

# **The Role of Recent Studies of the Molecular Pathology of the Thalassaemias in Their Control and Management**

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## **Introduction**

The thalassaemias, globally the commonest human monogenic diseases, were the first to be characterised at the molecular level and hence to disclose the extensive repertoire of mutations that underlie human genetic disease. They are still among the few disorders of this kind for which there is some understanding of the relationship between diverse clinical phenotypes and molecular pathology. For the population geneticist they have provided a remarkable model of how human diversity has been shaped by selection working through variation in susceptibility to lethal parasitic infections. They were the first diseases to be diagnosed prenatally by recombinant DNA technology, and as knowledge of their pathophysiology has increased over the years, there has been a major improvement in their symptomatic management.

Paradoxically, however, the thalassaemias are likely to pose an increasing world health problem in the next millennium. In emerging countries, as rates of neonatal and childhood mortality decline due to better sanitation, nutrition and control of infection, babies with serious genetic diseases can survive the first few years of life. Figures published by the World Bank in 1993 show that in the 30 years between 1960 and 1990, there was a dramatic fall in deaths during the first five years of life throughout the Middle East, the Indian subcontinent and Southeast Asia. Already, thousands of children are being born with different forms of thalassaemia in these regions, many of whom are already living long enough to require treatment. As conditions continue to improve in the next millennium, this problem will become even more acute, and it is likely that thalassaemia will pose a major public health problem for Southeast Asia.

The most important forms of thalassaemia are  $\alpha$  thalassaemia,  $\beta$  thalassaemia and haemoglobin E, a structural haemoglobin variant that is synthesised at a reduced rate and hence has the phenotype of a mild form of  $\beta$  thalassaemia. The interactions of these three major forms of the disease produce a bewilderingly complex series of clinical phenotypes that are known, collectively, as the thalassaemia syndromes. Here, I shall review briefly current knowledge about the molecular pathology, pathophysiology, control and management of the more important varieties of these diseases.

## **Molecular Pathology of the Thalassaemias**

### ***The $\alpha$ Thalassaemias***

The  $\alpha$  globin gene cluster is situated close to the tip of the short arm of chromosome 16, a location which has important implications for the understanding of its molecular pathology. The cluster contains two functional  $\alpha$  genes,  $\alpha 2$  and  $\alpha 1$ , and a functional  $\zeta$  gene, the embryonic counterpart of the adult  $\alpha$  genes. This cluster, which also contains a number of pseudogenes, is highly polymorphic; hence, it is usually

possible to determine the parental origin of particular  $\alpha$  globin genes. Because there are two  $\alpha$  globin genes per haploid genome, the normal genotype is written  $\alpha\alpha/\alpha\alpha$ .

There are two main forms of a thalassaemia:  $\alpha^0$  thalassaemia and  $\alpha^+$  thalassaemia. In the  $\alpha^0$  thalassaemias, both linked pairs of  $\alpha$  globin genes are lost; hence, the homozygous genotype is written  $--/--$ , while heterozygotes are represented as  $\alpha\alpha/--$ . In the  $\alpha^+$  thalassaemias one of the linked pairs of a globin genes is lost; hence, the homozygous genotype is written  $-\alpha/-\alpha$  if the genes are lost by deletion, or  $aaT/aaT$  if they are inactivated by a point mutation or similar defect.

There are two important clinical types of a thalassaemia: the haemoglobin Bart's hydrops syndrome and haemoglobin H disease. The former represents the homozygous state for  $\alpha^0$  thalassaemia, while the latter usually represents the compound heterozygous state for  $\alpha^+$  and  $\alpha^0$  thalassaemia ( $--/a-$ ), although it may sometimes arise from the homozygous state for non-deletion forms of  $\alpha^+$  thalassaemia ( $\alpha\alpha^T/\alpha\alpha^T$ ).

Both the  $\alpha^0$  and  $\alpha^+$  thalassaemias are extremely heterogeneous at the molecular level. It is now clear that at least three different classes of deletions result in  $\alpha^0$  thalassaemia. First, there are deletions that involve the  $\alpha$  globin gene cluster, the commonest being those that are found among Mediterranean or Oriental populations,  $--_{MED}$  and  $--_{SEA}$ . The second, and much rarer, type of deletion is one involving the HS40 regulatory region; loss of this region completely inactivates the  $\alpha$  globin gene cluster in *cis*, that is, on the same chromosome. Finally, small subtelomeric deletions may result in  $\alpha^0$  thalassaemia. Deletion forms of  $\alpha^+$  thalassaemia are caused by unequal crossing over events at the linked  $\alpha 2$  and  $\alpha 1$  genes. The two most common events of this type lead to the production of chromosomes containing a single  $\alpha$  globin gene due to a deletion of either 3.7 kb or 4.2 kb, depending on the site of crossing over; the opposite chromosome contains triplicated  $\alpha$  genes. Because the site of crossing over that generates the 3.7 kb deletion varies, there are several different subtypes of chromosomes containing this deletion. Finally, the  $\alpha^+$  thalassaemias also result from point mutations that involve either the initiation, translation or termination of the  $\alpha$  globin genes; the commonest non-deletion form of a thalassaemia is that associated with Hb Constant Spring, which results from a point mutation in the  $\alpha 2$  globin gene termination codon, which leads to read-through of messenger RNA that is not normally utilised and to the inefficient synthesis of an elongated  $\alpha$  globin chain.

## **b Thalassaemia**

Well over 100 different mutations have been found to cause the clinical phenotype of  $\beta$  thalassaemia. Those that produce  $\beta^0$  thalassaemia usually involve nonsense or frameshift mutations, while the  $\beta^+$  thalassaemias result from mutations involving the splice junctions or the activation of new splice sites within exons or introns. Another class of mutations involve the promoter regions of the  $\beta$  globin genes. Apart from a deletion that involves the 3' end of the  $\beta$  globin gene that is common in certain Indian populations, deletions are not commonly found in  $\beta$  thalassaemia. There is, however, one interesting family of  $\beta$  globin gene deletions that involve the 5' end of the  $\beta$  gene but which leaves the  $\delta$  globin gene intact; these result in the phenotype of  $\beta$  thalassaemia with an unusually high level of Hb A<sub>2</sub>.

### ***The $\delta\beta$ Thalassaemias and Hereditary Persistence of Fetal Haemoglobin (HPFH)***

These conditions are of less clinical importance than the  $\alpha$  and  $\beta$  thalassaemias, but their co-inheritance with  $\beta$  thalassaemias may produce milder phenotypes and an understanding of their molecular basis has been important for shedding some light on the mechanisms of persistence Hb F production in thalassaemia. In fact, they can be regarded as a group of extremely mild forms of  $\beta$  thalassaemia in which defective  $\beta$  chain production is either almost or completely compensated for by persistent  $\gamma$  globin chain synthesis.

The  $\delta\beta$  thalassaemias and deletion forms of HPFH are due to a series of different sized deletions involving the  $\gamma\delta\beta$  globin gene cluster. The associated phenotype seems to depend on the precise extent of the deletions. Some forms of HPFH are due to point mutations in the  $^G\gamma$  or  $^A\gamma$  gene promoter regions that allow high level expression of  $\gamma$  chain production in adult life. A heterogeneous group of conditions is associated with the persistent production of lower levels of Hb F, at least some of which seem to be encoded outside the  $\beta$  globin gene cluster, notably on either the X chromosome or chromosome 6.

### **Phenotype/Genotype Relationships**

#### ***The $\alpha$ Thalassaemias***

As mentioned above, the homozygous state for  $\alpha^0$  thalassaemia almost invariably results in the haemoglobin Bart's hydrops syndrome. It seems likely that this condition can also arise from the compound heterozygous state for  $\alpha^0$  thalassaemia and a severe form of  $\alpha^+$  thalassaemia. There is increasing evidence that the forms of Hb H disease that result from the compound heterozygous state for  $\alpha^0$  and  $\alpha^+$  thalassaemia of the non-deletion variety

( $-\alpha/\alpha T\alpha$ ) are more severe than those that result from compound heterozygosity for  $\alpha^0$  thalassaemia and the deletion forms of  $\alpha^+$  thalassaemia ( $--/-\alpha$ ).

There is another family of  $\alpha$  thalassaemias which follow a completely different form of inheritance. These disorders are all associated with varying levels of mental retardation. The  $\alpha$  thalassaemia/mental retardation syndrome (ATR) results from either long subtelomeric deletions of chromosome 16 (ATR-16) or mutations of a gene on the X chromosome that has recently been identified as *XH2*, a DNA helicase that is widely distributed during early development and which may be a transcription factor that is important in the activation of many different genes including the  $\alpha$  globin genes. This latter disorder is called ATR-X.

#### ***The $\beta$ Thalassaemias***

The  $\beta$  thalassaemias also show a remarkable clinical heterogeneity. In the homozygous or compound heterozygous states, the phenotype may range from an extremely severe form of anaemia requiring transfusion from early life through milder forms of anaemia with occasional transfusional requirements to a completely asymptomatic state; the latter forms of  $\beta$  thalassaemia go under the general descriptive heading of  $\beta$  thalassaemia intermedia. Even the heterozygous states for  $\beta$  thalassaemia,

although usually asymptomatic, may occasionally be so severe so that the disorder is inherited in a dominant fashion, i.e., heterozygotes have symptomatic disease with anaemia and splenomegaly. On the other hand, the carrier states may be completely silent with no haematological abnormalities.

Although there is some knowledge of the molecular basis for this remarkable heterogeneity, many questions remain. The severe homozygous or compound heterozygous forms of the disease may be modified by co-existent  $\alpha$  thalassaemia or persistent  $\gamma$  chain production. It is now clear that even homozygotes for  $\beta^0$  thalassaemia may follow a mild clinical course because of an increased propensity for  $\gamma$  chain synthesis. The genetic mechanisms involved are not clear, although at least one determinant has been found to be encoded on chromosome 6. By and large, promoter mutations and some of the splicing mutations of the  $\beta$  globin genes tend to be associated with milder clinical phenotypes, although it is still difficult to predict the likely clinical output for any particular molecular lesion.

The dominant forms of  $\beta$  thalassaemia are nearly all due to mutations in exon 3 which result in the synthesis of long, unstable  $\beta$  globin gene products that co-precipitate with  $\alpha$  chains in the red cell precursors and lead to ineffective erythropoiesis, anaemia and splenomegaly. The silent forms of  $\beta$  thalassaemia are nearly all due to promoter mutations upstream from the  $\beta$  globin genes.

It is becoming clear that one of the major problems that still requires resolution in the thalassaemia field is a better understanding of the reasons for the heterogeneity of  $\beta$  thalassaemia. This is particularly important in the case of haemoglobin E thalassaemia, probably the commonest symptomatic form of  $\beta$  thalassaemia in the world population. Although the clinical picture associated with this genetic combination is well described, very little is known of the reasons for its remarkable heterogeneity; what little information is available suggests that, like all the other forms of  $\beta$  thalassaemia, the major factor in determining the clinical outcome is the level of Hb F that is synthesised. Unfortunately, there is no way of predicting whether an individual patient will respond in this way.

## **Control and Management**

### ***Avoidance***

Since it is easy to identify heterozygotes, the thalassaemias have been obvious candidates for prenatal diagnosis and termination of pregnancies that carry affected babies. First by fetal blood sampling and *in vitro* globin-chain synthesis, and more recently by direct fetal DNA analysis, programmes have been established in several countries for prenatal detection of thalassaemia, in some cases with quite spectacular results. It is now at least theoretically possible to identify thalassaemia mutations by pre-implantation DNA analysis.

### ***Management***

There is now abundant evidence that patients with  $\beta$  thalassaemia who have been maintained on adequate blood transfusion with regular iron chelation using continuous infusions of desferrioxamine can grow and develop relatively normally and have a good

long-term prognosis; the reduction in iron-related endocrine, liver and cardiac disease has been clearly related to the level of compliance in this complex and sometimes distressing treatment regimen. Long-term complications of desferrioxamine therapy, which are being recognised with increasing frequency, include ocular abnormalities, acoustic nerve conduction defects, bone disease and shortness of stature. Transfusion-related infection, notably with HIV, hepatitis B and C, and other forms of viral hepatitis, are an increasing problem. The role of interferons in the management of viral hepatitis in thalassaemic children, particularly those with associated iron loading, remains to be clarified.

### ***Recent Developments in Treatment***

The role of bone marrow transplantation in  $\beta$  thalassaemia is gradually becoming clearer. It appears that if this is carried out early in life, before there is marked iron loading, the results may be extremely good. The role of the newer or oral chelating agents, notably L1, still remains to be clarified. This drug is undoubtedly effective, although it causes neutropenia in a small proportion of cases and a reversible arthritis in others. Some patients appear to become refractory to the use of this agent; the mechanism remains to be clarified. A number of agents have been administered in attempts to raise fetal haemoglobin levels, the most promising being butyrate analogues. Although there have been some spectacular successes, overall it is still not clear whether it will be possible to develop regimens that will significantly raise fetal haemoglobin levels in the bulk of patients with  $\beta$  thalassaemia. This, and specific somatic gene replacement therapy, probably offer the best long-term possibilities for treating the disease in those in whom marrow transplantation is not possible.

### **The Future**

Because of the massive increase in the number of children that will be born with thalassaemia who will survive to require treatment throughout Southeast Asia in the next millennium it is essential that countries develop a programme for the avoidance and management of these diseases. Because it is unclear whether prenatal diagnosis will be acceptable on religious or other grounds in many countries, it is vital that work directed towards simpler and more humane forms of treatment be encouraged. Recent evidence indicates that the thalassaemias have been maintained at their high level by resistance to different forms of malaria; this disease is increasing in its severity, but even if it were controlled it would be many generations before the current gene frequency of the thalassaemias starts to decline.

### **FURTHER READING**

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