THALASSAEMIA INTERMEDIA:
CELLULAR AND MOLECULAR ASPECTS
D. J. WEATHERALL
Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford.

The intermediate forms of β thalassaemia, that is those which are more severe than the trait but do not require regular transfusion to maintain life, which characterises the major forms of the illness, are extremely heterogeneous. They encompass many of the different interactions between β thalassaemia and structural haemoglobin variants, the homozygous and compound heterozygous states for the δβ thalassaemias, and some cases of homozygosity of compound heterozygosity for the haemoglobin Lepore disorders. The same clinical picture is encountered in the more severe forms of α thalassaemia, notably haemoglobin H disease. However, none of these conditions are common and their molecular and cellular features have been well characterised. Here I shall focus on the problem of β thalassaemia intermedia, that is the cellular and molecular basis for diseases which result from the inheritance of a β thalassaemia allele from each parent and yet are not which are not fully transfusion dependent. These conditions form a wide clinical spectrum ranging from disorders which are almost as severe as β thalassaemia major to conditions which are compatible with a completely normal life. I shall also briefly discuss the problem of the compound heterozygous state for β thalassaemia and haemoglobin E, Hb E β thalassaemia, which is the most important intermediate form of β thalassaemia in the world population. These topics are covered in detail in a recent monograph, and are summarised briefly in the sections that follow.
Cellular pathology of the \(\beta\) thalassaemia intermedias

Like all the \(\beta\) thalassaemias the fundamental defect in \(\beta\) thalassaemia intermedia is imbalanced \(\beta\) globin chain synthesis and the deleterious effects of the excess of \(\alpha\) chains on red cell maturation and survival. Overall, there is excellent evidence that these conditions are milder than the major forms of \(\beta\) thalassaemia because there is relatively less globin chain imbalance and therefore fewer excess \(\alpha\) chains, with the result that damage to the developing and mature erythrocyte is less severe. The mechanisms whereby excess \(\alpha\) chains cause intramedullary destruction of red cell precursors or the premature destruction of their progeny in the peripheral blood has been reviewed recently. Indeed, a great deal of this work has been carried out using blood cells from patients with intermediate forms of \(\beta\) thalassaemia.

In short, excessive \(\alpha\) chain production leads to haemichrome formation with subsequent damage to the red cell membrane. This damage is further mediated by the degradation products of \(\alpha\) chains, notably haem, haemin and free iron. The presence of these potentially toxic by-products of \(\alpha\) chain degradation has important pathological implications for the red cell membrane. In short, they cause partial oxidation and defective function of band 4.1 and a reduction in the spectrin/band 3 ratio. These changes are associated with increased apoptosis. In erythrocytes the formation of membrane-bound haemichrome creates a copolymer of macromolecular dimensions which promotes clustering of band 3 in the membrane. It has been suggested that these clusters are opsonised with autologous IgG and complement, and hence are removed by macrophages. As well as these more subtle changes it is also likely that mechanical damage to red cells in the peripheral blood is mediated through the presence of inclusion bodies formed of excess \(\alpha\) chains. The end result is a dehydrated, rigid red cell with a markedly shortened survival.

These changes are mirrored by an abnormal distribution of membrane phospholipids, with most of the phosphatidylinositol choline on the inner bilayer leaf and phosphatidyl-ethanolamine on the outer leaflet. These changes have been associated with an increased pre-coagulant activity on the surface of \(\beta\) thalassaemic red cells which may accelerate thrombin generation in vivo and in turn, trigger platelet activation. This may be an important mechanism in the hypercoagulable state of \(\beta\) thalassaemia intermedia.

Molecular mechanisms

The varying severity of the phenotype of \(\beta\) thalassaemia intermedia, and the reasons for this condition being generally milder than thalassaemia major are beginning to be understood, although many questions remain.

It seems likely that both genetic and environmental factors are involved in modifying the \(\beta\) thalassaemia phenotype. The genetic factors have been recently subdivided into primary, secondary and tertiary modifiers.

Primary modifiers

The primary genetic modifiers of the \(\beta\) thalassaemia phenotype are the many different alleles at the \(\beta\) globin gene locus. While many of these cause a complete or marked reduction in \(\beta\) chain production there are milder alleles, some of which may be silent in the heterozygous state. Thus a wide range of \(\beta\) thalassaemia phenotypes can result from compound heterozygosity for severe and milder alleles, with varying effect on \(\beta\) chain production.

Secondary modifiers

The secondary modifiers of the \(\beta\) thalassaemia phenotype are those which have a direct action on modifying the magnitude of excess of \(\alpha\) globin that is produced in the face of defective \(\beta\) globin chain synthesis. The two main modifiers of this type are the \(\alpha\) and \(\gamma\) globin genes. There is now extensive evidence that the co-inheritance of \(\alpha\) thalassaemia with \(\beta\) thalassaemia can cause a reduction in the phenotypic severity of the latter. Since there is a wide spectrum of \(\alpha\) thalassaemia mutations, resulting in a variable reduction in the output of \(\alpha\) globin chains, this interaction alone can cause broad phenotypic diversity of \(\beta\) thalassaemia. Similarly, there are a number of different genetic determinants, either encoded within the \(\beta\) globin cluster or on other chromosomes, which can modify the amount of haemoglobin F which is produced in patients with \(\beta\) thalassaemia.

Tertiary modifiers

The tertiary modifiers are polymorphisms at loci unconnected with the globin genes which modify various different complications of \(\beta\) thalassaemia. Although they are probably of relatively little importance in establishing the basic \(\beta\) thalassaemia phenotype, they may have important effects in determining the severity of associated complications, both in thalassaemia major and intermedia. They include polymorphic variation at loci involved in bone metabolism, iron metabolism, and bilirubin metabolism. It is also becoming apparent that, because there are many different polymorphic systems which have developed in response to malarial infection, and that these differ from race to race, individual patients’ response to infection may vary very considerably between different populations in which \(\beta\) thalassaemia occurs at a high frequency.

Acquired and environmental factors

There is a variety of acquired factors which may modify the \(\beta\) thalassaemia phenotype. For example, progressive splenomegaly may change the clinical
picture from thalassaemia intermedia to thalassaemia major. Folic acid deficiency has a similar effect. The importance of the environment in modifying the thalassaemia phenotype has been neglected. For example, it seems likely that chronic exposure to infections such as malaria may play an important role. Similarly, social conditions, nutrition, and the availability of medical care may have a profound effect on the phenotype.

Comment

Although a great deal of progress has been made towards an understanding of the genetic modifiers which may convert β thalassaemia major to intermedia, much remains to be learnt. Despite these sophisticated advances it is still very difficult to give a precise prognosis for an individual patient based on their genotype, and, as mentioned earlier, the phenotype may change from thalassaemia intermedia to major over time particularly if hypersplenism or related complications occur. The important practical implication of these observations is that when β-thalassaemia is diagnosed early in life it is vital not to start transfusion too early and to observe development, particularly growth, feeding and activity, and not to transfuse the infant until it is absolutely clear that it is a major form of the disease.

References


MILD BETA THALASSEMIA INTERMEDIA-MAJOR GENETIC DETERMINANTS IN THE PHENOTYPE SEVERITY

M.L. RIBEIRO AND C. BENTO
Unidade de Hematologia Molecular, Centro Hospitalar de Coimbra, Portugal.

The term β-thalassemia intermedia has been used to designate a broad spectrum of clinical phenotypes ranging from the asymptomatic β-thalassemia carriers to the transfusion dependent thalassemia major. At one end of the spectrum patients remain symptom free with Hb levels as high as 8 to 12 g/dL whereas at the other end patients are just able to maintain the Hb level at about 6g/dL without transfusion, however their growth and development may be retarded, they may have skeletal deformities, chronic leg ulcers and progressive splenomegaly. The knowledge of the β-globin gene mutations responsible for the phenotype of thalassemia interme-

dia may explain this large clinical spectrum, but in general it also involves the interaction between different molecular defects and factors.

The basic pathophysiological phenomenon in β-thalassemias is the globin chain imbalance with precipitation of the non-assembled α-globin chain, in the form of inclusions, which damage the erythroid precursors, causing ineffective erythropoiesis. Any conditions that reduce or increase the α/ non-α-globin chains imbalance ameliorate or aggravate the clinical phenotype. The coinheritance of α-globin gene abnormalities and the presence of genetic determinants that modulate the synthesis of Hb F are major genetic factors affecting clinical severity of the thalassemia intermedia phenotype.

Mutations in the β-globin gene may result in defects in transcription, RNA processing (splicing, polyadenylation), mRNA translation or in abnormal genes that code for very unstable globin chains. Depending on their nature, the effect ranges from total loss (β0) or only a mild (β+silents) impairment in the β-globin chain synthesis. Usually β+ and βsilents mutations occur in the promoter region, in the consensus splice junctions or in 5′ and 3′ untranslated regions (UTR).

The β-thalassemias caused by mutations in the promoter region of the β-globin gene provided the basis for some of the mildest forms of β-thalassemia. Nucleotide substitutions in conserved sequences, such as the distal and proximal CACCC boxes, the CCAAT and TATA boxes, result in a 10 to 25 % reduction in transcription, confirming their importance in the regulation of transcription, likely as binding motifs for trans-acting factors.

Only one mutation, –101 (C → T), was found in the distal CACCC box. The heterozygotes have a “silent” β-thalassemia phenotype, with normal hematological parameters, and high-normal Hb A2 levels. The combination of an allele carrying this mutation with one having a classical β-thalassemia, results in a relatively mild thalassemia intermedia phenotype. In vitro functional studies demonstrate a negative effect of the mutation on the transcription activity efficiency. Other experiments showed binding of erythroid specific factors to a fragment of the human β-globin gene promoter that include the distal CACCC sequence (nt –128 to –98). The difference in the binding capacity between the proximal and the distal CACCC boxes may reflect a different transcriptional activity, as evidenced by the mild β-thalassemia phenotype of the heterozygotes for point mutations in the proximal CACCC box, in contrast with the “silent” phenotype of the –101 (C → T) carriers. The –101 (C → T) mutation is a relatively frequent cause of β-thalassemia in the Italian population, and several studies demonstrated that it leads to a more severe deficiency in β-globin chain production in infancy than in adulthood. Nine mutations in the proximal CACCC box have been iden-
tified in association with mild phenotypes. Interesting to notice that, unlike other mild β-thalassemia mutations, these are associated with high Hb A2 levels in the heterozygotes; apparently they change the binding of the transcriptional factors, leading to an increased transcription of the β-gene in cis. The –90 (C → T) mutation, first described in a Portuguese family, is associated with a mild β-thalassemia intermedia phenotype in the homozygous state. Several mutations have been described in the TATA box. The –29 (A → G), together with –88 (C → T), are the most common mutation among the black population, homozygotes having a mild thalassemia intermedia. To date no mutations were described in the CCAAT box.

The first nucleotide (+1) of the transcript, the Cap site, is located 50 nucleotides 5’ to the initiation codon. An Asian Indian individual, homozygous for the +1 (A → C) point mutation, has a β-thalassemia trait phenotype. This mutation could have an effect on transcription or on capping, with a secondary effect on translation.

Two point mutations and two small deletions had been described in the S’ UTR. A G → A substitution at position +22 relative to Cap site creates a cryptic initiation codon, upstream to the normal one. The +33 (C → G) mutation was recently found associated with IVS-I-1 (G → A) in a Spanish woman with a moderately severe β-thalassemia intermedia phenotype. Carriers have normal red blood cell indices and normal Hb A2 level. In vitro experiences showed a reduced level of β-globin transcript (25-35% compared with normal) that is likely due to increased mRNA degradation. A + 43 to + 40 (−AAAC) deletion was described in a Chinese β-thalassemia carrier, however, in vitro studies failed to demonstrate that this deletion was responsible for the β-thalassemia phenotype.

Mutations at the donor or acceptor consensus splice junctions lead to the use of alternative splice sites and are associated with β-thalassemia phenotypes of variable severity. The presence of the IVS-I-6 (T → C) mutation, a common Mediterranean β-thalassemia mutation, results in a mild β-thalassemia, presumably due to the correct splicing of a substantial number of transcripts. Homozygotes have a moderate β-thalassemia intermedia phenotype that is known as the “Portuguese type” of β-thalassemia.

Another mechanism of abnormal splicing is the activation of the two cryptic donor splice sites within exon 1. Some mutations that change their consensus sequences appear to enhance their ability to compete with the normal site. Mutations in CD24, 26 and 27 activate the alternative splice site around CD 25, while mutations in CD 19 activate the silence splice site around CD 18. The CD 24 (T → A) substitution does not produce any amino acid change, however the CDs 19 (A → G), 26 (G → A) and 27 (G → T) mutations result in amino acid substitutions and, when the correct splicing site is used, the abnormal Hb variants: Hb Malay, Hb E and Hb Knossos, are synthesized. The overall reduction in splicing is the molecular basis for the mild β-thalassemia phenotype of these Hb variants. In contrast, Hb Aubenas and Hb Henri Mondor, despite having alterations on the same codon as Hb E, do not create an alternative consensus nucleotide sequence for splicing, and therefore the rate of synthesis of the variant chain is not affected.

RNA cleavage and polyadenylation are directed by several specific sequences, located near the 3’ cleavage site. The sequence AAUAAA is always present approximately 20 nts upstream from the cleavage site. Mutations at the polyadenylation signal (AATAAA) of the β-globin gene, interfere with the normal processing of the mRNA by reducing the cleavage and addition of poly A at the 3’ end of the RNA, which leads to the synthesis of an unstable elongated transcript. The normal polyadenylation site is not completely abolished. Transient expression studies showed that only 10-20% of the RNA transcripts are cleaved appropriately. Four point mutations and two small deletions have been identified, all associated with a β-thalassemia phenotype. Other sequences present in the 3’ UTR may influence the mRNA stability, possible by the binding of a protein that may protect it from degradation. A C → G mutation in nt 6 of the 3’ UTR region has been associated with silent β-thalassemia.

Mild β-thalassemia intermedia are usually due to homozygosity or compound heterozygosity for β+ or βθ-like mutations. The phenotypic variation associated with many of the alleles is generally uniform but in some cases it varies significantly in response to other modifying genetic factors. It was noticed that the same mutation in different haplotype backgrounds could differ in their phenotypic expression, particularly at the Hb F level. A polymorphism (C → T) at position −158 bp 5’ to the Cap site of the 5’-globin gene, which creates a restriction site for Xmn I, has been associated with higher production of Hb F under conditions of erythropoietic stress. A good example is the –29 (A → G) mutation that in the black population is often associated with the Xmn I (+) polymorphism, homozygous having a mild thalassemia with Hb levels of 10.6 g/dL, and more than 60% of Hb F; a Chinese homozygous for the same mutation, but on a Xmn I (−) allele, had a transfusion-dependent thalassemia major. This is also observed in Hb Lepore Baltimore carriers who are Xmn I positive and have a significantly higher level of Hb F (4.9%) than the Hb Lepore Washington-Boston carriers (2.8%), negative for that restriction site.

The Hb chain imbalance in β-thalassemia intermedia is also ameliorated by the co-inheritance of α-thalassemia, conversely, additional α-globin genes
may increase the severity of heterozygous β-thalassemia. In a recent study of 144 β-thalassemia patients the authors found that phenotypic severity could be predicted from the nature of the β-globin gene mutations and the interaction with α-globin gene lesions in about 70% of the cases; however α-thalassemia mutations and Xmn1 polymorphism failed to explain several mild clinical conditions, suggesting the presence of other still unidentified factors.

The T → C substitution at nt 6 of IVS-I, the second most common mutation in Central Portugal, is associated with a mild β-thalassemia phenotype, due to the considerable β-globin chain synthesis by the mutated allele. The homozygotes have a mild β-thalassemia intermediate phenotype, with low Hb F (10-20%) and high Hb A 2. Among the 23 patients that regularly attend the out-patient clinic of the Serviço de Hematologia in Centro Hospitalar de Coimbra, the phenotypic variations can not be fully explained on the basis of the haplotype or the number of α-genes. An attempt to identify factors contributing to this variability, suggests a major role for unknown genetic loci, not linked to the β-globin gene cluster, in controlling the γ-chain synthesis. In summary: identification of the β-globin gene mutations and the modifying genetic factors in β-thalassemia syndromes, can provide useful information for a more accurate prognosis, a more adequate patient management and contribute to the development of new therapeutic approaches such as the pharmacological induction of Hb F synthesis and an effective gene therapy.

When a β-thalassemia is diagnosed, family studies should be performed in order to identify other carriers, to provide the genetic counseling and, if necessary, to offer the prenatal diagnosis. The great majority of the individuals can be screened by measuring their red blood cell indices, the Hb A2 and F levels and by Hbs electrophoresis; the problem occurs when one member of a couple is clearly a β-thalassemia carrier and the other has a borderline MCV, MCH and Hb A2; or a has a thalassemia phenotype with normal Hb A2.

The mild β-thalassemia conditions, known as “silent” β-thalassemia, are difficult to detect in heterozygotes, because only a slight imbalance in the in vitro chain synthesis can be observed. “Silent” β-thalassemia carriers are usually identified in parents of patients with β-thalassemia intermedia due to the interaction of a classical high Hb A2; β-thalassemia allele and a silent type of β-thalassemia.

The classical β-thalassemia minor phenotype may be modified by the coinheritance of an α-thalassemia gene, which may normalize the red blood cell indices; or a δ-thalassemia gene, in cis or in trans, which, by decreasing δ-chain production, may prevent the increase in HbA2. A 7.2 kb deletion, which removes part of the δ-gene leaving the β-gene intact, accounts for about one third of the normal Hb A2; β-thalassemia trait in the Greek population and has also been described in two Italian individuals associated with a δβ CD39 (CAG → TAG) in trans. Hypochromic microcytic individuals with normal or slightly decreased Hb A2 levels and increased Hb F levels (5-20%) may carry a β-thalassemia trait.

In all couples with thalassemic heterozygosity and normal parameters, the identification of the underlying mutations should be performed to avoid misdiagnosis and incorrect genetic counseling. The genetic background of their ethnic descent should be considered to facilitate the diagnosis.

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LA TALASEMIA INTERMEDIA EN ESPAÑA
M. BAIGET Y E. DEL RÍO
Servicio de Genética. Hospital de la Santa Creu i Sant Pau. Barcelona

Introducción
El término talasemia intermedia se utiliza para describir los hallazgos clínicos y hematológicos de pacientes cuyas manifestaciones no son tan severas como las que caracterizan a una talasemia mayor, pero sí más importantes que las que corresponden al portador heterozigoto de un gen talasémico. Algunos autores definen como talasemia intermedia al síndrome talasémico que cursa con anemia, ictericia y esplenomegalia y sin requerimientos transfusionales. A pesar de esta definición, estos pacientes pueden precisar transfusiones sanguíneas cuando la anemia, habitualmente bien tolerada, se agrava debido a un proceso intercurrente. Weatherall sugiere que una talasemia con unos niveles de hemoglobina persistente por debajo de 90-100 g/l, si se acompaña de esplenomegalia, debe catalogarse de talasemia intermedia. Asimismo considera que pacientes con un síndrome talasémico que presenten unos niveles de Hb entre 60 y 90 g/l que se desarrollan bien y llegan a la vida adulta, precisando sólo transfusiones ocasionales, deberían también considerarse como talasemias intermedias.

En realidad, no existe una definición adecuada y tazrar una barrera entre la talasemia intermedia y la talasemia mayor es, en ocasiones, muy difícil. Sin embargo, no hay duda de que el cuadro clínico de talasemia intermedia es el resultado de interacciones entre múltiples genes talasémicos o entre éstos y distintos genes de hemoglobinopatías estructurales.

Las bases genéticas de la talasemia intermedia
Bajo un fenotipo de talasemia intermedia subyacen genotipos muy heterogéneos. En el último registro efectuado en España, de casos con talasemia homozigota se observa que un 15% de dichos pacientes presentan un fenotipo de talasemia intermedia. De ellos, 66% son homozigotos para β-talasemia, 13% son homozigotos para δβ-talasemia, 11% dobles heterozigotos β-talasemia/Hb Lepore, 5% dobles heterozigotos β-talasemia/δβ talasemia y 5% dobles heterozigotos β-talasemia/hemoglobinopatía estructural.

Mutaciones en el gen de la globina beta (β-talasemias)
En población española se han identificado distintas mutaciones puntuales en el gen de la globina beta (tabla 1) que conllevan: a) alteraciones del procesamiento del ARNm por su localización en regiones de "splicing" o en zonas intrónicas; b) síntesis de una proteína truncada por tratarse de mutaciones que crean un codón de parada prematuro o porque se altera el molde de lectura, y c) modificaciones cuantitativas en la expresión debido a mutaciones en las regiones promotoras y.

En pacientes con una talasemia intermedia únicamente se ha identificado la presencia de tres de dichos alelos en estado homozigoto: IVS1nt6 y CD8/9 + G o de doble heterozigoto: IVS1nt6/ IVS1nt110. Villegas et al señalan que los niveles de Hbf (90%) que presentaban los pacientes homozigotos CD8/9 + G explicarían el fenotipo de talasemia intermedia, por lo que los genes de globinas γ podrían considerarse como genes modificadores del fenotipo talasémico.

Deleciones en el cluster de las globinas tipo β (δβ talasemias)
La δ-β talasemia española es el resultado de una deleción de gran tamaño (> de 100 kb) cuyo extremo 5' se sitúa en una región con repeticiones del tipo AluI, entre los genes 5' y 6 que se extiende hasta 11 y 17 Kb más allá del extremo 3' de las delecciones características de la HPFH-1 y HPFH-2, respectivamente.

En la δβ-talasemia homozigota, la total ausencia de síntesis de cadenas de globina β y δ causadas por la deleción, se compensa por un aumento de la expresión de los genes de las globinas γ y que, al combinarse con las globinas α dan lugar a la Hb F y determina en estos pacientes una expresión fenotípica de talasemia intermedia. Los pacientes españoles con δβ-talasemia homozigota son originarios del sureste peninsular, por lo que es posible inferir que fue en esta área geográfica donde apareció la mutación española.

Doble heterozigocia β/δβ talasemia
En nuestro país se han descrito escasísimos casos de pacientes con este genotipo que tienen una ex-
presión clínica de talasemia intermedia y en aquellos
en que se han efectuado los estudios para identificar
la naturaleza de las mutaciones subyacentes se tra-
taba de ββ talasemia española en combinación con
la mutación CD39 en el gen β.

**Doble heterozigocia β talasemia/Hb Lepore**

La Hb Lepore se caracteriza por estar formada por cadenas α normales que se unen a cadenas de
globina híbridas ββ y en función de la estructura de
estas últimas se definen tres variantes. En España
se ha descrito únicamente la presencia de la
variente Lepore Boston y en los pacientes que han
heredado dicha hemoglobina junto a un gen β-ta-
lasémico, es éste el que determina si presentarán un
cuadro de talasemia intermedia o de talasemia ma-
yor. El análisis genotípico de uno de estos casos11
demostró la doble heterozigocia CD39 en el gen
β/Hb Lepore.

**Doble heterozigocia hemoglobopatías
estructurales/β talasemia**

Se han descrito un gran número de pacientes con una doble heterozigocia β-talasemia/variante estructural de la hemoglobina cuyas manifesta-
ciones clínicas componen un cuadro de talasemia intermedia. De todas ellas, únicamente se han descrito en España la asociación del gen talasémi-
co con la Hb S, con la Hb C o con la Hb D Pun-
jab12-15. La mayoría de estos estudios, realizados en los años 80, señalan: a) la ausencia de Hb A que
indica la presencia de un gen β-talasémico con una
mutación que conlleva ausencia de síntesis de glo-
binas β; b) en algunos casos, la presencia de Hb F es un factor determinante de la severidad del cuad-
dro (genes de las globinas γ como genes modifi-
cadores).

**Mutaciones en los genes de globina α (α-talasemias)**

En base al hecho de que existen dos genes α por
genoma haploide, las α-talasemias se clasifican en
función de la producción relativa de ambos genes α. Cuando los dos genes α de un mismo cromoso-
ma no existen debido a una delección, se denomina
αα-talasemia o α-talasemia tipo 1, y el genotipo he-
terocígoto se expresa como -/-αα. Cuando sólo uno
de los genes es inactivo, se denomina α-α-talasemia
o α-talasemia tipo 2, y el genotipo heterozigoto se
expresa como -α/αα. Cuando el déficit de globinas α es debido a una mutación puntual (α-talasemia
delecional), los genotipos se expresan como α/αα/αα (mutación en el gen α2) o αα/αα/αα (muta-
ción en el gen α1). La patología molecular de la α-talasemia viene determinada por una serie heterogénea de delecio-
nes de diverso tamaño que, en el caso de αα-talasemia, implican todo el complejo de genes α globi-
na. En el caso de la αα-talasemia existen delecciones que eliminan uno de dichos genes o mutaciones
puntuales que causan su inactivación parcial o
completa.

El fenotipo de talasemia intermedia se asocia a la
denominada enfermedad por Hb H que correspon-
de a la herencia combinada de un alelo αα-talasemia con un alelo αα-talasemia (-/-αα), de modo que exis-
ta un solo gen α funcional. Las primeras descripcio-
nes de pacientes españoles con la enfermedad por
Hb H se efectuaron en el año 196616 y hasta la fecha
se han publicado alrededor de 30 familias con pa-
cientes que presentan esta forma de talasemia in-
termedia, siendo, por lo tanto una entidad poco fre-
cuente en nuestro país.

En la tabla 2 se muestran las delecciones y mutacio-
nes puntuales del cluster de globinas tipo α que se han
identificado en España. En la casi totalidad de los pa-
cientes españoles afectados de enfermedad por Hb
H, el alelo αα-talasemia se debe a la presencia de la
delección αα-3.7, mientras que el alelo αα-talasemia con el que se asocia es variable (αα-Med, αα-Span, αα-Brl, αα-Cal
y αα-MD)17-21.

**Tripletas de genes de globina α y su interacción
con alelos β o ββ-talasémicos**

El mecanismo de entrecruzamiento no homólogo en el cluster de las globinas tipo α entre dos cromo-
mosomas 16 mal alineados que origina las delecciones antes comentadas, da también lugar a que se origi-
nen cromosomas con una triplicación de genes α. En
población control española, este tipo de triplica-
ción ocurre con una frecuencia del 0,5% y es del tipo
ααα-3.7.

El delicado balance entre la producción de cade-
nas de globina de tipo α y de tipo β que existe en
una eritropoyesis normal se altera de forma signifi-
cativa cuando junto a un alelo β-talasémico se he-
reda un cromosoma 16 con tres genes α: al déficit de
síntesis de cadenas de globina β se añade una
producción excesiva de globinas α que agrava la dis-
entropoyesis y origina un cuadro clínico de talas-
emia intermedia. En estos casos el gen α extra actúa
como un gen modificador: su presencia en indivi-
duos normales no conlleva un efecto fenotípico
pero, en cambio, es capaz de modificar la expre-
sión clínica de un gen de globina β mutado. En los

**Tabla 2. Deleciones y mutaciones puntuales del cluster de
globinas tipo α que se han identificado en España**

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5 pb del IVS2 (Hph) ATG-ACG codón iniciación (Nco) 13 pb del cd 51-55 αα1
individuos heterozigotos para β-talasemia que presentan un fenotipo más severo que el habitual, debe considerarse la presencia de una triplicación de genes de globina α.

Los casos de talasemia intermedia deben a esta interacción en los que se ha efectuado estudio genotípico han identificado la mutación IVS1nt1 G-A en el gen de globina β y una triplicación del tipo ααα en el α2 gene.

Los genes α triplicados pueden heredarse, también, junto con un alelo β-talasémico y, aunque esta situación es poco frecuente, se demostró en un paciente español diagnosticado de heterozigoto para la β8-talasemia española que presentaba un cuadro de talasemia intermedia.

Consideraciones finales

En este trabajo se presenta un panorama representativo de los patrones genéticos que determinan el desarrollo de un cuadro clínico de talasemia intermedia en nuestro país y que corresponden a la interacción de mutaciones en diversos genes de las globinas. Los pacientes con talasemia intermedia no representan en España un problema clínico relevante de su baja frecuencia, pero, frente al desconocimiento del patrón genético subyacente pueden plantear serias dudas en lo que al consejo genético se refiere.

La talasemia intermedia no deja de ser un “experimento de la naturaleza” que nos muestra que incluye en una de las enfermedades monogénicas más conocidas, la talasemia, es difícil establecer la relación genotipo-fenotipo. Esta circunstancia es debida a la coexistencia de los genes determinantes de la enfermedad tanto en los genes denominados modificadores. Un gen modificador puede definirse como aquel gen mutado cuyas alteraciones no comportan ningún efecto fenotípico en individuos normales, pero que es capaz de modificar la expresión clínica de un gen determinante mutado. En el ámbito de la talasemia intermedia existen dos claros ejemplos de genes modificadores. El primero es el gen α extra, producto de una triplicación de los genes α, cuando se heredaron junto con un alelo β mutado agrava la expresión clínica moderada del individuo portador de β-talasemia y lo convierte en un paciente con un cuadro de talasemia intermedia. El segundo ejemplo son los genes γ que cuando se activa en el α₂ globina β se sintetiza hemo y, en consecuencia, el hígado es capaz de atenuar la severidad del cuadro clínico asociado de talasemia homozigota.

Pueden también considerarse como genes modificadores que actúan en el ámbito de los síndromes talasémicos, el gen HFE, cuyas alteraciones comportan una hemocromatosis hereditaria y el gen UGT1 que se asocia a la presencia del síndrome de Gilbert. Tanto un almacenamiento de hierro elevado como una alteración en la síntesis de bilirrubina son factores capaces de modificar la expresión clínica de los genes de las globinas.

Agradecimientos

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Bibliografía

THALASSAEMIA INTERMEDIA:  
CLINICAL ASPECTS AND MANAGEMENT  
M.D. CAPPELLINI, M. CERINO, S. MARELLI  
Y G. FIORELLI  
Centro Anemie Congenite, Department of Internal Medicine,  
Ospedale. Maggiore IRCCS, University of Milan-Italia  

Introduction  
Thalassaemia intermedia encompasses a much broader clinical spectrum than thalassaemia major. The designation itself is a label applied to thalassaemia patients with anaemia and variable degree of splenomegaly with a transfusion-independent clinical course whose severity is extraordinarily heterogeneous. At the severe end of the clinical spectrum, patients present between the ages of 2 and 6 years and, although they are just capable of surviving without regular blood transfusion, it is clear that growth and development are retarded. At the other end are patients who are completely asymptomatic until adult life with only mild anaemia, who may never undergo transfusions, with haemoglobin levels between 8 and 10 g/dl except during infections. In some of these patients splenomegaly develops caused by excessive red cell breakdown or red cell pooling leading to hypersplenism that may exacerbate anaemia and render patients transfusion dependent, however, this may be reversed by splenectomy.

The degree of globin-chain imbalance is the main determinant of the severity of thalassaemia syndromes. The absent or reduced production of $\beta$-globin leads to a relative excess of $\alpha$-chains, which are highly unstable and precipitate in the bone marrow erythroid precursors causing membrane damage and cell death. The degree of this “ineffective erythropoiesis” is the principal determinant of anaemia, while peripheral haemolysis of mature red cells and the overall reduction in haemoglobin synthesis per cell are secondary mechanisms. Any inherited or acquired factor capable of reducing the degree of globin imbalance may produce thalassaemia intermedia phenotype.

Thalassaemia intermedia patients are most commonly homozygotes or compound heterozygotes for $\beta$-thalassaemia having both $\beta$-globin loci affected. Less frequently only a single $\beta$-globin locus is mutated, the other being completely normal. The main factors responsible for thalassaemia intermedia phenotype in homozygotes or compound heterozygotes for $\beta$-thalassaemia are: a) presence of mild or silent $\beta$-thalassaemia alleles; b) coinheritance of $\alpha$-thalassaemia; and c) coinheritance of determinants associated with increased $\gamma$-chain production.

When a single $\beta$-locus is mutated the worsening globin chain imbalance may be due to coinheritance of triplicated $\alpha$ gene or compound heterozygosity with some forms of hereditary persistence of fetal haemoglobin.

The differentiation at presentation between thalassaemia intermedia and thalassaemia major is essential to design an appropriate treatment: the precise prediction of a mild thalassaemia may avoid needless transfusions and their complications, while the diagnosis of thalassaemia major will allow an early start of the transfusion program. Unfortunately, the accurate identification of these two phenotypes at the onset is sometimes difficult. Nevertheless, a careful analysis of the clinical, haematological, genetic and molecular data may allow a reasonable aptitude for treatment. When the presence of a mild to silent $\beta$-thalassaemia mutation is suspected, accurate methods for identification should be used. The identification of $\beta$-thalassaemia mutations and appropriate analysis of the $\alpha$ and $\gamma$-globin genes in the parents may be useful in genetic counselling, allowing prediction of the clinical phenotype. In Table 1 are reported some parameters which may help in differentiating thalassaemia major and intermedia.

Clinical manifestations and management  
Three major factors are responsible for the clinical manifestations of thalassaemia intermedia: a) ineffective erythropoiesis; b) chronic anaemia, and c) iron overload. The severity of these factors depends on the underlying molecular defects determining the relative excess of $\alpha$-globin chains. Because of the extraordinary heterogeneity of thalassaemia intermedia, some patients have very few clinical abnormalities, whereas others experience severe complications due to ineffective erythropoiesis and anaemia. Bone marrow expansion, a consequence of ineffective erythropoiesis, results in characteristic deformities of the skull and face, and causes cortical thinning and pathological fractures of the long bones. Chronic anaemia causes increased gastrointestinal iron absorption. While iron loading is less accelerated than transfusional iron accumulation in patients with thalassaemia major, patients with thalassaemia intermedia often develop cardiac disease, hepatic fibrosis, endocrine abnormalities, diabetes mellitus and other complications of iron overload. Moreover, patients with thalassaemia intermedia suffer from various complications which are very uncommon in optimally transfused thalassaemia major patients, such as folic acid deficiency, leg ulcers, gallstones and thrombosis.

Table 2 reports the prevalence of clinical complications in a group of thalassaemia intermedia patients compared to thalassaemia major patients.

Transfusion therapy  
The decision to initiate regular transfusions in thalassaemia intermedia is very difficult because of the
heterogeneity of the disease. Recurrent of persistent medical complications such as abnormal facies, delayed growth, increasing bone marrow expansion, pathological fractures or cardiac complications are required. There is not benefit to limiting the quantity or frequency of transfusion once they have begun, and this should not be attempted.

Patients with haemoglobin levels of 8 to 10 g/dl may need transfusions during infections and others acute conditions. The development of hypersplenism may render these patients transfusion-dependent, but this situation may be reversed by splenectomy5.

It has been observed that starting transfusions after the third year increases the risk of red cell alloimunisation. Thus, it has been suggested that the first transfusions should be undertaken in conjuc- tion with 3-5 days of steroid treatment to minimize this risk, although the effectiveness of this approach in unproven.

Oral folic acid supplementation is advisable (1 mg/day) since thalassaemia intermedia patients are at risk of relative folate deficiency.

Pregnant women with thalassaemia intermedia need to be carefully monitored and transfusions may be necessary.

**Splenectomy**

The principal indications for splenectomy are clinical signs of hypersplenism (enlargement of the spleen and a drop in mean Hb level), reductions in growth rate, and decreased feeling of wellness, in the absence of other possible interacting factors, such as infection. Should these indicators become apparent, splenectomy should be considered (remove without delay). However, as long as patients with thalassaemia intermedia remain well and have an acceptable Hb levem, there is no need for splenectomy.

Symptomatic thrombocytopenia and leucopenia generally appear late in the progression of hyper- splenism, but are also indications for splenectomy. The recommendations for management before and after splenectomy are the same as those for thalassaemia major. Splenectomy before the age of 5 carries a high infection risk and is therefore not generally recommended6.

Before or during splenectomy, the gallbladder should be checked for gallstones that are common in thalassaemia intermedia patients, and a cholecys-

| Table 1. Criteria of differential diagnosis between thalassaemia major and thalassaemia intermedia |
|------------------------------------------------------|-------------------------------------------------|
| Clinical                                              | Haemotatological                                |
| Presentation (years)                                  | HbF (%)                                         |
| < 2                                                   | > 50                                            |
| Hb levels (g/dl)                                      | HbA₂ (%)                                        |
| < 7                                                   | < 4                                             |
| Liver/spleen enlargement                              | 10-50 (may be up to 100)                        |
| Moderate to severe                                    | > 4                                             |
| Genetic                                               | Genetic                                         |
| Both carriers of high                                 | Both carriers of high                           |
| HbA₂ beta-thalassaemia                                | HbA₂ beta-thalassaemia                         |
| Parents                                               | Parents                                         |
| High HbF beta-thalassaemia                            | Borderline HbA2                                 |
| Molecular                                             | Molecular                                       |
| Type of mutation                                      | Type of mutation                                |
| Severe                                                | Severe                                           |
| Mild/silent                                           | Mild/silent                                      |
| Coinheritance of α-thalassaemia                       | Coinheritance of α-thalassaemia                 |
| No                                                    | No                                              |
| Hereditary persistence of fetal haemoglobin           | Hereditary persistence of fetal haemoglobin     |
| No                                                    | No                                              |
| β-β-thalassaemia                                      | β-β-thalassaemia                                 |
| No                                                    | No                                              |
| δγδγ-polimorphism                                     | δγδγ-polimorphism                               |
| No                                                    | No                                              |

<table>
<thead>
<tr>
<th>Table 2. Percentage of complications in Thalassaemia Syndromes</th>
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<tr>
<td><strong>Thalassaemia intermedia</strong> (n = 63)</td>
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<tr>
<td>26 (17-37)</td>
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<td>Splenectomy</td>
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Personal data.
tectomy should be performed if necessary. A macroscopic liver examination and liver biopsy at the time of splenectomy provides the opportunity to evaluate liver histology and iron content.

Iron chelation therapy
Iron overload occurs even in untransfused thalassaemia intermedia patients because of ineffective erythropoiesis, peripheral red cell breakdown and increased intestinal iron absorption. Iron loading secondary to these causes is less accelerated than that associated with transfusional iron overload in transfusion-dependent thalassaemia patients. Nevertheless, the clinical consequences are similar, although this occurs later in life. An iron balance study reported in patients with thalassaemia intermedia indicates that iron loading may be on the order of 2-5 g iron per year in these patients.

Older patients with thalassaemia intermedia may have the same risk for iron-induced hepatic, cardiac and endocrine dysfunction as patients with thalassaemia major. In thalassaemia intermedia patients, elevated concentration of liver iron (LIC) have been observed despite a slight increase in serum ferritin. For these reasons, measurement of liver iron concentrations or initiation of iron-chelating therapy should be seriously considered in thalassaemia intermedia patients who have even moderately increased ferritin. Transferrin saturation may be more reliable than ferritin in thalassaemia intermedia, but a direct determination of iron burden by measuring liver iron concentration in a biopsy or by non-invasive methods is recommended.

Patients with thalassaemia intermedia should be advised to avoid eating foods that are particularly rich in iron (e.g., liver), and to avoid ‘health’ foods and drinks with amounts of iron supplements. Drinking black tea with meals reduces the absorption of non-heme iron.

Chelation therapy should be started when iron overload is determined by biochemical or histochmical testing. Desferrioxamine given subcutaneously 2 or 3 days a week may be sufficient. Treatment should be monitored in the same way as for patients on regular transfusion.

Osteoporosis
Almost all patients with thalassaemia intermedia exhibit spinal bone mineral density (S-BMD) values at or near the fracture threshold. Because osteoporosis is a progressive disease, prevention and early diagnosis are more effective than attempting to treat the established disease. Osteopenic patients should be encouraged to performed active exercise, to increase their dietary intake of calcium and to avoid smoking. They may also benefit from oral calcium and vitamin D supplementation. Recently, biphosphonates have been successfully used in the treatment of post menopausal osteoporosis. Their role in thalassaemia patients remains to be validated.

Extramedullary erythropoietic masses
Hyperplastic bone marrow leads to the formation of extramedullary erythropoietic tissue, mainly in the thorax and paraspinal region. This can cause neurological complications due to compression of the spinal cord. The presence of the masses can be documented by X-ray or, more precisely, by MRI. A hypertransfusion regimen usually reduces these masses. Radiotherapy may be necessary in some cases, when neurological lesions are already documented. Recently, some cases have been successfully treated with hydroxyurea; however, no controlled study data are available at present.

Leg ulcers
Leg ulcers, common complication in adult thalassaemia intermedia patients, are very difficult to treat. In persistent cases, regular transfusions provide some relief. Simple measures, such as keeping the legs and feet raised above the level of the heart for 1 or 2 hours during the day when possible and sleeping with the end of the bed raised by about 10 cm are advisable. Hydroxyurea, alone or in combination with erythropoietin, has been used in sporadic cases with some benefit.

Thrombophilia
Patients with thalassaemia intermedia have an increased risk of thrombotic events as compared to the general population. Several mechanisms could be involved to explain these patients’ thrombophilic status, including procoagulant activity of damaged circulating red cells, and possible co-inheritance of coagulation defects. Although there is not, at present, a consensus on prophylactic treatment, a platelet anti-agregant is recommended when there is thrombocytosis, and anti-coagulant treatment (e.g, low molecular weight heparin) is given to patients undergoing surgical operations or when thrombosis is documented. Anticoagulant therapy must be monitored carefully. Transfusions before surgery may reduce the procoagulant activity of thalassaemic erythrocytes.

Acknowledgments
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References