Molecular basis of α -thalassaemia

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Abstract

α -thalassaemias is an autosomal recessive disorder, in which there is impaired production of the α -globin chains of haemoglobin. It is associated with microcytic hypochromic anaemia, and a clinical phenotype varying from almost asymptomatic to a lethal haemolytic anaemia. It is probably the most common single gene disorder worldwide, and is especially frequent in populations originating from the Mediterranean region, SE Asia, Africa, Middle East and Indian subcontinent.

Introduction

This presentation will give an overview on two main aspects of the molecular basis of α -thalassaemia: i) the molecular basis of common forms of α -thalassaemia, which provides information for clinical applications, including definitive diagnosis of patients and carriers, patient management, family counseling and prevention; ii) rare and sporadic molecular defects associated with α -thalassaemia, which potentially support a deeper understanding of the regulation of globin gene expression, many aspects of which are also potentially applicable to comprehending the molecular genetics of many other human diseases, too.

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Discussion

The molecular basis of common forms of α -thalassaemia

The α -globin gene cluster

The synthesis of α -globin chains is directed by the duplicated α -globin genes located in the α -globin gene cluster near the telomere of the short arm of chromosome 16 (16p13.3, GenBank NG 000006). The $\alpha 1$ and $\alpha 2$ globin genes (HBA1 OMIM 141800 and HBA2: OMIM 141850, respectively) are almost identical, although the $\alpha 2$ globin gene normally produces 2 to 3 times more α globin chain than the $\alpha 1$ gene.

The cluster contains one embryonic ζ globin gene ($\zeta 2$), two α globin ($\alpha 2$, $\alpha 1$) genes, three pseudogenes ($\psi\zeta 1$, $\psi\alpha 2$, $\psi\alpha 1$) and the θ globin gene of undetermined function, arranged from telomere to centromere (5' $\zeta 2$ - $\zeta 1$ - μ - $\psi\alpha$ - $\alpha 2$ - $\alpha 1$ - θ 3'). The α -globin gene cluster is also surrounded by many widely expressed genes.

The tissue- and developmental-specific co-ordinated expression of the genes in both clusters is dependent on the presence of four highly conserved noncoding sequences (called multispecies conserved sequences, MCS), located in cis, approximately 25-65kb upstream to the α -globin genes. All are implicated to play a role in the regulation of the genes in the α -cluster, with MCS-R2 (also known as HS-40) likely the most important.

Molecular basis: introduction

More than 80 different mutations causing α thalassemia have been described worldwide (<http://globin.cse.psu.edu/hbvar/menu.html>). The majority of most common α thalassemia determinants are due to deletions that remove part, or all, of the α globin gene cluster. Less commonly the defects are point mutations or very small deletions within either of the duplicated α globin genes (point mutations, also known as non deletion mutations). Mutations which partially abolish the synthesis of α -globin chains by the affected chromosome are known as α^+ thalassemia mutations (or α thalassemia 2), and those that totally abolish synthesis of α -globin by the affected chromosome are known as α^0 thalassemia mutations (or α thalassemia 1). Traditionally, to symbolize the types of mutations, " $\alpha\alpha$ " represents a normal allele with both α globin genes intact, " $-\alpha$ " represents a deletion of a single α gene, " $-$ " represents deletion of both α genes from the same allele, and " $\alpha^T\alpha$ " or " $\alpha\alpha^T$ " represent non deletion alleles, where the point mutation affects the $\alpha 2$ or $\alpha 1$ gene, respectively. A superscript is often added to indicate, for example, the size of the deletion or the nature of the point mutation or the population in which the mutation was first described e.g. $-\alpha^{3.7}$ indicating the α^+ deletion of 3.7kb, or $-\alpha^{SEA}$, indicating a common α^0 deletion in SE Asian populations, or $\alpha^{PolyA}\alpha$ which represents a point mutation in the $\alpha 2$ globin gene which affects the polyadenylation site.

New nomenclature for mutations underlying genetic diseases follows recommendations and guidelines of the Human Genome Variation Society (HGVS, <http://www.hgvs.org/>), and all α -thalassaemia

mutations also have HGVS nomenclature (see HbVar - Globin Gene Server, globin.cse.psu.edu/hbvar/menu.html).

Molecular basis: common α -thalassemia deletions

The most common α thalassemia mutations are α^+ thalassemia deletions which leave a single functional α globin gene on the chromosome, removing either 3.7kb or 4.2kb ($-\alpha^{3.7}$ or $-\alpha^{4.2}$, respectively). These common α^+ -deletions are predisposed to arise due to the presence of duplicated homologous regions around the two α -genes, which leads to reciprocal recombination when the chromosome pairs misalign during meiosis. The reciprocal chromosome which arises following the deletion of a single α gene has a triplicated α gene arrangement ($\alpha\alpha\alpha$). There are over 250 million carriers of α^+ -thalassemia in the world, with the highest incidence found in the populations of India, Southeast Asia and Africa, and less commonly in the Mediterranean and Middle East.

α^0 thalassemia deletion determinants are caused by complete or partial deletion of both α -genes in *cis*, which abolish the α -chain synthesis directed by these chromosomes. Homozygotes for such deletions have the Hb Bart's Hydrops Foetalis Syndrome. Several deletions have been described which remove the ζ -globin genes at the 5' end of the cluster, as well as the α -genes. Although heterozygotes for such deletions appear to develop normally, it is unlikely that homozygotes survive even the early stages of gestation, since neither embryonic ($\zeta\gamma 2$) nor foetal ($\alpha 2\gamma 2$) haemoglobins can be synthesised. At the 3' end of the α -gene cluster many of these deletions include also the $\theta 1$ -gene whose function is unknown, but of note is that individuals with complete absence of $\theta 1$ -gene survive. Generally there are about 20 α^0 thalassemia deletions that tend to be recurrent in various populations, four of which are more commonly found: $-\text{Med}$ and $(-\alpha)^{20.5}$ deletions in Mediterranean populations, and $-\text{SEA}$ and $-\text{FIL}$ in South East Asia. The breakpoints of the more common α^0 thalassemia deletions lie within the α globin cluster the deletions have arisen through non-homologous recombination.

Molecular basis: point mutations

There are more than 40 α thalassemia defects resulting from point mutations, usually within the $\alpha 2$, and less commonly the $\alpha 1$ gene. About half of these mutations affect RNA processing or translation. In addition there is an interesting group of mutations which leads to post-translational instability of the globin polypeptide, mimicking an α thalassemia phenotype, as the synthesis of normal α -globin is effectively reduced. These latter variants are usually hematologically silent in carriers, but when they interact with other α thalassemia mutations, they cause a spectrum of phenotypes which include either Hb H disease or a condition comparable to thalassemia intermedia. In rare cases they have been described to cause Hb H Hydrops fetalis in the homozygous state or when co-inherited with severe synthesis-deficient α thalassemia determinants. Typical examples of these types of mutations include Hb Quong Sze ($\alpha 2$ cd125 CTG > CCG; Leu > Pro), Hb Agrinio ($\alpha 2$ cd29 CTG > CCG; Leu > Pro) and Hb Taybee ($\alpha 1$ cd38 or 39 delACC; Thr).

Phenotype-genotype correlations in α -thalassemia

According to the number of α genes with impaired function, four major hematological and clinical phenotypes can be characterized. In carriers of α^+ thalassemia (α thalassemia 2), one α gene is affected either though a single gene deletion or point mutation defect. Heterozygotes (who have 3 functional α globin genes) usually have minimal or no red cell abnormalities. Carriers of α^0 thalassemia mutations (who have 2 functional α globin genes) are classified as severe carriers (α thalassemia 1), and usually have hematological findings which include microcytic, hypochromic red blood cells, with normal or

borderline-normal hemoglobin levels. Homozygotes for α^+ thalassemia deletions (2 functional α globin genes) are usually phenotypically indistinguishable from α^0 -thalassemia carriers.

Coinheritance of α thalassemia mutations may lead to the expression of clinically relevant conditions, most notably Hb Bart's hydrops fetalis and Hb H disease. Hb Bart's hydrops fetalis usually results from the coinheritance of two α^0 thalassemia determinants and thus the complete absence of functional α globin genes and hence α chain production. Rare cases of Hb H hydrops fetalis with hyperunstable α globin variants in the homozygous state or interacting with α^0 thalassemia deletions have been reported. Fetal blood contains mainly Hb Bart's ($\gamma 4$), functionally useless for oxygen transfer, and small amounts of Hb Portland I and Portland II ($\zeta 2\gamma 2$, $\zeta 2\beta 2$), which support the survival of the fetus to late pregnancy. The severe fetal anemia leads to asphyxia, hydrops fetalis, and stillbirth or neonatal death. Prenatal diagnosis may avoid the severe toxemic complications that occur frequently in pregnancies with a hydropic fetus.

Hemoglobin H (Hb H) disease is the severest form of α thalassemia compatible with postnatal life, although it is clinically less severe and thus less important as a public health problem compared to the homozygous state for most forms of β thalassemia. It occurs when α thalassemia mutations interact to reduce α globin synthesis to levels approximately equivalent to the output of a single α globin gene. Hb H disease is so called because of the presence of Hb H (β globin tetramers, $\beta 4$) in peripheral blood, measurable by electrophoresis or chromatography. Predominant clinical features include chronic anemia, often with jaundice and hepatosplenomegaly and some cases may require blood transfusions and/or splenectomy. There is considerable variability in clinical and hematological severity, although there is good correlation between the severity of Hb H disease and the degree of α chain deficiency, and thus α thalassemia determinants. In most populations Hb H disease is most commonly caused by the interaction of α^0 with α^+ determinants and less commonly by the interaction of severe nondeletion α^+ thalassemia determinants. Hb H disease patients with nondeletion α thalassemia defects ($-\alpha^T\alpha$ or $\alpha^T\alpha/\alpha^T\alpha$), especially those causing hyperunstable α thalassemia globin variants, tend to be more severe than patients with deletion determinants ($-\alpha$).

The homozygous state for some hyperunstable α globin variants, or their coinheritance with typical α^+ thalassemia determinants may cause a condition which, based on phenotypic findings alone, is often misdiagnosed as β thalassemia intermedia. The laboratory and clinical characteristics include a moderate microcytic, hypochromic anaemia (Hb75-95g/L), without detectable abnormal haemoglobin fractions, with normal HbA2 and borderline/normal HbF. Such patients have features of dyserythropoiesis including erythroblasts, some cases have peripheral haemolysis, and they also may have splenomegaly, and occasionally thalassaemic facies and bone changes. Finally they are cases often have β -thalassaemia-like biosynthesis ratios, and definitive diagnosis is only clarified based on DNA analysis of the α -globin genotype. These cases are relatively rare and examples include patients with Hb Taybee ($\alpha 1$ cd38 or 39 delACC, Thr), Hb Heraklion ($\alpha 1$ cd36/7 delCCC, Thr) and Hb Questembert ($\alpha 1$ cd131 TCT > CCT, Ser > Pro).

Molecular basis: clinical utility

Although α -thalassaemia is considered to be less of a health problem compared to the β -thalassaemia syndromes, the knowledge of the molecular basis supports both optimal patient management and prevention. For example, prenatal diagnosis not indicated for most common forms of Hb H disease, but could be considered in families with rare genotype interactions associated with severe Hb H disease or Hb H hydrops foetalis. To this end, the application of an effective molecular diagnosis programme requires, in addition to an evaluation of phenotype-genotype correlations, knowledge of population-specific muta-

tions, along with the appropriate technological infrastructure and expertise.

Less common molecular defects associated with α -thalassaemia,

Molecular basis: rare α -thalassemia determinants in cis to cluster

Many less common and rare α^0 thalassemia deletion determinants extend beyond the α -gene cluster to include the flanking genes. Most affected individuals appear to be phenotypically normal, apart from haematological findings compatible with an α thalassemia carrier state. In patients with more extensive deletions of > 1Mb (meaning that they are monosomic for a large segment of 16p13.3), the α thalassemia is additionally associated with developmental abnormalities and mental retardation - a syndrome known as ATR-16.

There are also rare deletions, most of which appear to have arisen *de novo*, that cause α^0 -thalassaemia determinants by removing the upstream multispecies conserved sequences (MCS), but which leave the α -genes intact. This region composed of four, called MCS-R1 to R4, correspond to the previously identified erythroid-specific DNaseI hypersensitive sites referred to as HS-48, HS-40, HS-33 and HS-10. Of these elements, only MCS-R2 (HS-40), 40 kb upstream from the ζ globin mRNA capsite has been shown to be essential for α globin expression. Generally observations of rare natural variations have helped define the region of chromosome 16 which directs fully regulated expression of the α globin locus.

A rare and novel α^0 thalassemia allele was recently described in an individual with a deletion of approximately 18kb, which removed the $\alpha 1$ gene and θ gene, juxtaposing a truncated copy of another gene (LUC7L, which codes a putative RNA-binding protein). Due to the deletion, the RNA transcript from the truncated LUC7L gene expresses across the $\alpha 2$ gene and its CpG island. This mediates methylation of the $\alpha 2$ gene CpG island and leads to transcriptional silencing of $\alpha 2$ gene expression.

In addition another rare and novel mechanism underlying α^+ thalassemia was described caused by a single nucleotide polymorphism within the α globin gene cluster which caused the creation of a new promoter site motif for binding of α -gene specific transcription factors such as GATA1. This mutation results in transcriptional competition with the downstream α genes, causing a down-regulation of their expression and thus a phenotype of α^+ thalassemia, which in fact accounts for about 10% of α thalassemia alleles in the Melanesian population.

Unusual phenotypes involving α Thalassemia and trans-acting mutations

There are unusual forms of α thalassemia associated with mental retardation (ATR), known as ATR-16 syndrome (OMIM # 141750 - see previously) and ATR-X syndrome (OMIM # 301040). ATR-X syndrome is an X-linked disorder caused by mutations of ATR-X gene located on chromosome Xq 13.3. Affected males present a mild α thalassemia phenotype, severe mental retardation and characteristic facial, genital and other congenital abnormalities.

In addition there are acquired forms of α thalassaemia associated with myeloid malignancy (or myelodysplastic syndrome), known as AT-MDS [OMIM: 300448]. AT-MDS may be caused by either acquired deletions of the α globin gene cluster within the neoplastic clone or, more commonly, inactivating somatic mutations of the trans-acting chromatin-associated factor ATRX, which cause dramatic down-regulation of α globin gene expression.

ATRX is a chromatin-associated remodelling protein, influencing the expression of many other genes during development. To date more than 120 mutations in the ATRX gene have been described in patients,

clustering in the two active domains of the protein (the ADD domain or the helicase domain). However, the exact function of ATRX is still unknown and further progress is needed in order to identify other ATRX target genes besides the α -globin genes.

Conclusions

The characterization of mutations in carriers and patients with α -thalassaemia has supported the understanding its molecular basis, in turn supporting phenotype-genotype correlations, patient management, counseling and prevention when indicated.

Molecular studies have also supported the elucidation of the normal structure and variation of the most telomeric region of chromosome 16p around the α -globin gene locus, as well as many aspects that are involved in controlling the correct developmental and tissue-specific expression of the α -globin genes

An ultimate aim of scientists working in the thalassemia field is to fully understand the control of α -globin expression in order to identify novel approaches for the treatment of patients with thalassaemia.

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