transfusion-dependent.

6.3 Haemoglobin Bart's hydrops fetalis

In the vast majority of cases this syndrome is due to homozygosity for α^0 thalassaemia and it is seen almost exclusively in patients of south-east Asian (commonly

--SEA/--SEA) or Mediterranean (commonly --MED/--MED) origin¹. Affected infants die either in utero between 20 and 40 weeks of gestation, or soon after birth. The long-term follow-up of two affected infants that were delivered alive in North America at 28 and 32 weeks and were transfused and intensively nursed^(28,29) is awaited with interest. The usual clinical picture is of a pale oedematous infant with cardiac failure and signs of prolonged intrauterine hypoxia. The liver and spleen are grossly enlarged and there may be other congenital abnormalities. The haemoglobin level ranges from 3-10 g/dl, and the blood film shows large hypochromic irregular-shaped red cells, many of which are nucleated. The haemoglobin consists of about 80% Hb Barts, the remainder being haemoglobins H and Portland. Only Hb Portland is efficient in transporting oxygen; hence the severe fetal hypoxia.

Published information on the natural history of α thalassaemia hydrops fetalis is quite limited⁽³⁰⁾. At Siriraj Hospital in Bangkok there are about 20,000 deliveries a year. All still-births or neonatal births are examined by a pathologist, and when hydrops is present, a blood sample is sent to the haematology laboratory for definitive diagnosis. About 60% of hydropic fetuses prove to have α thalassaemia hydrops fetalis, and the incidence of Hb Bart's hydrops fetalis is 0.3/1,000, corresponding to 3% heterozygote frequency.

Of 65 affected infants examined⁽³¹⁾, 25% died in utero, 18% died during delivery, and 54% died at from 1-40 minutes after birth. Forty eight per cent required "assisted delivery" (Table 12). Contrary to expectation the diagnosis was not always obvious during pregnancy or even after delivery. Only a few mothers had polyhydramnios, and though many infants were premature, their maturity was over-estimated because of oedema, and the diagnosis of hydrops fetalis was not always made. Complications during pregnancy included severe pre-eclampsia in 44% of mothers, mild pre-eclampsia in 32%, eclampsia in 2%, severe antepartum haemorrhage in 9%, and severe post-partum haemorrhage in 2%. From the pathology observed, it seems reasonable to guess that when obstetric assistance is unavailable, 20-50% of mothers with a hydropic fetus might suffer lethal complications.

Pathological findings in the fetuses included gross enlargement of the heart, liver and spleen, as expected, and severe hypoplasia of the lungs, which no doubt accounts for death so soon after birth. One of the most remarkable findings was severe retardation in brain growth: many brains were only about 60% of the expected weight. However, the severity of pathological changes varies, and a few infants reached birth with less severe abnormalities.

7. ALPHA THALASSAEMIAS AND NATURAL SELECTION

It is thought that the α thalassaemias are so common, and their distribution overlaps that of the β haemoglobinopathies so closely, because both groups of mutation confer protection against the more severe consequences of falciparum malaria, the degree of protection probably being proportional to the degree of red cell microcytosis. Alpha thalassaemias can co-exist at high frequency with the β chain haemoglobinopathies because they do not interact to cause pathology (the effect being, if anything, the opposite).

The prevalence of the severe haemoglobinopathies (β thalassaemia, HbS and α^0 thalassaemia) is determined by a balance between their selective advantage, which increases the number of these genes in a population, and the death of homozygotes, which causes genes to leave the population. By contrast, mild forms of α thalassaemia are almost harmless in the homozygous state and so can reach a very high frequency. It therefore seems surprising that their frequency has not risen to 100% in populations exposed to malaria.

¹ However, there have recently been reports of hydrops fetalis with a very low level of α chain synthesis in three Greek and one Southeast Asian infants, resulting from interaction of α^0 thalassaemia with non-deletion mutations ($\alpha\alpha^T$). The latter were not characterized, but they probably resemble the $\alpha\alpha^T$ Saudi defect. The Greek mutation has been called $\alpha\alpha^T$ Karditsa (Annex Table 1). A recent survey in China revealed some hydropic babies with no α chain production and a non-deletion mutation, identifying a new type of $\alpha\alpha^T$ disorder.

There are several possible explanations. For example, homozygotes may have a selective disadvantage due to mild anaemia; or populations may derive most effective protection from maintaining the widest possible range of variation in red cell characteristics, so that the malaria parasite cannot adapt. Alpha and β thalassaemias, abnormal haemoglobins, G6PD deficiency and iron deficiency anaemia probably all combine to give a population optimal protection. The relatively small populations with more than 50% of α^+ thalassaemia carriers (e.g., Oman, some South Indian tribes, Vanuatu) may have reached this high frequency by genetic drift superimposed on natural selection⁽²⁰⁾.

Though α^0 thalassaemia trait is probably as protective to heterozygotes as β thalassaemia trait, it is much less common. This may be because, in addition to its lethality in homozygotes, α^0 thalassaemia trait constitutes a threat to the life of female heterozygotes (due to the obstetric complications of hydrops fetalis). This risk is proportional to their risk of marrying another carrier, i.e., to the square of the gene frequency. There is also a risk of relatively unfit offspring with HbH disease, proportional to the frequency of α^+ thalassaemia in the population concerned. The great selective disadvantage of the α^0 thalassaemia gene would be expected to hold it at a lower frequency than β thalassaemia in a given environment, and this appears to be generally true, but in north-west Thailand and southern China the prevalence of α^0 thalassaemia exceeds that of β thalassaemia.

The practice of consanguineous marriage, by increasing the frequency of matings between carriers of α^0 thalassaemia trait, increases both the disadvantage due to intrauterine death of homozygotes, and that due to maternal mortality and morbidity. Therefore, α^0 thalassaemia should be almost completely excluded from areas where consanguineous marriage is common, and this appears to be the case in India and the Middle East.

8. CONCLUSIONS AND RECOMMENDATIONS

The α -thalassaemias are globally very common and cause much pathology, especially in Asia where the severe α^0 thalassaemia gene is common. In the homozygous form this leads to α thalassaemia hydrops fetalis. Affected infants always die in utero or immediately after birth, and the mother may suffer severe complications during pregnancy and delivery. The combination of a severe with a mild α^+ thalassaemia gene leads to Hb H disease, which can be debilitating, and often causes anxiety and misdiagnosis; but most people with HbH disease can lead an essentially normal life. It is necessary to develop strategies for diagnosis, information, and optimal management for the α thalassaemias, and for prenatal diagnosis when indicated.

Carrier detection and the offer of prenatal diagnosis and selective abortion is indicated for α^0 thalassaemia hydrops fetalis, for both obstetric and genetic reasons. The desirability of prenatal diagnosis for HbH disease is much less clear. Probably most couples at risk for the common deletional form would prefer neonatal diagnosis and protection of children with HbH disease, but couples at risk for the more severe Saudi Arabian type might be more interested in prenatal diagnosis. Parental choice is likely to be influenced by the severity of the mutations involved, their previous history (e.g., two or three previous children with HbH disease) and the society in which they live.

Screening and genetic counselling for α thalassaemia is automatic wherever there is a β -thalassaemia control programme, as in Europe and the Mediterranean area. However, in south-east Asia where α^0 thalassaemia really is a public health burden, and in the Middle East where it forms part of a particularly complex picture of haemoglobinopathies, no haemoglobinopathy control programmes yet exist.

For HbH disease, it is necessary to define the natural history in relation to the types of α thalassaemia gene present; define the role of hyperplenism in its pathophysiology; identify appropriate treatment; provide correct diagnosis, genetic counselling and assistance with social integration; and generate information for the community.

For α^0 thalassaemia, it is necessary to develop methods for carrier screening and genetic counselling, especially during pregnancy, because of its direct relevance for the

health of the pregnant women. If it becomes possible to identify carriers by a specific screening test, this could be a convenient first step to population-screening for haemoglobinopathies in parts of Asia.

For severe α^+ thalassaemia it is necessary to define the distribution, interactions and clinical picture in order to provide a basis for accurate genetic counselling in the future.

Fortunately the DNA methods that are essential for accurate diagnosis are becoming simplified to such an extent that it is realistic to introduce them into the developing countries where the haemoglobinopathies are such an important problem.

An α -thalassaemia control programme cannot be developed on its own, but should be an integral part of a comprehensive anaemia control programme.

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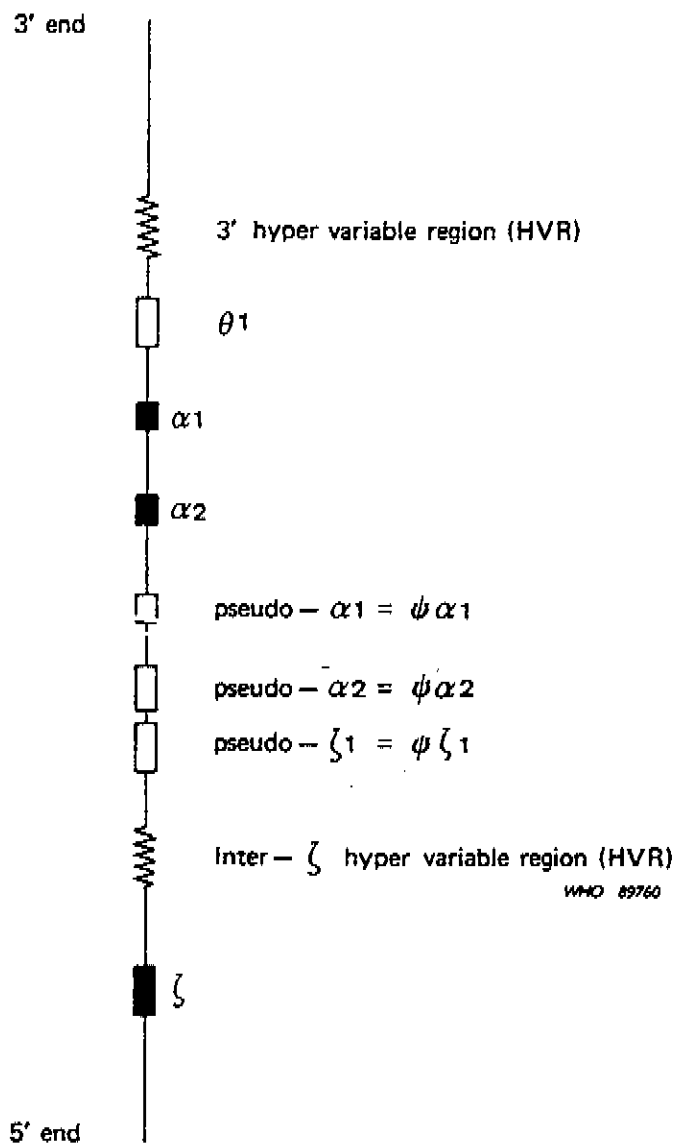
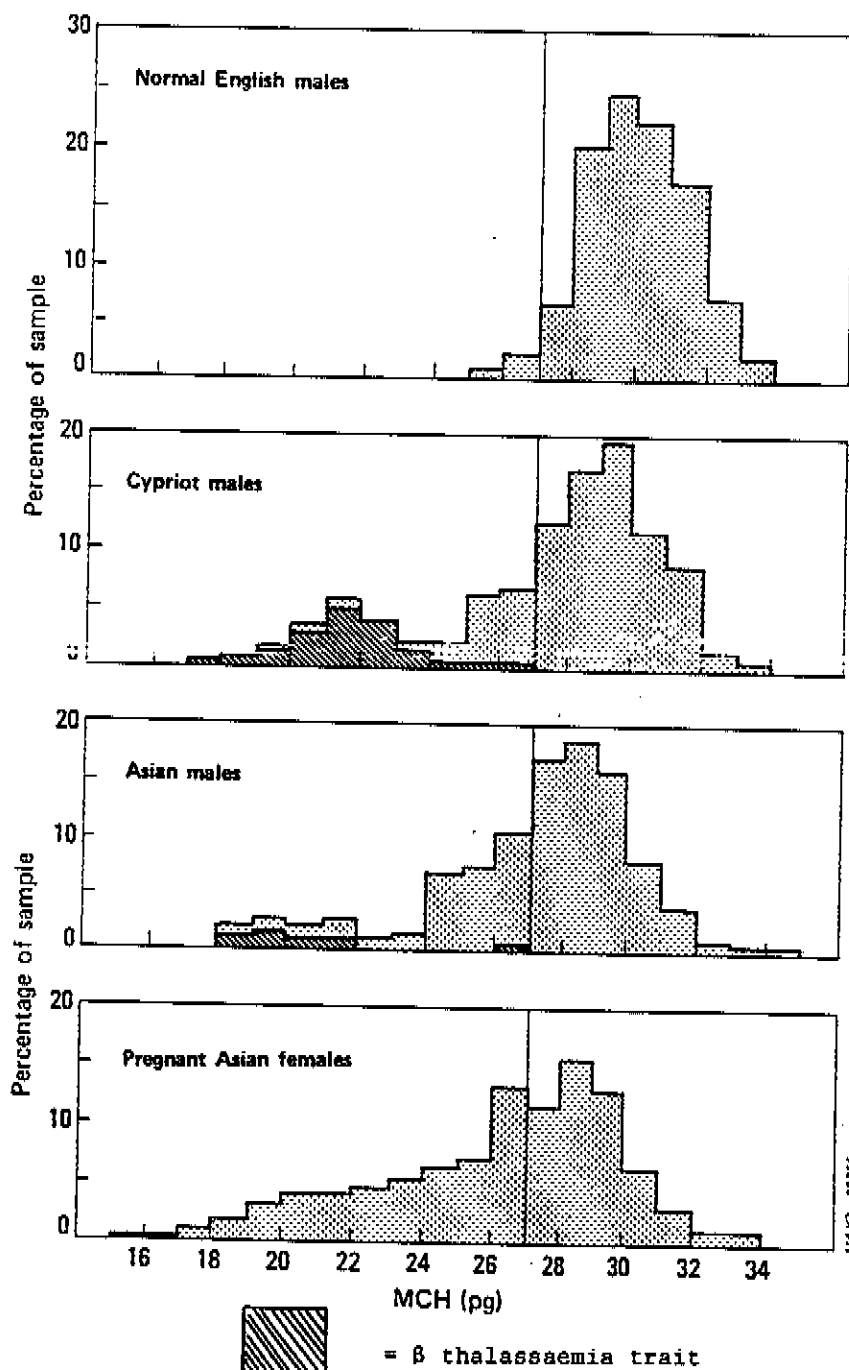


FIG. 1

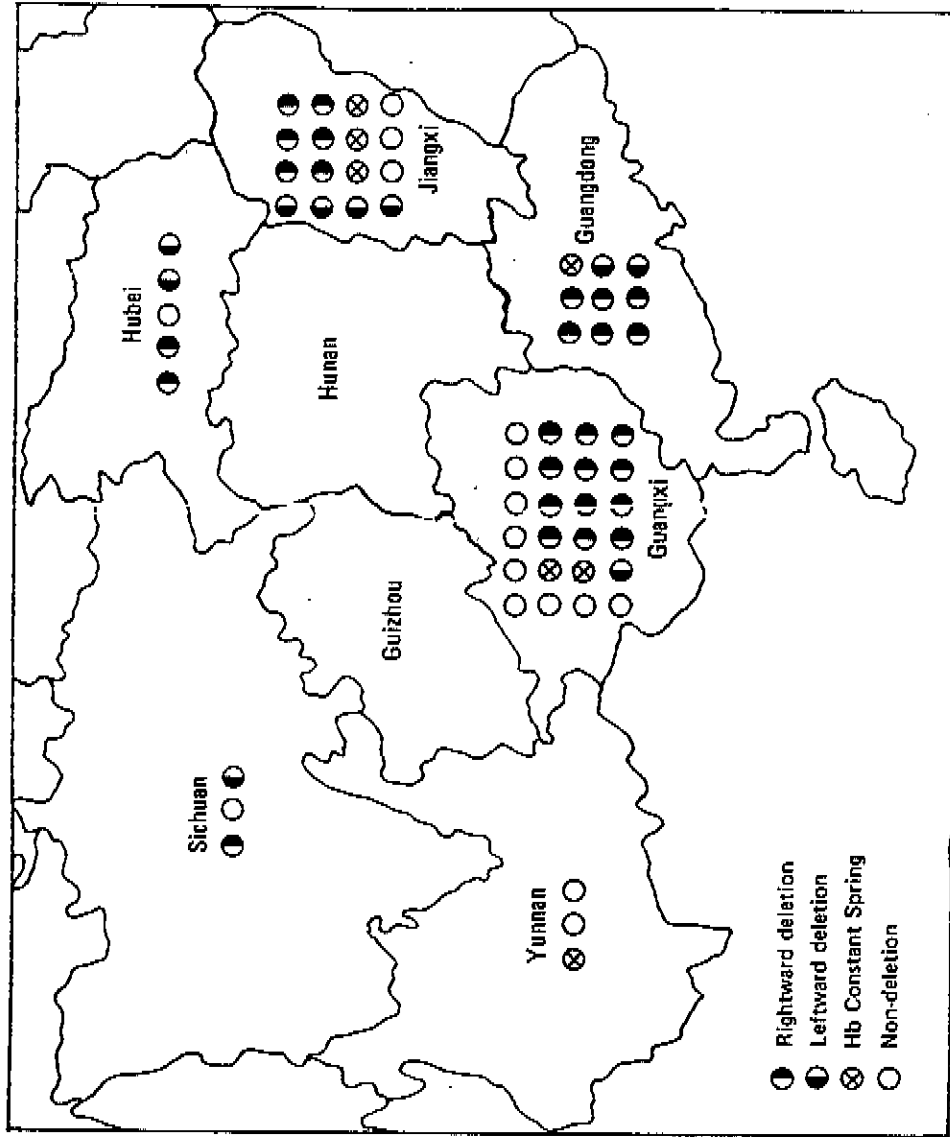
Diagram (low resolution) of the α -globin gene cluster. Functional globin genes are shown as black boxes. Non-functional "pseudo-genes" with sequences homologous to the functioning genes, but with numerous defects that prohibit functioning, are shown as empty boxes. Their significance is not known. The Thera (θ) gene is probably not a globin gene, and it is uncertain whether it produces a protein transcript or not.

FIG. 2
THE MCH DISTRIBUTION IN DIFFERENT POPULATION GROUPS
IS INFLUENCED BY THE PREVALENCE OF α AND β
THALASSAEMIA AND IRON DEFICIENCY



In males, the shift in MCH distribution below 27 pg is mainly due to the presence of α^+ thalassaemia in the population. In female Asians, it is due to both iron deficiency and α thalassaemia. Comparing the distribution of the MCH in males and females allows an evaluation of the prevalence of iron deficiency among females.

FIG. 3
GEOGRAPHICAL DISTRIBUTION OF VARIOUS TYPES OF Hb H DISEASE IN
SOUTH CHINA: THE ASSOCIATED FORM OF α^+ THALASSAEMIA IS SHOWN



WHO 89/62

(from Y.T Zeng, S-Z Huang 1985)

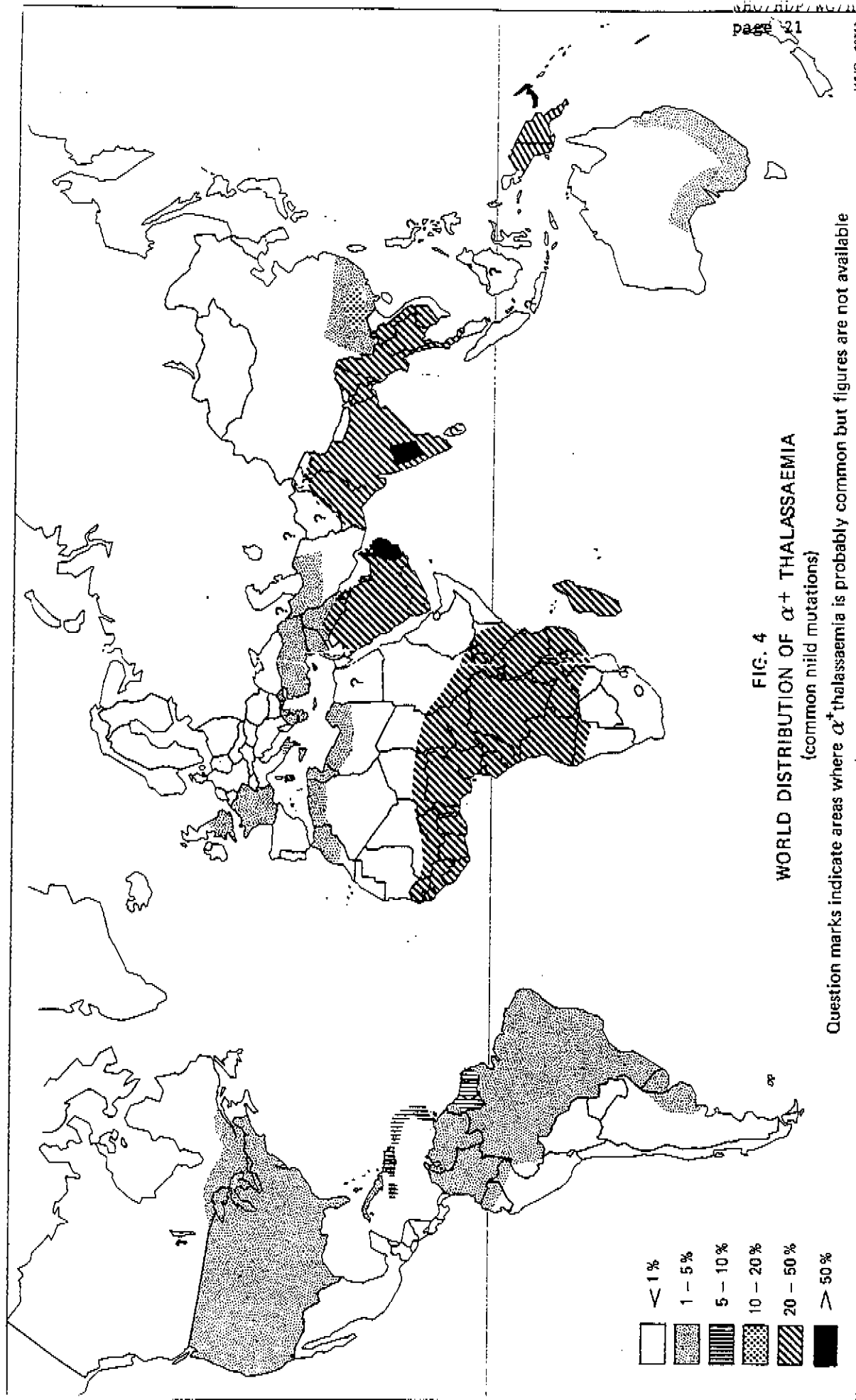


FIG. 4
 WORLD DISTRIBUTION OF α^+ THALASSAEMIA
 (common mild mutations)

Question marks indicate areas where α^+ thalassaemia is probably common but figures are not available

- $< 1\%$
- 1 - 5%
- 5 - 10%
- 10 - 20%
- 20 - 50%
- $> 50\%$

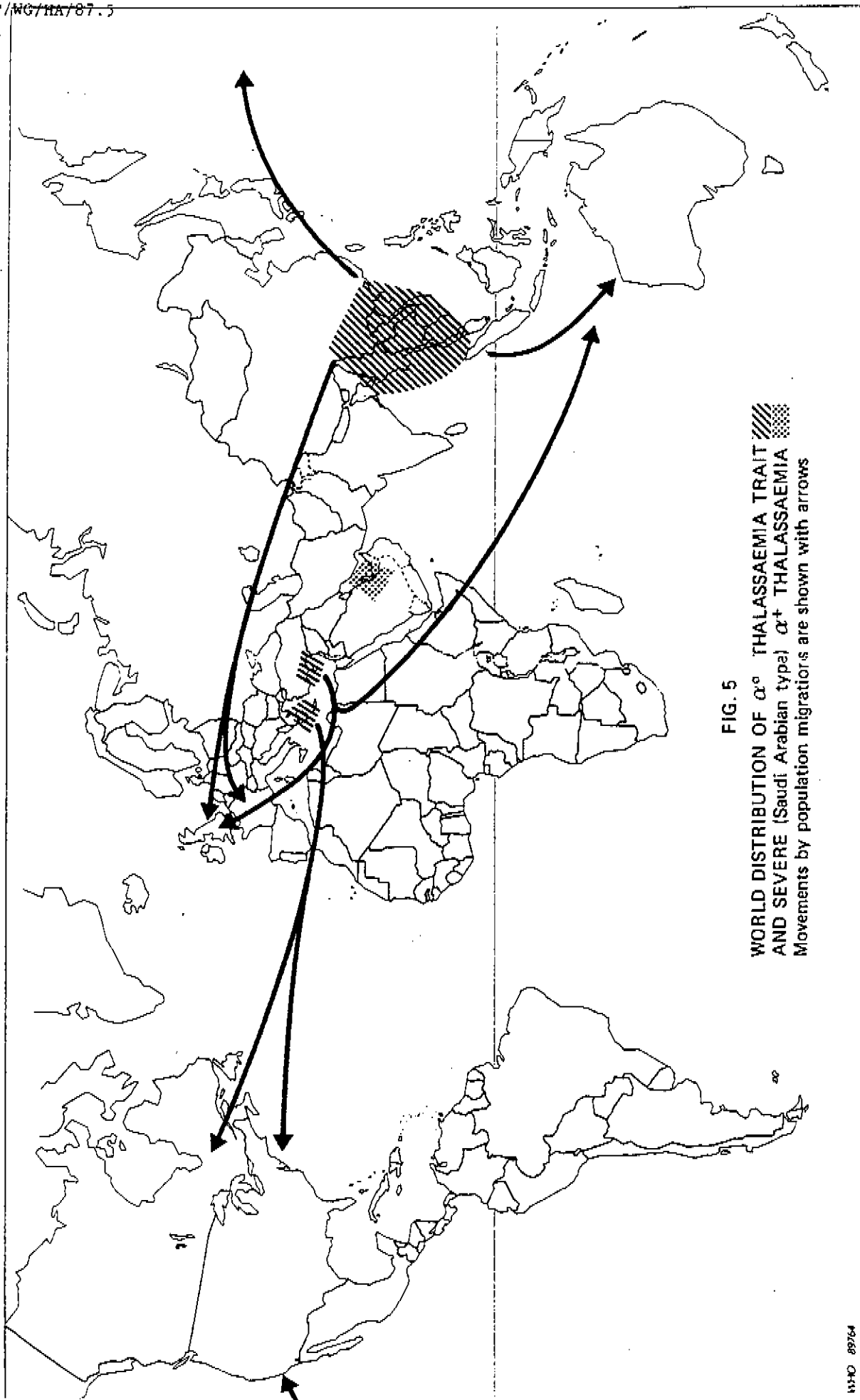


FIG. 5
WORLD DISTRIBUTION OF α^0 THALASSAEMIA TRAIT
AND SEVERE (Saudi Arabian type) α^+ THALASSAEMIA
Movements by population migration:s are shown with arrows

TABLE 1
Composition and Developmental Sequence of Normal Human Haemoglobins

" α " chain (Chromosome 16)	"non α " chain (Chromosome 11)	Structure	Hb Name	Found
ζ	ϵ	$\zeta 2\epsilon 2$	Gower 1	First 6 weeks of embryonic life.
α	ϵ	$\alpha 2\epsilon 2$	Gower 2	First 6 weeks of embryonic life.
ζ	γ	$\zeta 2\gamma 2$	Hb Portland	First weeks of embryonic life, and in α -thalassaemia hydrops fetalis.
α	γ	$\alpha 2\gamma 2$	Hb F	Predominant Hb from 6 weeks of embryonic life to term. <1% in normal adults.
α	β	$\alpha 2\beta 2$	Hb A	Up to 10% in a normal fetus from 10 weeks of embryonic life. Predominant normal adult Hb.
α	δ	$\alpha 2\delta 2$	Hb A ₂	Minor Hb associated with and produced at 1/40 the level of Hb A. <3% in normal adults.

TABLE 2

Recognized forms of alpha-thalassaemia

	HAPLOTYPE	HETEROZYGOTE
Normal	$\alpha\alpha$	$\alpha\alpha/\alpha\alpha$
α^0 thalassaemias (no α chain produced)		
<u>Deletional</u>		
both α genes deleted (12 types)	--	--/ $\alpha\alpha$
<u>Non-deletional</u>		
1 α gene deleted, 1 non-functional (2 types)	-(α)	-(α)/ $\alpha\alpha$
α^+ thalassaemias (reduced α chain production)		
<u>Deletional</u>		
α 1 deleted (leftward)	$-\alpha^{4.2}$	$-\alpha^{4.2}/\alpha\alpha$
$\alpha 1/\alpha 2$ hybrid (usually) (rightward)	$-\alpha^{3.7}$	$-\alpha^{3.7}/\alpha\alpha$
other	$-\alpha^{3.5}$	$-\alpha^{3.5}/\alpha\alpha$
<u>Non-deletional</u> (α genes intact)		
Mutation reduces α globin production by 1 gene, e.g.,	$\alpha^{nCol}\alpha$	$\alpha^{nCol}\alpha/\alpha\alpha$
Mutation reduces α globin production by both genes, e.g.,	$\alpha^T SAUDI\alpha$	$\alpha^T SAUDI\alpha/\alpha\alpha$
Chain termination mutation e.g., Hb Constant Spring	$\alpha^{CS}\alpha$	$\alpha^{CS}\alpha/\alpha\alpha$
Unstable haemoglobins, e.g.,	Hb Quang Sze	-

TABLE 3

For clinical purposes, alpha thalassaemia genes fall into two groups according to their severity

GROUP I

Lethal or potentially lethal
in homozygotes: compound
heterozygotes with a group
group II gene have Hb H disease

α^0 thalassaemias

severe α^+ thalassaemias
e.g. α^+ SAUDI

GROUP II

Homozygotes are healthy
Compound heterozygotes with
a group I gene have Hb H disease

mild α^+ thalassaemias
(e.g. deletional forms)

TABLE 4
Haematological indices in Alpha Thalassaemia (Adults)
(Means, with standard deviation in brackets)

Genotype	Number Observed	Males	Hb	Females	MCV fl	MCH pg	HbH %	α/β chain synthesis ratio
$\alpha\alpha/\alpha\alpha$	-	15.5 (1.0)	14.0 (1.0)	90 (5)	30 (2)	0	1.06 (0.11)	
$-\alpha/\alpha\alpha$	>67	14.3 (1.3)	12.6 (1.3)	81.5 (7.2)	26.2 (2.4)	0	0.88 (0.12)	
$\alpha^+\alpha/\alpha\alpha$	>18	14.5 (0.9)	12.6 (0.6)	74.9 (4.9)	24.6 (1.7)	0	0.75 (0.12)	
$-\alpha/-\alpha$	>23	14.4 (1.1)	12.0 (1.1)	71.8 (4.4)	23.0 (1.3)	0	0.72 (0.12)	
$--/\alpha\alpha$	>41	13.9 (1.1)	12.1 (1.0)	69.0 (5.0)	21.7 (1.8)	0	0.66 (0.11)	
$\alpha^T\alpha/-\alpha$	9	12.4 (1.1)	10.5 (0.6)	66.1 (3.5)	21.2 (1.6)	0	0.72 (0.18)	
$\alpha^T\alpha/\alpha^T\alpha$	5	11.2 -	9.3 -	60.6 -	18.7 -	13.2	0.47	
$-\alpha/--$	>55	10.8 (0.8)	9.3 (1.1)	64.6 (7.6)	19.3 (2.3)	6.4 (4.4)	0.43 (0.19)	
$\alpha^T\alpha/--$	6	10.7 -	8.7 -	69 -	19.6 -	22.9 -	0.32	

From Ref. 4

TABLE 5

Summary of genotype and blood picture in the various forms of α thalassaemia

Phenotype	Equivalent Number of Functional α -Genes	Level of Hb Barts at Birth ¹	Hb H Inclusions	MCV ² (fl)	MCH ² (pg)	α/β Globin Chain Synthesis Ratio	Interacting Haplotypes	Most Frequently Encountered Genotypes
Normal	4	0 ³	0 (none)	85-100	~28	~1.0	α/α	$\alpha\alpha/\alpha\alpha$
α^+ Thalassaemia Trait	3	0-2	0 (rare)	75-85	~24	~0.8	α^+/α	$-\alpha/\alpha\alpha$
α^0 Thalassaemia Trait	2	2-8	0 (occasional)	65-75	~20	~0.6	α^0/α or α^+/α^+	$-\alpha/\alpha\alpha$ $\alpha\alpha^T/\alpha\alpha$ $-\alpha/\alpha$
Hb H Disease	1	10-40	2-40% (many)	55-65	~20	~0.3	α^0/α^+ or α^+/α^+	$-\alpha/\alpha$ $-\alpha/\alpha^T$ $\alpha\alpha^T/\alpha\alpha^T$
Hb Barts Hydrops Fetalis	0	~80	present	110-120	reduced	0.0	α^0/α^0	$-\alpha/\alpha$ $-\alpha/\alpha^T$

1 Hb Barts gradually disappears from peripheral blood in the 3-6 months following birth.

2 These values vary with age. The figures given here are representative values for adults.

3 Very small amounts of Hb Barts (<0.5%) have been detected in normal newborns. Also, not all infants with α thalassaemia produce detectable Hb Barts.

TABLE 6

Methods for diagnosis of α thalassaemia, and their limitations.
For correct diagnosis, all the methods may be needed.

METHOD	LIMITATION
In Adults	
Microcytosis α^0 picture (MCH<25) α^+ picture (MCH 24+)	may represent $-/\alpha\alpha$ or $-\alpha/-\alpha$ extensive overlap with normal
Low or normal Hb A ₂	extensive overlap with normal may represent "normal A ₂ " β thalassaemia
Globin biosynthesis $\alpha<\beta$	unreliable for less severe forms, except in very expert hands
DNA studies:	
restriction fragment length oligonucleotide probes DNA sequencing	does not detect non-deletional forms detect only known mutations needed to define new non-deletional forms
In Newborns	
Hb Bart's on electrophoresis microcytosis DNA studies	absent in 30% of $-\alpha^{3.7}/\alpha\alpha$ overlap with normal range as above

TABLE 7

Lengths of restriction fragments produced in different forms of α -thalassaemia, by the enzymes Bam HI and Bgl II, detected by α and ζ probes.

Haplotype	α -probe		ζ probe	
	Bam HI	Bgl II	Bam HI	Bgl II
$\alpha\alpha$	14	<u>12.6</u> , 8.0	10.5, 5.9	<u>12.6</u> , 11.0*
$-\alpha^{3.7}$	10.5	<u>16.0</u>	10.5, 5.9	<u>16.0</u> , 11.0*
$-\alpha^{4.2}$	10.0	<u>8.0</u>	10.5, 5.9	<u>8.0</u> , 11.0*
..SEA	-	-	~20, 5.9	10.5, 10.5*
..MED	-	-	5.9	13.5

* band size may vary due to the presence of the interzeta hypervariable region

Underlined fragments are the same fragments identified by either α or ζ probes.

TABLE 8
Estimated approximate global numbers (millions) of heterozygotes carrying α -thalassaemia

	Population (millions)	% of the Population Heterozygote for:		Number of People (millions) Heterozygous for:		Comments
		GpI (severe)	GpII (mild)	GpI (severe)	GpII (mild)	
AFRICA						
North	116	-	2.3	-	3	α -thalassaemia very rarely causes pathology
North-east	38	-	-	-	-	
East	82	-	ca 30	-	25	
South	34	-	-	-	-	
Islands	11	-	ca 30	-	3	
Sub-Saharan	212	-	ca 30	-	64	
Total	493	-	ca 19	-	95	
AMERICA						
North	257	rare	3.4	+	9	Pathology is uncommon; it occurs only where α^0 thalassaemia genes have been brought from S.E. Asia
Central	97	-	-	-	-	
South	252	rare	7.2	+	18	
Caribbean	31	rare	ca 30	+	9	
Total	637	+	ca 6	+	36	
ASIA						
North-west	161	0 - 2	ca 10	0.16 ??	16	α -thalassaemia is an important public health problem
South	924	-	ca 40	-	370	
North-east	915	-	-	-	-	
South-east	663	2.0	ca 30	13.3	200	
Total	2,663	0.5	ca 22	13.5	586	
EUROPE						
North-west	237	occurs	1.5	+	3.5) As above for America) α^0 thalassaemia is endemic at low frequency in parts of south Europe
North-east	79	-	-	-	-	
South	172	1-2% locally	0 - 30	+	ca 1	
USSR	270	?	?	?	?	
Total	758	+	min 0.5	+	min 3.5	
OCEANIA						
Total	28	very rare	5	+	1	As above for America
GLOBAL TOTAL	4,579	0.3	16	>13.5	720	

TABLE 9
Relative birth incidence of infants with α^0 hydrops fetalis
and Hb H disease in Bangkok and Singapore

Place	α^0 thalassaemia trait	Prevalence of α^+ thalassaemia trait	Birth Incidence per 1,000 of Hydrops Fetalis	Hb H Disease	Ratio Hb H Disease/ α thalassaemia hydrops
Singapore	3.5	3.5	0.3	0.6	2
Bangkok	4.0	16.0	0.4	3.2	5

TABLE 10

Estimates of the number of α -thalassaemia heterozygotes, and the birth-rate of affected infants (α -thalassaemia hydrops fetalis and Hb H disease) in South-east Asia

Country	Population (millions)	Birth Rate 1,000	Annual Births 1,000	% of Population Heterozygous for:		Births/1,000 of infants with:		Numbers/year born with:	
				$\alpha^0\alpha^0$	$\alpha^+\alpha^+$	Fetalis $\alpha^0\alpha^0$	Hb H Harmless $\alpha^+\alpha^+$	α -thal Hydrops	Hb H Disease
China ¹ :									
Guangdong	59.3	25.0	1,482	3.5	3.5	0.3	0.6	445	890
Guangxi	36.4	27.3	994	8.8	14.2	1.9	6.2	1,890	6,160
Brunei	0.2	29.8	6	2	?	?	?	?	?
Burma	35.9	38.6	1,386	?	8?	?	?	?	?
Kampuchea	7.0	30.9	216	4	16	0.4	3.2	86	690
East Timor	0.8	44.1	35	2?	?	?	?	?	?
Hong Kong	5.2	16.5	86	3.5	3.5	0.3	0.6	26	52
Indonesia	153.0	33.6	5,142	?	0.5?	?	?	?	?
Laos	3.9	44.1	69	4?	20	0.4	4.0	28	280
Malaysia	14.8	33.1	489	3.5	5.0	0.3	0.9	147	440
Philippines	50.7	36.6	1,837	2.0	?	0.1	?	>184	?
Singapore	2.5	17.2	42.5	3.5	3.5	0.3	0.6	13	26
Thailand	48.5	32.3	1,565	4.0	16.0	0.4	3.2	630	5,010
Viet Nam	56.2	40.1	2,254	?	?	?	?	?	?
TOTAL	474.4	33	15,604			0.2	0.87	>3,450	>13,550

¹ Information based on adequate screening is available only for Guangxi (personal communication with Dr Wu Guanyun) and Guangdong. Therefore the figures quoted are minimum figures only.

TABLE 11

Correlation of phenotype with genotype in 21 Greek patients with hemoglobin H disease

Patient	Age at Diagnosis	Spleen Enlargement cm	Hb g/dl	Hb H %	Clinical Phenotype	Genotype
AG	65 y	4-5	10.0	3.2	mild	--MED/-α3.7
SA	40 y	5	8.5	3.4	mild	--MED/-α3.7
PQ	27 y	2	11.6	8.3	mild	-(α)20.5/-α3.7
MN	4 m	2	9.5	4.2; HbS 12%	intermediate	--MED/-α3.7
SK	13 y	3	7.5	5.0	intermediate	--MED/-α3.7
AK	4 y	2	9.0	9.4	intermediate	--MED/-α3.7
KT	5 y	3	9.5	6.7	intermediate	--MED/-α3.7
KK	4 y	0	9.5	8.0	intermediate	--MED/-α3.7
ML	6 y	3	8.5	6.8***	intermediate	--MED/-α3.7
KA	4.5 y	3	9.3	1.9	intermediate	-(α)20.5/-α3.7
KR	3.5 y	0	9.3	1.8	intermediate	-(α)20.5/-α3.7
AB	2.5 y	2	10.6	3.7	intermediate	-(α)20.5/-α3.7
BE	2 y	2	9.5	6.8	intermediate	-(α)20.5/-α3.7
FO**	12 m	3-4	7.8	21.6	severe	-(α)20.5/ααT
BI**	2.5 y	2	7.7	18.5	severe	--MED/ααT
PL	9 m	5-6	7.6	23.7***	severe	--MED/ααT
PTa*	7 m	Splenectomy	6.4	14.8	severe	--MED/ααT
PTb*	5 m	7-8	4.2	17.9	severe	--MED/ααT
IA**	9 m	4	8.0	20.0	severe	--MED/ααT
TS**	3 m	3	7.5	8.0***	severe	ααT/ααT
AL**	12 m	3-4	7.9	16.8	severe	ααT/ααT

* Regular transfusion

** Occasional transfusion

*** HbH + Hb Barts

TABLE 12

Type of assistance required at delivery by 63 women with Hb Bart's hydropic fetuses

	Number	Normal Delivery	Breech Assisted	Forceps	Caesarian section	Embryotomy
Gestational age known	32 ¹	15	4	4	7	2
Gestational age not known	31	18	5	6	2	0
Total	63	33	9	11	9	2
X of Total	100	52	14	17	14	3

¹ Mean gestational age at delivery was 33.5 weeks, SD 4.1 weeks

ANNEX 1

MOLECULAR BASIS FOR THE ALPHA THALASSAEMIAS

The α -globin gene cluster

Figure 1 shows that each α -gene is located within a region of homology approximately 4 kb long, interrupted by two short non-homologous regions. The homologous regions probably arose from an ancient duplication event, and subsequently became divided by insertions and deletions, leaving three large homologous subsegments (the X, Y and Z "boxes"). The homologous Z boxes are 3.7 kb apart, but the greater length of the non-homologous segment in the $\alpha 2$ domain causes the X boxes to be 4.2 kb apart. Within the α globin gene cluster there are two hypervariable regions (see Fig. 1 in main text). They lead to considerable variation in the structure of the α -globin gene complex in normal individuals, with no apparent effect on the expression of the α -globin genes.

Although most non-thalassaemic individuals have four α -globin genes ($\alpha\alpha/\alpha\alpha$), about two percent of individuals in most populations have five α genes ($\alpha\alpha\alpha/\alpha\alpha$). Individuals with five or even six α genes may produce excess α globin, but their haematological picture is essentially normal. It is not known whether the triple α gene has a selective advantage, but certainly its relatively common occurrence indicates a high general frequency of unequal crossing over between the 2 α -gene loci.

Mutations of both non-deletional and deletional types that cause decreased function of the α -globin genes, i.e., α -thalassaemias, are very common. Deletions seem to have occurred particularly frequently through unequal cross-over events due to misaligned pairing of the two α genes.

Alpha-plus thalassaemia due to deletions

The mechanism by which the α^+ thalassaemia deletions occur is determined by the molecular structure of the α -globin gene complex. Misalignment and crossing-over between the "wrong" Z and X boxes at meiosis can give rise to chromosomes with either single ($-\alpha$) or triplicated ($\alpha\alpha\alpha$) α globin genes (Fig. 2). Crossing-over between misaligned Z boxes usually involves exchange between the $\alpha 2$ and $\alpha 1$ genes. It deletes 3.7 kb of DNA and leaves a single $\alpha 2$ or $\alpha 1/\alpha 2$ hybrid gene on the chromosome. This is referred to as a rightward deletion, $-\alpha^{3.7}$. Crossing-over between misaligned X boxes does not involve exchange between the $\alpha 1$ and $\alpha 2$ genes. It deletes 4.2 kb of DNA, and leaves a single $\alpha 1$ gene on the chromosome. This is referred to as a leftward deletion $-\alpha^{4.2}$. (The corresponding triplicated α gene arrangements are $\alpha\alpha\alpha^{\text{anti } 3.7}$ and $\alpha\alpha\alpha^{\text{anti } 4.2}$). These deletions can be identified relatively easily since they remove some restriction-enzyme sites, and so cause DNA restriction fragments carrying deletional α -thalassaemia genes to have characteristic lengths, and to travel in characteristic positions on electrophoresis, where they can be located by using α -gene probes.

Rearrangements involve the longer Z box more often than the shorter X region. Recently it has been possible to subdivide the common Z box rearrangements into three subtypes ($-\alpha^{3.7I}$, $-\alpha^{3.7II}$, $-\alpha^{3.7III}$) (fig. 3) depending on the position of the cross-over site relative to three restriction enzyme sites that differ between the $\alpha 2$ and $\alpha 1$ Z boxes. In general the frequency of these subtypes and $-\alpha^{4.2}$ seem to be simply related to the length of homology within each subsegment, suggesting that crossing over is equally likely to occur at any point within the DNA segment. Y box crossing-over has not yet been identified. An unexpected $-\alpha^{3.5}$ deletion has recently been found in an Asian Indian patient. Determination of the sequence of this breakpoint will show if it involves a homologous (as in the $-\alpha^{3.7}$ and $-\alpha^{4.2}$) or an illegitimate recombination event.

The level of expression of the single remaining α gene ($-\alpha$) can be assessed by the effect on the blood picture (see text Table 3), and more directly by measuring the production of α -specific mRNA from such chromosomes. All five deletions ($-\alpha^{3.1}$, $-\alpha^{3.7II}$, -

$\alpha^{3.7111}$, $-\alpha^{4.2}$, $-\alpha^{3.5}$) reduce α chain production from the affected chromosome to a similar extent, the remaining α gene being expressed at a level intermediate between that of a normal $\alpha 1$ and $\alpha 2$ gene. The red cell characteristics of homozygotes for the $-\alpha^{4.2}$ determinant, in whom only $\alpha 1$ genes are present, and homozygotes for the $-\alpha^{3.7111}$ determinant, in whom only $\alpha 2$ genes remain, are also very similar. This suggests that removal of the more active $\alpha 2$ gene results in a partial, compensatory increase in the expression of the remaining $\alpha 1$ gene on the $-\alpha^{4.2}$ chromosome.

Alpha-zero thalassaemia due to deletions

To date, 12 deletions have been described that involve both α genes and thereby abolish α chain production from the affected chromosome (Fig. 4). Most are large (5.2 kb to 62 kb) and the mechanism by which they have been generated is poorly understood. Detailed analysis suggests several general principles. Firstly, several 3' breakpoints fall within a 6-8 kb region at the 3' end of the alpha globin complex, suggesting a breakpoint cluster region similar to those observed in the chromosomal translocations associated with certain malignant diseases. In a subset of the deletions ($-\text{MED}$, $-\text{SEA}$, $-\text{20.5}$, $-\text{SA}$, $-\text{BRIT}$) the 5' breakpoints are staggered at approximately the same distance apart, and in the same order along the chromosome, as their respective 3' breakpoints, resembling a group of deletions in the β globin gene-cluster. Such staggered deletions may result from illegitimate recombination events that delete an integral number of chromatin loops during DNA replication.

One deletion ($-\text{MED}$) involves a more complex rearrangement that introduces a new piece of DNA between the two breakpoints in the α gene cluster. The new DNA originates from upstream of the α cluster and has been replicated into the junction in a manner suggesting that the upstream segment of DNA also lies at the base of a replication loop - which may lie close to the bases of the proposed replication loops involved in the group of clustered deletions described above. Sequence analysis shows that members of the dispersed family of Alu repeats are often found at or near the breakpoints of these deletions, and in one case ($\alpha\alpha^{\text{RA}}$) the deletion resulted from simple homologous recombination between two Alu repeats that are normally 62 kb apart. Possibly Alu family repeats will be found to play a part in similar recombination events elsewhere in the genome.

Some ζ gene activity persists when the α genes are deleted, as in α^0 -thalassaemia hydrops fetalis, where a significant proportion of Hb Portland is found together with Hb Barts ($\gamma 4$) at birth. Traces of ζ gene product may also be found in heterozygotes for α^0 thalassaemia, indicating that this deletion allows some derepression of the ζ locus.

Deletional α^0 thalassaemia gives no band with an α -probe but a ζ -gene probe may be used to confirm that DNA digestion has occurred correctly.

By contrast with the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ defects, the α^0 deletions have a limited geographical distribution, and there is evidence that each one has arisen only once during evolution.

Non-deletional α -thalassaemias

Analysis of DNA from patients with non-deletional α thalassaemias reveals no gross abnormality. Present information on the location and nature of these mutations is summarised in figure 5. Most are due to single or oligonucleotide mutations at regions of the α -gene sequence that are critical for normal expression. Similar mutations in the β -globin gene are much more common.

Most of the known non-deletional mutants affect the $\alpha 2$ gene, possibly because the $\alpha 2$ gene is the more active of the two, so its mutations have more effect on red cell characteristics and presumably a greater selective advantage. It is possible that mild forms of non-deletional α^+ thalassaemia affecting the less active $\alpha 1$ gene and causing minimal microcytosis and no detectable $\delta 4$ in the newborn, are common but have so far evaded detection. By contrast with deletional defects, expression of the $\alpha 1$ gene is not increased when the $\alpha 2$ gene is inactivated by a point mutation.

Like the deletions that cause α^0 thalassaemia, molecular lesions leading to the non-deletional α thalassaemias have arisen relatively infrequently and each one seems to be quite limited in its geographical distribution. As with the β thalassaemias they are classified according to the level of gene expression that they permit.

Two non-deletion mutants that affect processing of the primary mRNA transcript have been identified. One is a pentanucleotide deletion involving the invariant GT donor splicing sequence (GGT GCT) of IVS I of the $\alpha 2$ globin gene (α^{Hph}), which prevents the normal removal of IVS I during processing. The second, $\alpha\alpha^{\text{T SAUDI}}$, involves the poly (A) addition signal (AATAAA \rightarrow AATAAG) of the $\alpha 2$ gene and interferes with 3' end-processing and possibly, with termination of transcription. Apparently failure to correctly terminate transcription of the $\alpha 2$ gene also down-regulates the linked $\alpha 1$ gene, causing a particularly severe α^+ thalassaemia.

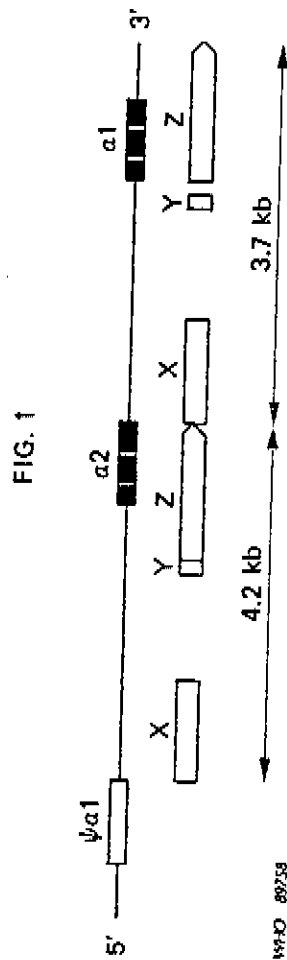
A second group of mutations exert their effect by interfering with the translation of mature mRNA. In one case (α^{Nco}) the initiation codon is completely inactivated by a T \rightarrow C transition (CATGG \rightarrow CACGG), and in another the efficiency of initiation is reduced by a dinucleotide deletion in the consensus sequence around the start signal (CACCATG \rightarrow CCCGATG). Four mutations specifically change the termination codon (TAA) and prevent normal termination of translation, each giving rise to elongated abnormal α -globin chains: Hb Constant Spring (α^{CS}), Hb Icaria (α^{I}), Hb Koya Dora (α^{KD}), and Hb Seal Rock (α^{SR}). Another mutation identified in a Black patient from Mississippi (α^{MS}), causes premature termination of translation by changing codon 116 in Exon III to an in-phase terminator (CAG \rightarrow UAG). Hb Quong Sze (α^{QS}), Hb Suan Dok ($\alpha\alpha^{\text{SD}}$), Hb Patah Tikvah ($\alpha\alpha^{\text{PT}}$) and Hb Evanston are structural mutations that cause α -thalassaemia by giving rise to highly unstable α -globin chains.

Among the many non-deletion α thalassaemia mutations that remain to be characterized, those associated with the Hb Bart's hydrops fetalis syndrome are particularly interesting. They may turn out to be due to a Saudi Arabian type defect, or may be even more severe.

Acquired lesions of the α -globin genes

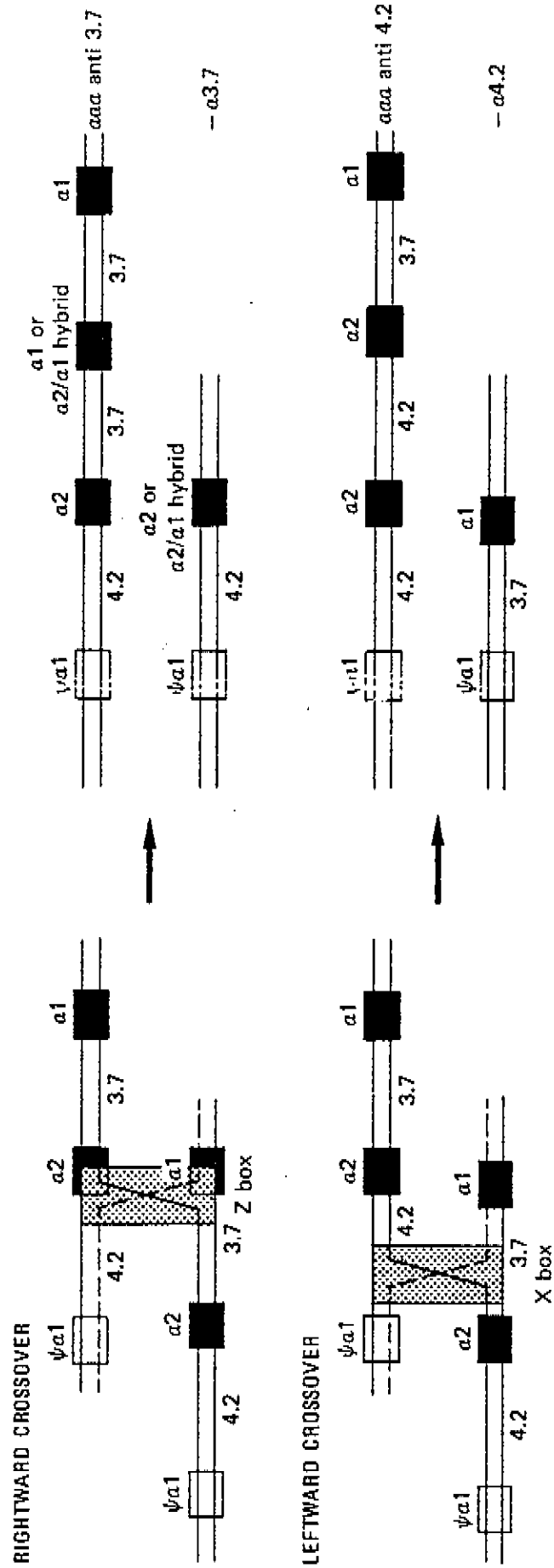
Hb H disease can occasionally be acquired by previously completely normal individuals who develop a myeloproliferative syndrome. The structure of the α -globin gene complex appears to be normal, but production of α -specific mRNA and α -globin chains is severely reduced. There seems to be an acquired defect in transcription of the α -genes, although the precise molecular mechanism and its relationship to the hematological malignancy is not yet known. This form of α thalassaemia is more frequent in males than females.

A second unusual type of α thalassaemia is associated with mental retardation. Family studies show that neither parent carries a severe α thalassaemia determinant, but in every case at least one chromosome 16 of the patient has been affected by a new mutation that causes α thalassaemia, and is also associated with mental retardation (IQ range <50 - 76) and other developmental abnormalities including microcephaly, hypogonadism, hypotonia, telocanthus, and mild skeletal abnormalities. Cytogenetic analysis has revealed no gross abnormality of chromosome 16 in any of these cases. In two cases a de novo deletion extending for at least 27 kb and involving the entire α gene complex has been found, but in other cases there is no detectable rearrangement around the α -gene complex. In cases where the α genes are deleted, other critical neighbouring genes may also be removed, giving rise to the associated developmental abnormalities. In cases where the α gene complex appears to be intact, expression of the α -genes may be affected by deletions of neighbouring genes that do not extend into the α -complex. Other well-documented large deletions of the α and β globin gene complexes apparently affect only globin gene expression, so in this syndrome we might expect very large deletions indeed.



Regions of homology in the α -globin gene cluster. The homologous X, Y and Z boxes are separated by non-homologous regions of different lengths. Thus unequal crossing-over may delete different lengths of DNA. The α -globin genes each have 2 introns. The $\alpha 2$ globin gene produces 2-3 times more mRNA than the $\alpha 1$ gene. Most known non-deletional mutations involve the $\alpha 2$ gene.

FIG. 2



WHO 89759

Methods of formation of the two main types of α -thalassaemia genes. Unequal crossing over involving the Z boxes (above) leaves a single hybrid α globin gene. Unequal crossing over involving the X boxes (below) leaves a single, usually $\alpha 1$ globin gene.

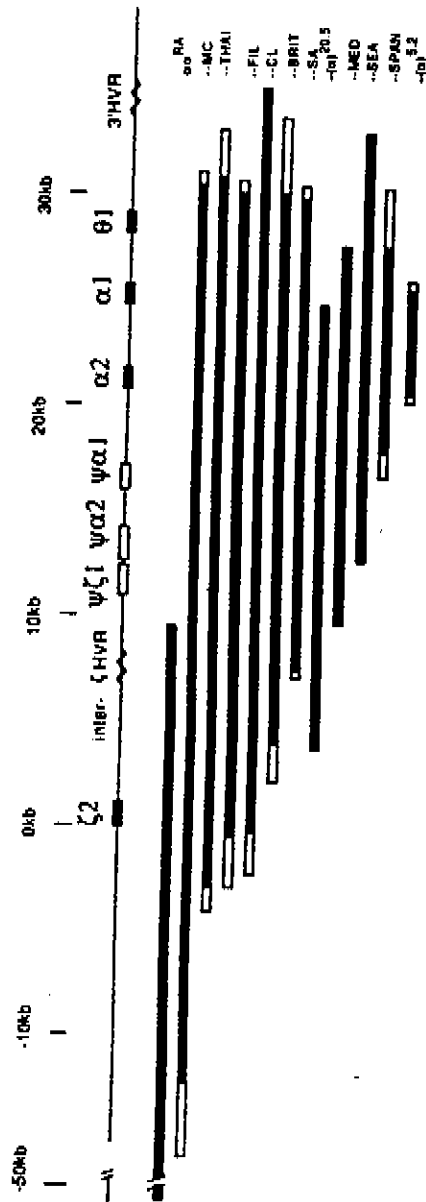
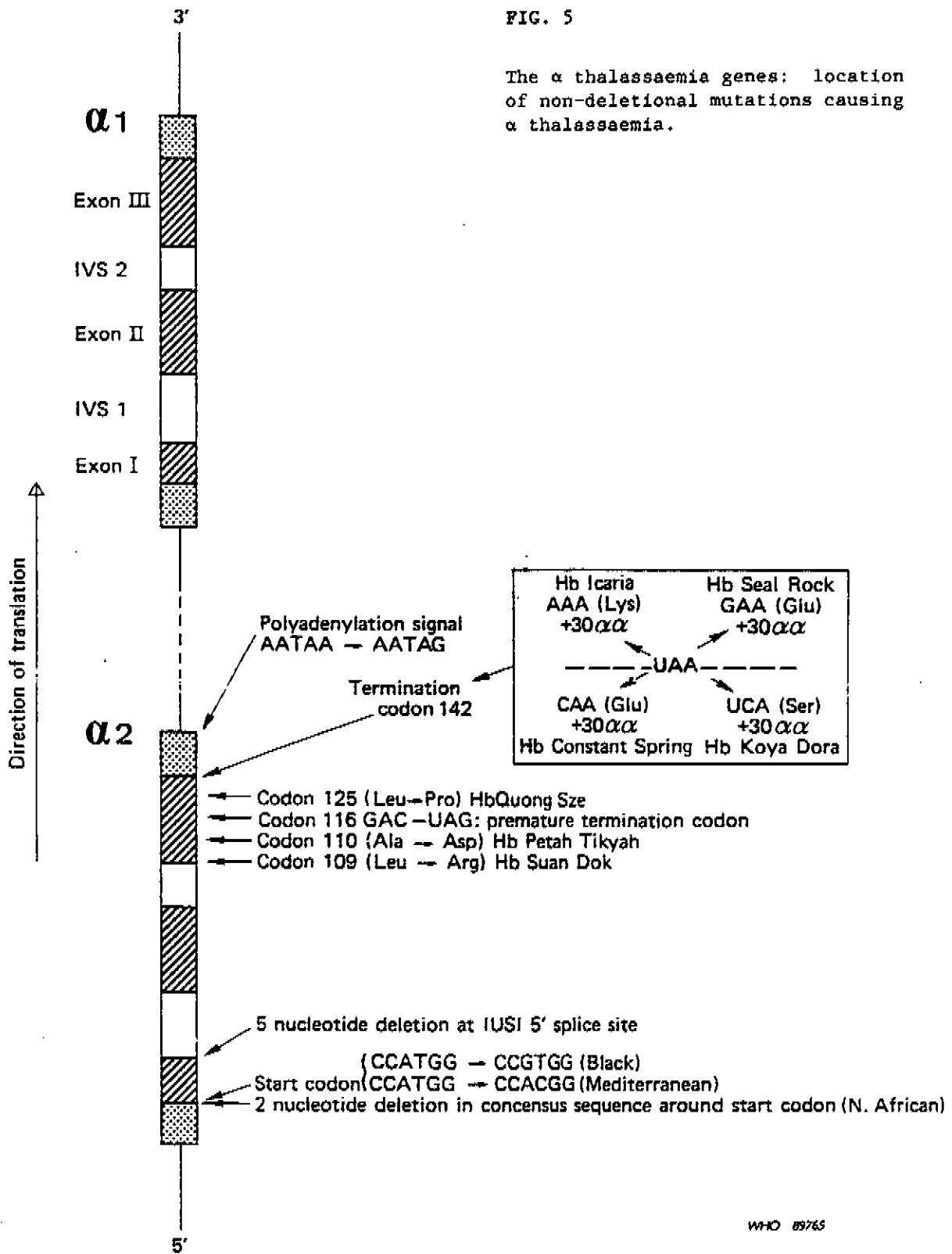


FIG. 4

Known extent of deletions leading to α^0 thalassaemia.



ANNEX 2

Educational Booklet for Genetic Counselling for Alpha Thalassaemia Trait

Producing educational materials is a difficult task requiring collaboration between doctors and specialists in health education. It is necessary for doctors to write the information that should be conveyed, and for specialist health educators to adapt it for the general public, with reference to local needs.

The draft that follows represents stage one of this process and is intended only as a basis for further work by local health educators.

WHAT YOU NEED TO KNOW ABOUT ALPHA THALASSAEMIA TRAIT

Dear Reader,

There are several kinds of thalassaemia. This booklet is for people who have had a blood test that shows they carry alpha thalassaemia trait (this is usually written α thalassaemia trait).

α thalassaemia trait is not an illness, and will not affect your health.

It is not the same as beta thalassaemia trait (written as β thalassaemia trait).

There are two kinds of α thalassaemia trait:

(a) Alpha-plus (α^+) thalassaemia trait is very common, and is almost always harmless.

(b) Alpha-zero (α^0) thalassaemia trait is uncommon, and could be a problem for your children. So if you have α thalassaemia trait it is important to know which type.

This booklet gives information about both types.

Do not forget that you carry α -thalassaemia trait. Keep the blood test report or thalassaemia card permanently with your medical record.

If you want more information after you have read this booklet, ask your doctor to arrange a visit to a genetic counsellor.

Take this booklet with you if you go to see your doctor about your α -thalassaemia trait.

WHAT IS "THALASSAEMIA"?

Thalassaemia is a peculiarity of the blood that is common among people originating from the Mediterranean area, the Middle East, or Asia. It is rare in North Europeans.

There are two main forms of thalassaemia: alpha thalassaemia and beta thalassaemia (α thalassaemia and β thalassaemia).

When people talk about thalassaemia, they usually mean β thalassaemia, because it causes problems more often than α thalassaemia. You can obtain a separate booklet about β thalassaemia - "Educational Materials on Thalassaemia" (WHO/HDP/EMT/90.1), available free of charge in English (Arabic, Chinese, French, Russian and Spanish translations will be available in autumn 1990) from the Hereditary Diseases Programme, Division of Noncommunicable Diseases and Health Technology, World Health Organization, 1211 Geneva 27, Switzerland.

When a person carries α thalassaemia, they are said to carry α thalassaemia trait.

There are two types of α thalassaemia trait:

(a) Alpha plus (α^+) thalassaemia trait is extremely common, and nearly always completely harmless.

It is carried by about

- one third of people originating from Africa.
- half the people of India and Pakistan.
- many people from the Mediterranean area, particularly Cyprus, Sardinia, Greece or South Italy.
- many people from the Middle East.

(b) Alpha zero (α^0) thalassaemia trait is quite uncommon.

It does no harm to the people who carry it, but it could affect the health of their children.

It is carried by about

- one in thirty people originating from south-east Asia (south China, Hong Kong, Singapore and Thailand).
- one in a hundred people originating from Cyprus or parts of Greece.

One of the problems with α thalassaemia is that it can be quite difficult to distinguish α^+ and α^0 thalassaemia trait.

HOW CAN I BE SURE IF I HAVE α^0 OR α^+ THALASSAEMIA?

If you, or your ancestors, come from Africa, India or Pakistan, you will have α^+ thalassaemia. You are extremely unlikely to have α^0 thalassaemia, and you have nothing to worry about.

But if you have α thalassaemia and you or your ancestors come from Cyprus, Greece, the Middle East, south-east Asia (Thailand, Vietnam, Kampuchea, Laos), South China or Singapore, you could have α^0 thalassaemia trait. This would not do you any harm, but it could affect your children. You may be advised to bring your partner for a test before you have children. If your partner does not have any type of α thalassaemia, there will be no risk for your children, and you have nothing to worry about. But if your partner's blood test result shows any peculiarity, you should see an expert in haemoglobin disorders for further testing, and advice.

If you are in any doubt about the type of α thalassaemia you carry and you need to find out, go to see your doctor, and take this booklet with you.

BLOOD AND ANAEMIA

To explain about thalassaemia, we need to talk a little about normal blood and about anaemia.

What is blood made of?

Blood is made up of a lot of red blood cells in a clear, slightly yellow liquid called plasma. Blood is red because the red blood cells contain a substance called haemoglobin. Haemoglobin is very important because it carries oxygen from your lungs to wherever it is needed in your body. It contains a lot of iron. In fact, the main reason why people need iron in their food is to make haemoglobin.

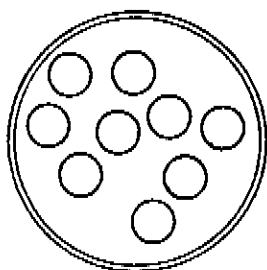
What is anaemia?

Some people have too little haemoglobin in their blood. These people have anaemia. There are many different kinds of anaemia. The most common kind is iron deficiency anaemia. This happens when people are not eating enough of the foods that contain iron. Some people who carry thalassaemia have a very mild anaemia, but it has nothing to do with the amount of iron you are getting from your food. It is inherited.

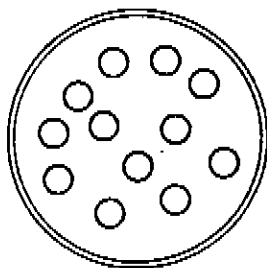
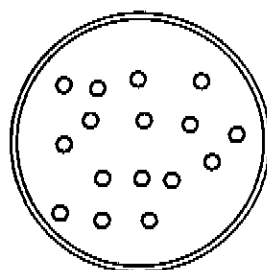
WHAT DOES ALPHA THALASSAEMIA TRAIT MEAN FOR ME?

People with α thalassaemia trait are perfectly healthy: only a few have a slight anaemia. That is why most people with α thalassaemia trait do not know they have it. They only discover it through having a special blood test.

The red blood cells of people with α thalassaemia trait are smaller than the usual kind of red cells. In α^+ thalassaemia the red-cells are about three-quarters the usual size, in α^0 thalassaemia they are about two-thirds the usual size.



Usual red cells

 α^+ thalassaemia red cells α^0 thalassaemia red cells

α thalassaemia trait is present in the fetus before birth, it remains the same throughout life, and can be handed from parents to children. That is, it is inherited.

WHY DO YOU NEED TO KNOW IF YOU CARRY α THALASSAEMIA TRAIT?

Sometimes people with α^0 thalassaemia trait can have babies born with a very severe anaemia. If you have α^0 thalassaemia trait it is important to know about this risk.

A very few people with α^+ thalassaemia trait can have children with a milder anaemia called Hb H disease. If you have α^+ thalassaemia trait, you have a very small risk of having children with anaemia.

IS A THALASSAEMIA CARRIER ILL?

No, so there is no need for any medical treatment.

ARE THERE ANY OTHER PROBLEMS?

No. Thalassaemia carriers are not more likely to get any other illnesses, nor are they weak in any way, or limited in their choice of job.

CAN ANY TREATMENT CHANGE α THALASSAEMIA TRAIT?

No. If you are born with thalassaemia trait, you will always have it.

CAN α THALASSAEMIA TRAIT TURN INTO A SEVERE FORM OF THALASSAEMIA?

No. It cannot.

DO α THALASSAEMIA CARRIERS EVER NEED IRON?

Yes, they sometimes do, but it is important that you only have iron medicine if you really need it. The best way to be sure a thalassaemia carrier needs iron is by a blood test

to measure the amount of iron in your blood. If you do not have this test, the doctor may think that you are short of iron simply because you have small red blood cells and a slight anaemia, and may advise you to keep taking extra iron even when you do not need it. This will do you no good, and in the long-run it could be harmful.

WHAT ABOUT PREGNANT WOMEN?

Pregnant women with thalassaemia trait need extra iron just as much as other pregnant women.

WHY IS α THALASSAEMIA TRAIT FOUND IN CERTAIN COUNTRIES?

People with α thalassaemia trait are less likely to die if they catch malaria. In the past, in countries where malaria was common, α thalassaemia trait was an important advantage because people with α thalassaemia trait survived malaria where other people died of it. These people passed the trait on to their children, so as time passed, it became more common in malarial parts of the world. But now we can usually cure or prevent malaria and thalassaemia trait is no longer an advantage. As it is inherited, it does not go away from a population when malaria disappears.

In every country where malaria is or was common a large number of people have α thalassaemia trait.

OTHER FORMS OF THALASSAEMIA TRAIT

This booklet is about α thalassaemia trait. It is important not to get it mixed up with other forms of thalassaemia.

Beta (β) thalassaemia trait is common in many of the places where α thalassaemia trait occurs. It has a similar effect on the people who carry it, but it causes rather more risk for their children. It is described in a separate booklet "Everything you need to know about thalassaemia trait".¹

Delta-beta- ($\delta\beta$)-thalassaemia trait and haemoglobin Lepore trait are both forms of β thalassaemia trait.

There are also four main types of abnormal haemoglobins. These are:

- HbS
- HbC
- HbD
- HbE

If someone has α thalassaemia trait and chooses a partner who has β thalassaemia trait, $\delta\beta$ thalassaemia trait, haemoglobin Lepore trait, or haemoglobin S, C, D or E, there is no risk that their children could have a severe anaemia. This problem can only ever arise if one α thalassaemia carrier chooses another α thalassaemia carrier as a partner. Even then, problems are not very common.

¹ See WHO information booklet on educational materials on thalassaemia, WHO unpublished document WHO/HDP/EMT/90.1, pages 2-9. Available free of charge in English (Arabic, Chinese, French, Russian and Spanish translations will be available in the autumn of 1990) from the Hereditary Diseases Programme, Division of Noncommunicable Diseases and Health Technology, World Health Organization, 1211 Geneva 27, Switzerland.

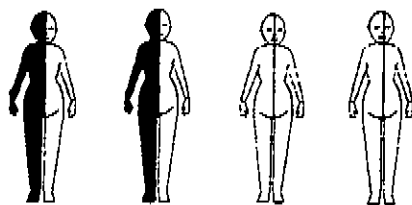
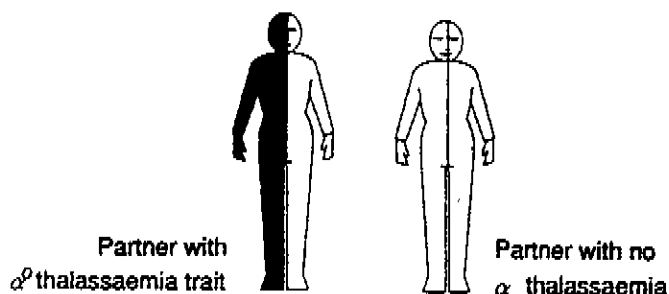
WHAT COULD MY α THALASSAEMIA MEAN FOR MY CHILDREN?

The risk is quite different for carriers of α^+ and α^0 thalassaemia trait. Most problems can arise for carriers of α^0 thalassaemia trait, so we will discuss this first.

WHAT ARE THE POSSIBLE RISKS FOR CARRIERS OF α^0 THALASSAEMIA TRAIT?

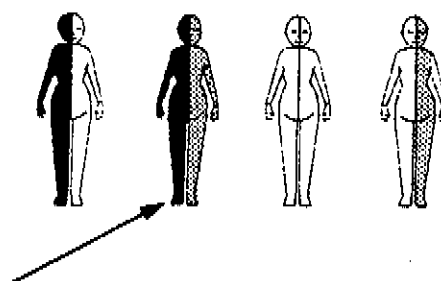
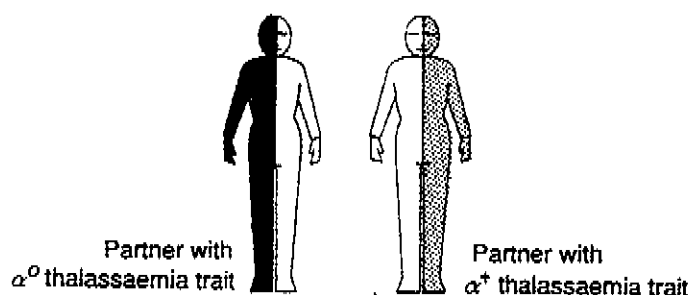
To answer this question, we must see how α thalassaemia is passed on from parents to their children. Let us consider three sorts of couples.

1. If a carrier of α^0 thalassaemia trait chooses a partner who carries no α thalassaemia at all, on average half the children will carry α^0 thalassaemia trait and half will have the usual type of blood. None of them will be ill with an important α thalassaemia. There is no risk for an α^0 thalassaemia carrier whose partner is not a carrier.



Half the children will carry α^0 thalassaemia, and half will not. All are healthy.

2. Sometimes a person with α^0 thalassaemia trait chooses a partner with α^+ thalassaemia trait. Most of their children will be completely healthy (half will carry α^+ thalassaemia and a quarter will not carry any type of thalassaemia). But a quarter (25%) will inherit α^0 thalassaemia from one parent and α^+ thalassaemia from the other. This leads to a type of anaemia called "haemoglobin H disease".



One out of 4 children (on average) may inherit α^0 thalassaemia from one parent and α^+ thalassaemia from the other. This child will have haemoglobin H disease. All the others are healthy.

What is haemoglobin H disease?

Children with HbH disease are anaemic: they have a haemoglobin level of 8-9 grams per decilitre (8-9 g/dl). The normal level is about 11-14 grams per decilitre (11-14 g/dl). So their haemoglobin level is lower than normal.

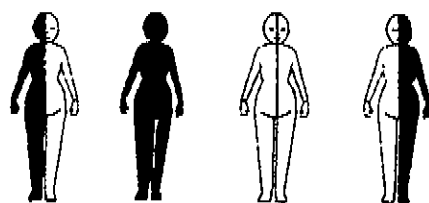
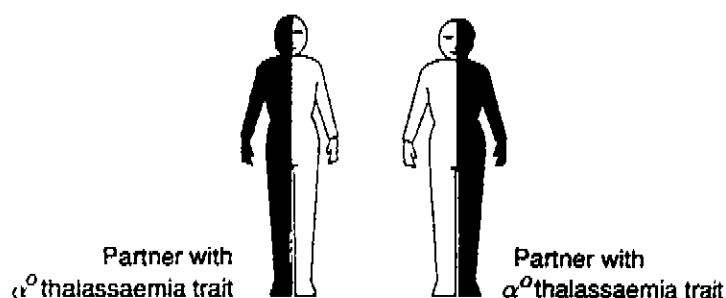
But people with haemoglobin H disease are usually quite well, and can work and have children like other people.

Once couples who could have children with HbH disease understand the situation, they are not really worried.

They usually ask to have the baby tested as soon as it is born, so that they can know the situation.

If the baby does have haemoglobin H disease, the parents are advised to attend a regular paediatric clinic a few times a year, just to check that the baby is developing well, and to make sure that there are no problems.

3. If by chance (rarely) a carrier of α thalassaemia trait chooses a partner who also carries α^0 thalassaemia trait, most of their children will be healthy. (They may carry α^0 thalassaemia trait, or they may have completely normal blood). But a quarter (25%) will inherit α^0 thalassaemia from both parents. They will have α thalassaemia major.



This fetus will be affected by α^0 thalassaemia hydrops fetalis.
All the other children will be healthy.

In each pregnancy there is a one in four (25%) chance that the child will have normal blood and two in four (50%) chance that the child will have α^0 thalassaemia trait. There is a 1 in 4 (25%) chance that the fetus will have α^0 thalassaemia major.

WHAT IS α^0 THALASSAEMIA MAJOR?

Another name for α^0 thalassaemia major is α thalassaemia hydrops fetalis.

This is a very serious anaemia that develops in the fetus. It can only happen when both parents carry α^0 thalassaemia trait.

The fetus cannot make enough haemoglobin, because its bone marrow cannot produce enough red blood cells. The red blood cells that are produced are nearly empty. As a result, the fetus becomes very anaemic and weak and its heart is not able to pump blood around properly.

The pregnancy seems to go normally up to about five months, sometimes for longer, but then the baby stops growing normally, and the mother may develop high blood pressure. An ultrasound examination may be done. This usually shows that the baby is "oedematous" - which means that it is puffed up, with too much water in it.

Usually the mother starts labour early, between 28 and 36 weeks of pregnancy, and the baby is dead or dying when it is delivered.

There is one in four (25%) chance of the same thing happening in any further pregnancies, so this is one condition that people are very eager to avoid.

This is why it is so important for people to know if they have α^0 thalassaemia trait, and whether their partner also carries it, before they decide to have a family.

CAN α^0 THALASSAEMIA MAJOR BE TREATED?

There is no treatment for α^0 thalassaemia major.

CAN α^0 THALASSAEMIA MAJOR BE PREVENTED?

When both partners carry α^0 thalassaemia trait, there are several ways to avoid having a stillborn baby. It is possible to tell very early on indeed in a pregnancy whether the fetus will be healthy, or suffers from α^0 thalassaemia major. Most couples who both carry α^0 thalassaemia trait ask the doctors to test each pregnancy to find out if the baby has α^0 thalassaemia major. This test can be done any time after 8 weeks after the last period. When the fetus is affected, it has no hope of a normal life, so parents usually wish to have the pregnancy terminated. Then they start again with another pregnancy, hoping to have a healthy child next time. Remember, there is a three quarters (75%) chance of a healthy child in each pregnancy!

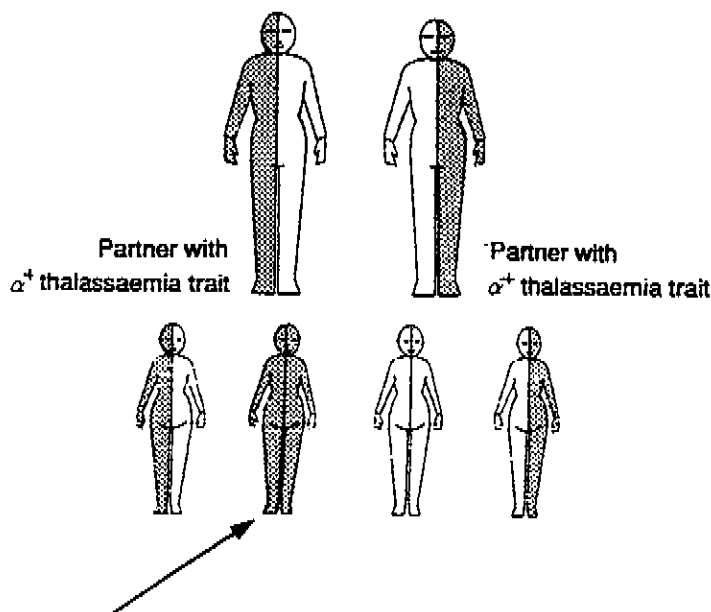
There are several other ways to avoid having children with α thalassaemia major. To find out more, ask your doctor to arrange for you to visit a genetic counsellor.

RISKS FOR CARRIERS OF α^+ THALASSAEMIA TRAIT

The most important risk for carriers of α^+ thalassaemia trait is the risk of a mistake. They could be told they carry α thalassaemia, and then people might think it is the severe form of α^0 thalassaemia.

There is a very small risk indeed that a carrier of α^+ thalassaemia trait will have children with anaemia. Let us consider several situations.

1. A carrier of α^+ thalassaemia chooses a partner who does not carry any form of thalassaemia. On average half the children carry α^+ thalassaemia and half will not, and none should suffer from a severe inherited anaemia.
2. A carrier of α^+ thalassaemia chooses a partner who also carries α^+ thalassaemia. In this case, a quarter (25%) of the children will inherit α^+ thalassaemia from both parents. However, α^+ thalassaemia is so mild, this simply leads to slightly smaller red blood cells, and the person is still perfectly healthy.

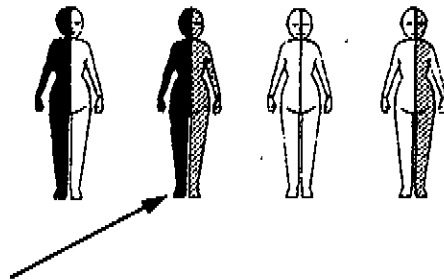
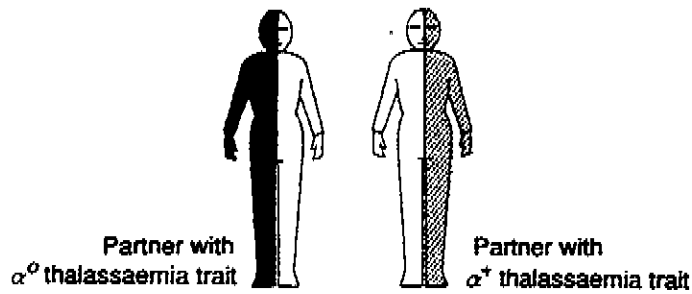


One out of 4 children (on average) may inherit α^+ thalassaemia from both parents. This child will be perfectly healthy.

All the other children will be healthy also.

3. Rarely a person with α^+ thalassaemia trait chooses a partner who has α^0 thalassaemia trait. Most of their children will be completely healthy (half will carry α^+ thalassaemia and a quarter will not carry any type of thalassaemia). But a quarter (25%) will inherit α^0 thalassaemia from one parent and α^+ thalassaemia from the other. This leads to a type of anaemia called "haemoglobin H disease" (see pages 50-52).

So in conclusion, most people who carry α^+ thalassaemia trait have no need to worry. They should think of themselves as normal in every way.



One out of 4 children (on average) may inherit α^0 thalassaemia from one parent and α^+ thalassaemia from the other. This child will have haemoglobin H disease. All the others are healthy.
