Thalassaemia

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Thalassaemia is one of the most common genetic diseases worldwide, with at least 60 000 severely affected individuals born every year. Individuals originating from tropical and subtropical regions are most at risk. Disorders of haemoglobin synthesis (thalassaemia) and structure (eg, sickle-cell disease) were among the first molecular diseases to be identified, and have been investigated and characterised in detail over the past 40 years. Nevertheless, treatment of thalassaemia is still largely dependent on supportive care with blood transfusion and iron chelation. Since 1978, scientists and clinicians in this specialty have met regularly in an international effort to improve the management of thalassaemia, with the aim of increasing the expression of unaffected fetal genes to improve the deficiency in adult β-globin synthesis. In this Seminar we discuss important advances in the understanding of the molecular and cellular basis of normal and abnormal expression of globin genes. We will summarise new approaches to the development of tailored pharmacological agents to alter regulation of globin genes, the first trial of gene therapy for thalassaemia, and future prospects of cell therapy.

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Introduction

Abnormalities in the structure and synthesis of the α-like and β-like globin chains that form tetramers of haemoglobin ($\alpha_2\beta_2$) lead to the most common forms of inherited anaemias.[1] In thalassaemia, there are defects in the production of either the α-like (α-thalassaemia[2]) or the β-like (β-thalassaemia[3]) globin chains. From the 1970s, these diseases, which specifically affect red blood cells, were among the first to be analysed with the use of molecular biology. Their detailed characterisation has established many of the general principles supporting our understanding of human molecular genetics.[4] Furthermore, research into globin genes has greatly contributed to the understanding of how human gene expression is activated and silenced during differentiation and development.[5–8] Despite these advances, manipulation of globin gene expression to ameliorate or potentially cure the common disorders of these genes is not yet possible. Every 2 years since 1978, leading research groups have met at the Hemaglobin Switching Conference to report and discuss progress. In this Seminar we will summarise the understanding of the molecular and cellular pathophysiology, epidemiology, and management of β-thalassaemia, which is the main clinical problem in this specialty.[1] We also review the developments reported at the 17th Hemaglobin Switching Conference in Oxford, UK, which offer renewed hope for novel approaches to treat these disorders.

Production of red blood cells

To fully understand the pathophysiology and management of thalassaemia, how red blood cells are normally produced (erythropoiesis), and how the globin genes are normally expressed at each stage of development should be considered. First, a transient cohort of embryonic red blood cells originate in the blood islands of the yolk sac. Definitive haemapoietic stem cells (HSCs), which persist throughout fetal and adult life, then emerge from the ventral wall of the dorsal aorta. These cells migrate from the ventral wall to the fetal liver and, by about 60 days of gestation, the first fetal red blood cells are released into the circulation to replace embryonic red blood cells. During fetal development, HSCs migrate to the bone marrow, which is the site of erythropoiesis for the rest of normal adult life. In early postnatal life, adult red blood cells from the marrow replace the fetal cells.[9,10] At all stages of development, senescent red blood cells are continually replaced with new blood cells. These new cells are derived from HSCs, which differentiate into mature red blood cells via erythroid progenitors and precursors (erythroblasts). For an adult to maintain a normal red blood cell count, about 2 million to 3 million new cells must be produced every second. For severe forms of thalassaemia, in which many erythroblasts and mature red blood cells are damaged, erythropoiesis can be increased by 20–30 times.[11,12] However, erythropoiesis is ineffective in severe cases of thalassaemia because the increased numbers of erythroid precursors fail to develop into mature red blood cells.

Normal expression of globin genes

Changes in the sites of erythropoiesis are associated with changes in the types of haemoglobin produced. At the molecular level, haemoglobin synthesis is controlled by two multigene clusters on chromosome 16 (encoding the α-like globins) and on chromosome 11 (encoding the β-like globins). In the human clusters, the genes are arranged along the chromosome in the order by which

Search strategy and selection criteria

We searched PubMed using the terms "thalassaemia" in combination with "molecular basis" or "treatment" or "pathophysiology". We mostly selected publications from from June 2006, to June 2011, but did not exclude frequently referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected the most relevant ones. Review articles and book chapters are cited to provide readers with more details and more references than can be addressed in this Seminar.

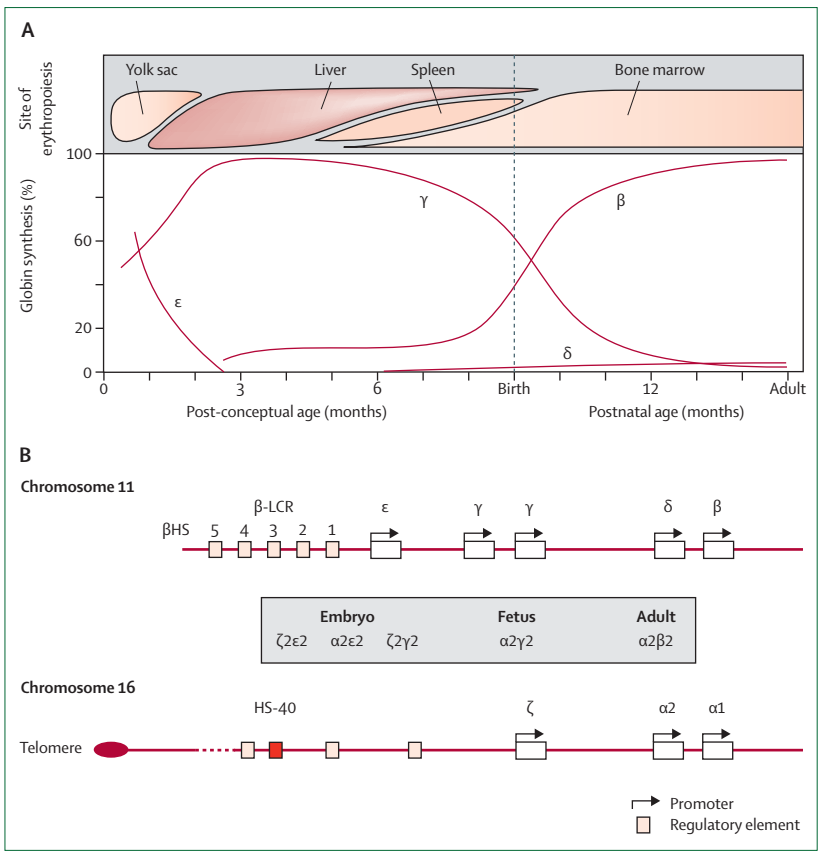

Figure 1: **Normal developmental switches in globin expression**
(A) The sites of haemopoiesis at different stages of development and the levels of expression of the embryonic, fetal, and adult globin chains at various gestational ages are shown. (B) The structure of the α-like and β-like globin gene clusters are shown together with the types of haemaglobin produced at each developmental stage. The promoters are regions that bind transcription factors, including polymerase II, which transcribes the gene into RNA. Enhancers and related regulatory elements also bind transcription factors and interact with promoters to increase the levels of RNA transcribed. HS=major upstream regulatory element. β-LCR=β-locus control region.

they are expressed during development to produce different haemaglobin tetramers: embryonic (Hb Gower-I [$\zeta_2\varepsilon_2$], Hb Gower-II [$\alpha_2\varepsilon_2$], and Hb Portland [$\zeta_2\gamma_2$]), fetal ($\alpha_2\gamma_2$), and adult ($\alpha_2\delta_2$ and $\alpha_2\beta_2$). In the late 1980s, expression of the globin genes was found to be dependent on remote regulatory elements upstream of the clusters. In the β-globin cluster there are five such elements that are collectively referred to as the β-globin locus control region (β-LCR).[5,6] There are four elements in the α cluster, of which one (HS-40) seems to have the dominant role (figure 1).[8]

Research has established how the globin genes are activated in erythropoiesis. The key chromosomal elements involved in this process are the upstream regulatory elements and the promoters of the globin genes themselves (figure 1). Each element is bound by either repressing or activating proteins (transcription factors). As HSCs become committed to the erythroid lineage, the upstream elements are bound by activating transcription factors, some of which are expressed specifically in erythroid cells (eg, *GATA1*, *KLF1*). The associated chromatin then becomes activated. These changes might be associated with alterations in DNA methylation, with repressed genes being methylated and active genes being unmethylated.[13–15] Associated changes also occur in post-translational modifications of the histones, which can indicate activation (eg, acetylation and methylation of specific lysine residues) or repression (eg, methylation of other lysines). Individual promoters and their associated chromatin might also become activated or repressed by binding different transcription-factor complexes.[15–17] In the late stages of erythropoiesis, the upstream elements and activated promoters seem to physically interact via a looping mechanism, which is mediated by the proteins bound to these elements (figure 2). The interaction between upstream elements and specific promoters of the globin genes recruit and activate RNA polymerase II, which is needed to transcribe the gene into RNA.[18] This nuclear RNA is processed into messenger RNA, transported into the cytoplasm of erythroblasts, and translated into the globin chains.

Interpretations have suggested that the molecular switches between embryonic, fetal, and adult globins rely on competition between the globin promoters for access to their activating upstream regulatory elements. The ability of the promoters to compete might in turn rely on changes in the activating or repressing transcription factors that they bind (figure 2). Protein complexes that are assembled at these elements might change the affinity of interactions between the upstream regions and the various globin gene promoters during development—eg, in fetal life, the γ-globin promoter binds an activating complex that preferentially interacts with the upstream elements. By contrast, in adult life, the γ genes are bound by repressive factors, whereas the β genes are now bound by activating factors (figure 2). This simple description belies the complexity of this process, which involves hundreds of proteins for whose involvement there is good genetic and experimental evidence.[19–21] The activity of the enhancer and promoter interactions should be contained within the globin cluster and not propagated into flanking genes. The activity of such loci is delimited by chromosomal boundary elements flanking the globin clusters.[22] Although the precise role of such elements is still under investigation, they have proven to be helpful in strategies to develop gene therapy.

Variants that alter expression of globin genes

More than 200 β-thalassaemia alleles have been described in the database of human haemaglobin variants and thalassaemias, which involve mutations in any of the stages from transcription to RNA processing and translation of β-globin mRNA (figure 3).[23] These mutations are detectable by DNA analysis and provide the basis for genetic counselling.[24] Although most β-thalassaemias are caused by point mutations in the gene or its immediate flanking region, small deletions removing the β gene can also occur. When expression of

For the **database of human haemoglobin variants and thalassaemias** see http://globin.cse.psu.edu/hbvar/menu.html

β globin is abolished by the mutation it is referred to as β^0-thalassaemia, whereas reduced output of normal β chains produces β^+-thalassaemia. Some structural variants (particularly the β^E mutation) might also lead to a thalassaemic effect because they are produced at reduced levels, and their interactions with β^0 and β^+ thalassaemia (eg, β^E/β^T) lead to many forms of clinically severe β thalassaemia.[25]

Some rare forms of β thalassaemia result from deletions removing the upstream regulatory elements, but leaving all of the globin genes intact.[26,27] These deletions result in a substantial reduction in expression of all of the linked globin genes, and first indicated the importance of long-range regulatory elements in controlling expression of the β-like globin genes. Other rare cis-acting mutations (ie, mutations linked on the same chromosome) have been important in the development of our understanding of how the switch from γ-globin to β-globin gene expression is regulated. All these mutations result in variable levels of increased γ-globin expression and increased levels of fetal haemaglobin—so called hereditary persistence of fetal haemoglobin (HPFH). HPFH can arise from mutations in the γ-globin promoters affecting the binding of activating or repressive complexes (figure 3).[7,28,29] Other forms of HPFH result from deletions removing the adult δ-globin and β-globin genes, but leaving at least one γ-globin gene intact (figure 3). These deletions can lead to a moderate (δβ-thalassaemia) or considerable (HPFH) increase in γ-globin expression.[7,28,29]

Several models have been proposed to explain these effects (figure 3). First, if the γ and β genes normally compete for the activity of the β-LCR, removal of both adult globin gene promoters (δ and β) might abolish competition with the fetal γ-globin genes. Second, these deletions alter or remove binding sites for proteins that repress γ-globin expression. Third, the large HPFH deletions juxtapose new enhancer sequences (that normally lie downstream of the β cluster) next to the γ-globin genes. Such enhancers can activate expression from either side of a gene. Evidence suggests that all three mechanisms might have a role in increased γ-globin expression.[28]

In the past 10 years, several rare trans-acting mutations (ie, mutations that are not physically linked to the locus) have been identified that alter the pattern of globin gene expression. These mutations include those in general transcription factors and chromatin-associated factors, and in transcription factors that specifically affect the erythroid lineage. Some mutations affect expression of α globin (eg, *ATRX*[30]), whereas others mainly affect γ-globin or β-globin expression (eg, *GATA1* and *KLF1*).[31–33] Insights into the regulation of globin genes that are obtained from these sporadic mutations have been greatly enhanced by extensive observations from family studies, twin studies, and genome-wide association studies (GWAS). Findings from GWAS have identified other transacting factors that can normally regulate the patterns of globin gene expression.[20,34–37] Two new regulatory pathways that lead to increased γ-globin expression have been identified (*BCL11A* and *HBS1L-MYB*) (figure 4), and both seem to act by directly or indirectly affecting the production of repressor proteins that specifically target the γ-globin genes. These findings suggest that one or both of these pathways might provide targets that are useful for therapeutic intervention because patients with haemoglobinopathies who co-inherit particular DNA sequence variants in these pathways, or mutations in the regulatory protein-binding sites, have high concentrations of haemaglobin F and mild disease phenotypes.[38–40]

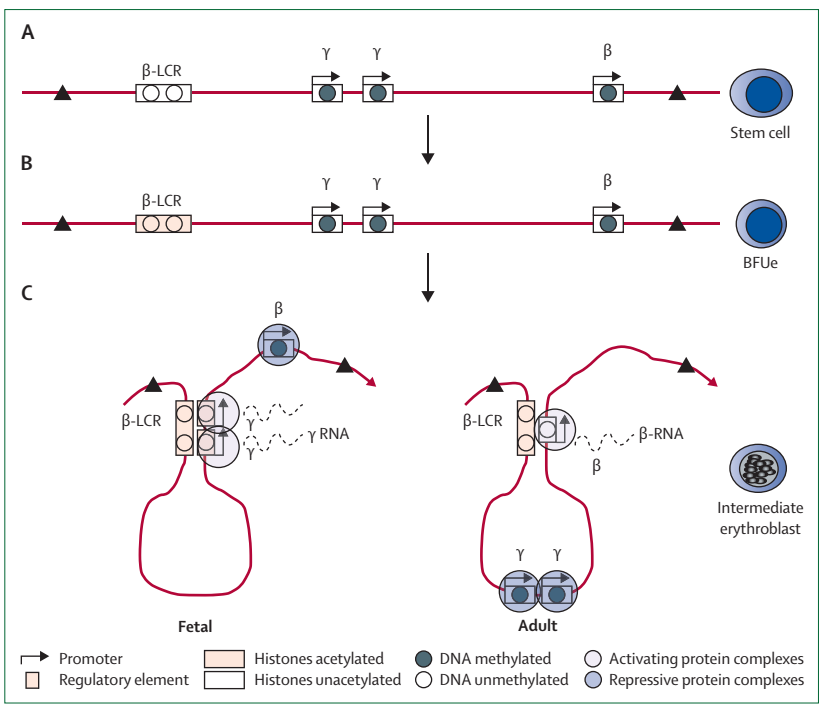

***Figure 2*: Expression of globin genes in fetal and adult life**
(A) In multipotent stem cells, the β-LCR and the genes are mostly unmodified and inert. (B) In early-committed progenitor cells, the chromatin associated with the β-LCR is acetylated and the underlying DNA becomes unmethylated. (C) In fetal erythroblasts the γ genes are activated and associate with active protein complexes that interact with the β-LCR while the β genes are silent. In adult erythroblasts the β genes are now activated and the γ-globin genes are silenced by repressive protein complexes. At each stage there might be competition between the γ and β genes for access to the β-LCR. β-LCR=β-locus control region. BFUe=erythroid burst-forming unit.

The molecular and cellular pathology of β-thalassaemia

At each stage of development, the production of α-like and β-like globins is balanced. β-globin synthesis is normally controlled by the two β genes (one on each copy of chromosome 11). A mutation affecting one gene (β/β^T or β-thalassaemia trait) usually causes no clinically significant problem, whereas patients who inherit deleterious mutations in both β genes (β^T/β^T) frequently have severe anaemia. The main pathophysiology in β-thalassaemia results from the synthesis of insufficient β chains to partner the α-globin chains to generate adult

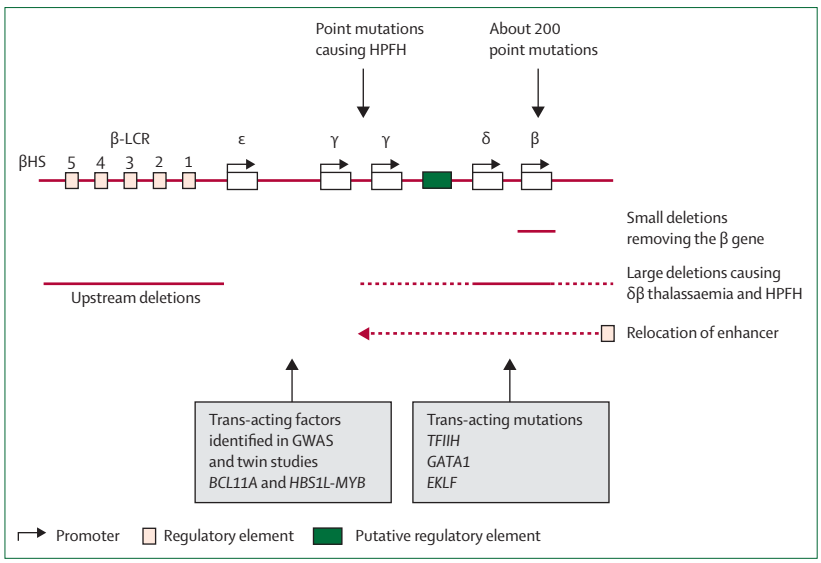

***Figure 3*: Mutations affecting the β-globin locus**
A summary of the mechanisms underlying β-thalassaemia and HPFH. Dashed lines represent variation in the amounts of flanking DNA that are removed by 25 different deletions, which underly δβ-thalassaemia and HPFH. Enhancers downstream of the β-globin cluster are relocated because of the associated deletions. βHS=upstream regulatory element. β-LCR=β-locus control region. HPFH=hereditary persistence of fetal haemoglobin. GWAS=genome-wide association study.

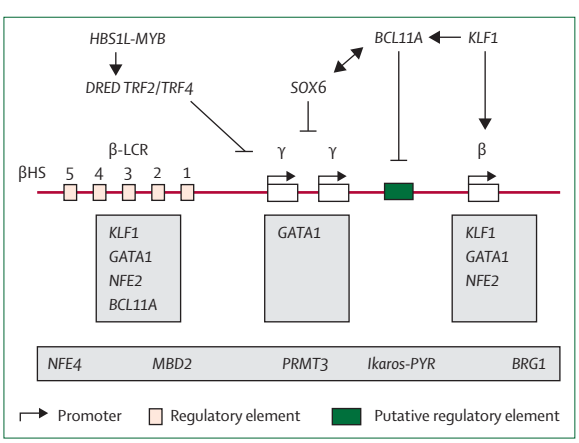

***Figure 4*: New pathways regulating the switch from γ globin to β globin**
Many factors are involved in this process but we emphasise two identified pathways. One pathway seems to result from variation in a DNA region (HBS1L-cMYB on chromosome 6), which is needed for increased expression of c-Myb—a proto-oncogene whose expression is crucial for erythropoiesis. Reduction of c-Myb in the affected cells reduces the synthesis of two nuclear receptors—TR2 and TR4—that are thought to repress the γ globins by binding to their promoters in the region where many HPFH point mutations occur. Some of the variation in fetal haemaglobin associated with *HBS1L-MYB* might be mediated via this pathway. A second pathway involves *BCL11A* (on chromosome 2). *BCL11A* normally cooperates with other repressors (eg, Sox6) to silence the γ-globin genes in erythroid cells. *BCL11A* binds to the β-globin locus within a large expanse of DNA containing putative regulatory regions (defined by many HPFH deletions) lying between the fetal and adult genes. These independant threads of information came together with the discovery that the expression of *BCLIIA* is regulated by the transcription factor *KLFI*. In this way another network of factors involved in regulating the γ-globin to β-globin switch was established. Predictions from this regulatory model have been noted in patients with altered globin switching in vivo. bHS=upstream regulatory element. β-LCR=β-locus control region. HPFH=hereditary persistence of haemoglobin.

haemaglobin ($\alpha_2\beta_2$). Excess α chains precipitate in erythroid precursor cells causing dyserythropoiesis, and in mature red blood cells causing membrane damage and haemolysis (figure 5). These primary changes in erythropoiesis lead to all the secondary changes (anaemia, splenomegaly, marrow expansion and bone deformities, hypermetabolic state, and iron accumulation) that lead to long-term organ damage.[12] Understanding how the products of α-chain degradation and iron result in oxidative damage to the tissues,[41] and how hypoxia and bone-marrow dysfunction interact to result in inappropriate levels of iron absorption is increasing.[42]

The severity of β thalassaemia is determined partly by the extent to which β-globin synthesis is reduced by the associated molecular defects (determined by the combinations of β^+ and β^0 thalassaemia). Nevertheless, for more than 25 years researchers have questioned why patients who are homozygous for identical molecular defects in the β-globin genes (β^T/β^T) can have such remarkably different phenotypes. Some patients need regular blood transfusion (β-thalassaemia major), whereas others are transfusion independent (β-thalassaemia intermedia). If we understood the genetic basis for this natural variation, patients' disease could be changed from a severe to a mild phenotype. Two important modifiers of β-thalassaemia have emerged from studies of clinical genetics. First, the co-inheritance of α-thalassaemia seems to be associated with a mild phenotype[43]—α-thalassaemia reduces the pool of free α chains and thereby reduces damage to the red blood cell and its precursors. Second, the co-inheritance of any disorder associated with increased synthesis of fetal γ-globin chains in adults (eg, HPFH) is associated with a mild phenotype.[12] In HPFH, additional γ-globin chains partner excess α chains to form fetal haemoglobin ($\alpha_2\gamma_2$), which increases the production of fully functional haemaglobin and thereby reduces the pool of free α chains.

Epidemiology

WHO has estimated that about 1·5% of the world's population might be carriers of β-thalassaemia (β/β^T) and that about 60 000 severely affected infants are born every year.[44] These individuals mostly originate from the Mediterranean, Middle East, central Asia, India, and southern China, which suggests that there could be a selective advantage to carrying such a mutation in these areas. Similar observations have been made for α thalassaemia,[1] which is even more widely distributed and more frequent than β-thalassaemia. Findings from extensive microepidemiological and case-controlled studies[45] strongly suggest that individuals with either α-thalassaemia or β-thalassaemia trait are somewhat protected in areas where *Falciparum malaria* is or has been endemic, thus explaining the high carrier frequency via natural selection. Frequent consanguinity might also contribute to the prevalence of thalassaemia in many of these areas.[1,46]

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Estimates of thalassaemia prevalence are based on incomplete data and, at best, provide a minimum estimate of the global problem. Most estimates of gene frequency were made from a small number of centres more than 20 years ago, when the geographical distribution of these disorders was not fully known. Furthermore, the extent to which the interaction between β thalassaemia and a structural variant β^E (which also affects β-globin synthesis) contributes to the range of patients who have serious forms of β thalassaemia was unclear. In fact, 50% of seriously affected patients with β thalassaemia have the β^E/β^T genotype.[1,47,48]

Two additional points further emphasise the current clinical importance of β-thalassaemia. First, thalassaemia originates mostly in low-income countries where infants would not have survived to be diagnosed or treated; however, many of these countries are rapidly undergoing improvements in all aspects of public health. The burden in treatment of such individuals with regular transfusion could overwhelm the health budget of a developing economy, as in Cyprus and Sardinia in the 1960s.[1] Second, with increasing migration and travel, a genetic disease that was rare in northern Europe, Australia, and North America is now becoming more common, and systems for genetic counselling, prenatal diagnosis, and life-long medical care are needed. According to the Thalassaemia International Federation, at least 200 000 patients with thalassaemia are registered as receiving regular treatment throughout the world. Although the true burden of thalassaemia is unknown, the actual number of patients worldwide is probably underestimated and many do not receive any treatment.

Clinical phenotypes and standard management

The three broad clinical phenotypes in patients with β thalassaemia are major, intermedia, and minor. These phenotypes are associated with more than 200 different mutations that either reduce (β^+-thalassaemia) or abolish (β^0-thalassaemia) expression of the affected β-globin genes. Thalassaemia major occurs in homozygotes (β^T/β^T) or compound heterozygotes (eg, β^T/β^E) for such mutations. Affected individuals usually present with pallor, hepatosplenomegaly, and failure to thrive in the first year of life when the change from fetal to adult haemoglobin is completed. The blood profile shows a severe anaemia (<80 g/L) with hypochromic (mean corpuscular haemaglobin [MCH] <20 pg) microcytic (mean corpuscular volume [MCV] <70 fL) red blood cells. Without blood transfusion, such children remain anaemic and develop increasing hepatosplenomegaly. They do not grow normally and show general features of a hypermetabolic state. Children with untreated or partially treated thalassaemia major die in the first or second decade of life.[3,12,49,50] The most widely accepted blood transfusion protocol for thalassaemia aims to increase the concentration of haemaglobin to 130–140 g/L after transfusion, and to maintain the concentration at more than 90–100 g/L at all times.[3,12,47,49] By about 10 years of age, children who are maintained on such a transfusion regimen develop the complications of iron overload, including cardiomyopathy, liver fibrosis, and endocrine dysfunction.

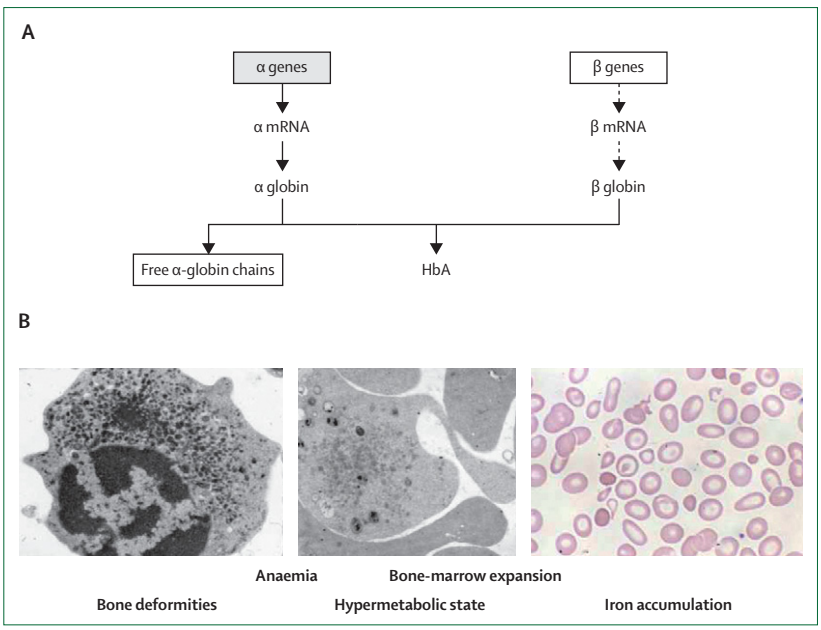

***Figure 5*: The pathophysiology of β-thalassaemia**
(A) Outline of abnormal globin synthesis in β thalassaemia; (B) electron micrographs showing precipitation of α-globin chains in an erythroblast (left), and a mature red cell (centre).The typical blood film of a patient with severe β thalassaemia (right).

Until about 10 years ago, iron overload could be reduced only by subcutaneous infusion of an iron chelator (desferrioxamine) administered five to seven nights per week. Administration of this drug had demonstrable benefits for morbidity and mortality.[51–53] Such benefits were particularly so when good adherence to iron chelation was achieved, with many patients with β thalassaemia surviving in good health to late adulthood. More recently, the orally active iron chelators (deferiprone and deferasirox) have been introduced. Deferiprone was licensed in Europe in 1999 for adults with thalassaemia major who could not be effectively treated with desferrioxamine but is not yet licensed in North America. Monotherapy with deferiprone controlled concentrations of liver iron in only a few patients, but when desferrioxamine was also given two to four times a week, more patients achieved iron balance.[54]

This regime also reduces myocardial iron more effectively than desferrioxamine monotherapy given five nights a week at standard doses.[50,51] The most serious drawback of deferiprone is the occurrence of agranulocytosis in about 1% of patients, necessitating weekly blood counts. Defarasirox has been licensed in North America and Europe since 2006 for the treatment of several disorders with transfusional iron overload, has been effective at improving liver iron, serum ferritin[55] and

For more on the **Thalassaemia International Federation** see http://www.thalassemia.org.cy

myocardial iron,[56] and has an acceptable tolerability profile in prospective studies involving more than 7000 patients.[57]

Although with good chelation, mortality from cardiac disease is now decreasing and many patients with severe thalassaemia now survive into late adulthood, for various reasons, many others still suffer the complications of iron overload. The contribution of different chelation modlities to improved outcomes has been debated and is difficult to define with retropsective analyses. Consequently, understanding of iron metabolism and improvements in iron chelation in thalassaemia are still important areas of future research.

Individuals with thalassaemia intermedia are also homozygotes or compound heterozygotes for β^+ and β^0 thalassaemia. No strict criteria are available for diagnosis of thalassaemia intermedia because this diagnosis is dependent on how a patient develops without transfusion. Patients with thalassaemia intermedia generally present later in life (aged 2–6 years) than do those with thalassaemia major. Some patients with thalasaemia intermedia will not grow and develop normally without regular blood transfusion and will therefore be categorised as having thalassaemia major. By contrast, other patients who present late might thrive without regular blood transfusion (thalassaemia intermedia). The blood profile shows a haemoglobin concentration of 70–100 g/L with an MCV of 50–80 fL and an MCH between 16–24 pg.

The iron status in all patients with thalassaemia intermedia should be carefully monitored (and chelation considered) because excess gastrointestinal absorption of iron almost always occurs as a direct result of ineffective erythropoiesis. Studies have also emphasised the occurrence of other complications with increasing age, including pulmonary hypertension, tumours resulting from extramedullary erythropoiesis, silent cerebral infarcts, thromboembolic disease, chronic leg ulcers, and renovascular disease.[58] Patients with either thalassaemia major or thalassaemia intermedia might benefit from splenectomy. In patients with thalassaemia major, splenectomy is indicated if the red blood cell requirements to maintain haemaglobin at more than 100 g/L exceed about 200 mL/kg of packed red blood cells per kg per year, with the assumption that other reasons for increased consumption of red blood cells (eg, immune haemolytic reactions) have been excluded. Other indications for splenectomy in thalassaemia major and intermedia include symptoms from splenic enlargement, leucopenia or thrombocytopenia, and increased iron overload despite iron chelation.[49] Any decision to undertake splenectomy in patients with thalassaemia should be balanced against the substantial risks associated with this treatment, particularly in patients with thalassaemia intermedia. These risks include sepsis, which might be reduced by pre-splenectomy immunisation against encapsulated bacteria, and the use of prophylactic antibiotics. Furthermore, there is a risk of thrombosis and possibly an increased incidence of pulmonary hypertension.[59,60]

Individuals who inherit a single β^+ or β^0 allele (β/β^T) generally have β-thalassaemia minor. Carriers of β thalassaemia typically have only a mild hypochromic microcytic anaemia with a raised concentration of the minor HbA_2. Such patients need no specific treatment, but should avoid unnecessary iron supplements.

Genetic testing and prenatal diagnosis

Initial screening of populations and identification of families at risk of producing infants who are affected by β thalassaemia has been achieved by examination of red-blood-cell indices and analysis of haemaglobin. The techniques to identify specific mutations underlying β thalassaemia in DNA from adults and fetuses are now well established and extensively applied to genetic counselling and prenatal diagnosis.[24] New non-invasive techniques to analyse fetal DNA in the maternal circulation are being developed.[61] Establishment of the range of mutations in specific geographical regions has enabled diagnostic centres to develop appropriate strategies for effective counselling and diagnostic testing. The application of such strategies in many regions of the world has had a substantial effect on the birth rate of infants with β-thalassaemia major and intermedia.[62]

Genetic counselling can be complicated when the clinical outcome cannot be accurately predicted from the β-globin genotype—eg, some patients with β^E/β^0 thalassaemia might have β-thalassaemia major, and others with exactly the same β genotype might have a very mild form of thalassaemia intermedia.[39] The accuracy of genetic counselling will rise as more genetic modifiers of β thalassaemia are identified, their effects on phenotype documented, and low-cost high-throughput DNA testing becomes available. Therefore, in countries with no ethical or religious objections, prenatal diagnostic programmes should be established.

Pharmacological agents used to treat thalassaemia

The aims of therapeutic interventions for β thalassaemia are to increase expression of γ globin or to decrease expression of α globin, thus restoring the balance between α-like and β-like globin chains. Much evidence from clinical genetic studies shows that either (or preferably both) of these manipulations would have substantial clinical belenfits in patients with β thalassaemia.[7,12,43,63] Pharmacological studies have all focused on increasing expression of γ globin, but have been based on an incomplete understanding of globin gene-regulation.[64–66] Various approaches have included use of cytostatic agents (eg, hydroxycarbamide) to increase fetal haemaglobin by altering the kinetics of erythropoiesis; DNA demethylating agents (eg, azacitidine and decitabine) to hypomethylate the γ-globin genes and increase their expression;

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deacetylase inhibitors (eg, sodium butyrate) to increase γ-globin expression via histone acetylation; and short-chain fatty acids related to butyrate. Although some of these experimental therapies have yielded notable results in early-phase clinical trials (including some substantial improvements) the results have not been sufficiently encouraging to develop large-scale trials.[64–66] Furthermore, despite extensive analysis, these agents have several effects, and how any of these drugs work to increase expression of fetal haemaglobin is not fully understood.

Improvement in the understanding of the transcriptional network causing the transregulation of the change from γ-globin to β-globin expression has provided many new potential targets for manipulation of this switch. As additional transcription factors, cofactors, and chromatin-associated factors in this newly discovered pathway are elucidated (figure 4), a detailed understanding of this switch might be forthcoming and translated into a mechanism-based approach to the reactivation of production of haemaglobin-F in β thalassaemia.

Stem-cell transplantation for thalassaemia

In the past 30 years, stem-cell transplantation has substantially advanced treatment of thalassaemia major.[67] Children who are identified before developing viral hepatitis or severe iron overload and who receive HLA-identical related donor stem-cell transplants have a very high likelihood of remission, with less than 10% mortality and minimal morbidity, apart from impaired fertility.[67] Most groups report event-free survival of 80–90% for β thalassaemia.[67,69–72] By contrast, event-free survival in adult patients with thalassaemia major who receive bone-marrow transplantation is less than 70%.[67,73] This finding is presumably because patients have already developed the complications of thalassaemia and iron overload, thus compounding the clinical problems associated with transplantation.

A major problem in development of a programme for stem-cell transplantation in young patients with β thalassaemia is that HLA-matched stem cells are often unavailable. Transplants from matched unrelated or haploidentical donors are associated with a substantially lower disease-free survival (20–70%) and a higher incidence of morbidity and mortality (25–30%) than are HLA-identical transplants from related donors.[74–78] Definitive HSCs can be obtained from cord blood and, since the 1990s, several studies[71] have assessed the use of cord-blood transplantation for patients with thalassaemia. The outcome of related cord-blood transplantation for the treatment for β thalassaemia is now approaching that for conventional bone-marrow transplantation, with rates of disease-free survival of about 90%. The main benefit from cord-blood transplantation will come from the use of unrelated transplants that are derived from a cord-blood bank; but, data are scarce.[79–81] Unpublished results from the Eurocord cooperative group suggest that transplantation of patients with cord-blood stem cells from an unrelated donor is feasible; however, the outcome is less succesful than that of patients given HLA-compatible cord-blood transplants from a sibling (Rocha V, personal communication).

The main complication of cord-blood transplantation is graft rejection, which can be reduced by modification of the protocols used for pretransplant immune suppression.[71,79] Stem-cell transplantation is an exciting development for the treatment of otherwise fit, young patients with thalassaemia. Nevertheless, a fine clinical judgment has to be made to balance a potential cure that has a risk of mortality and morbidity (including graft *vs* host disease), against a burdensome life-long

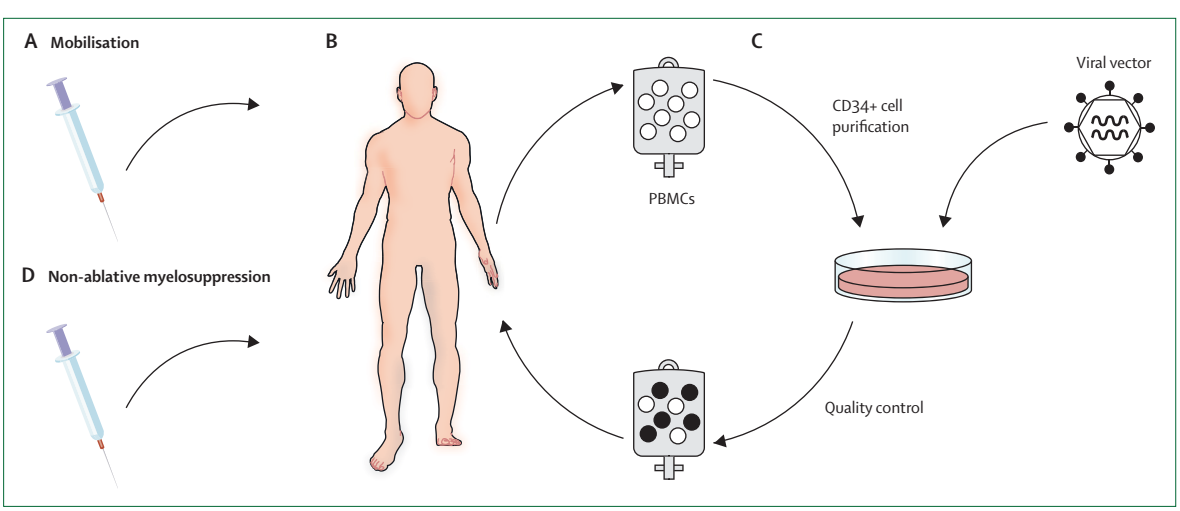

Figure 6: **An outline of the protocol used for gene therapy in patients with β thalassaemia**
(A) The patient is first treated with G-CSF to mobilise HSCs into the peripheral blood. (B) Peripheral blood mononuclear cells are then collected and CD34+ cells (enriched for HSCs) are harvested. (C) These cells are cultured with a viral vector that is designed to express high levels of normal human β globin. (D) The patient then undergoes non-ablative myelosuppression and is engrafted with the virally-modified HSCs (filled circles). PBMC=peripheral blood mononuclear cell. G-CSF=granulocyte colony-stimulating factor. HSC=haemopoietic stem cell.

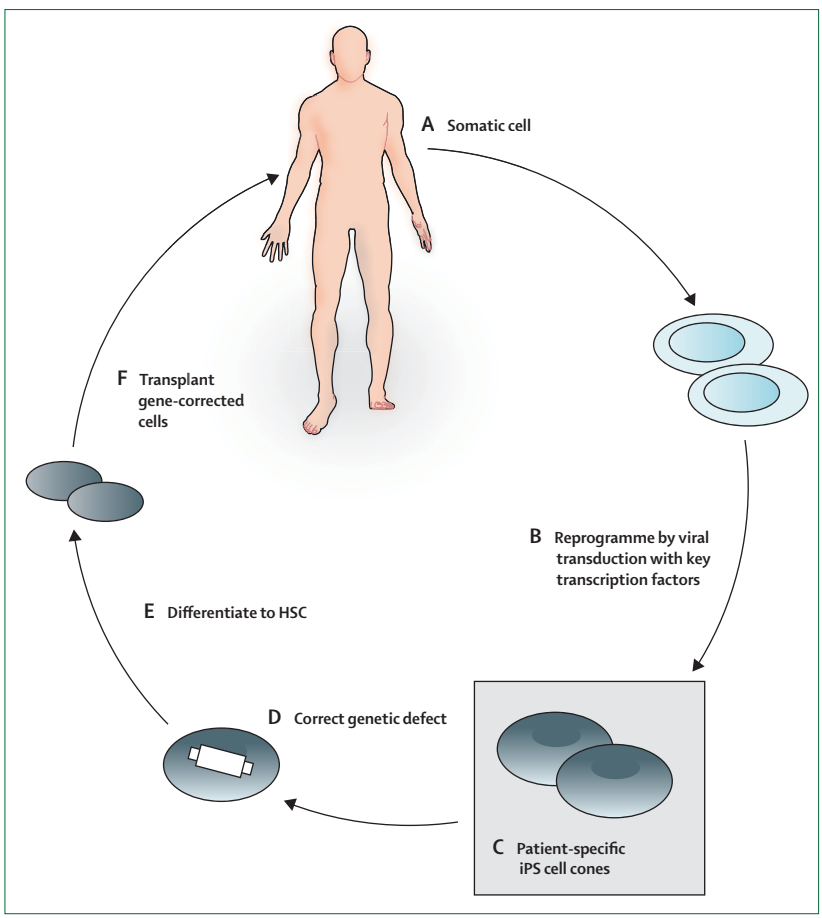

Figure 7: **An outline of the proposed use of iPS cells for therapy in patients with β thalassaemia**
Somatic cells from the patient (A) are reprogrammed by expression of key transcription factors that are associated with pluripotency (B) to make patient-specific iPS cells (C). In thalassaemia, the defect in the β-globin gene is then corrected (D). These corrected iPS cells are then differentiated into haemopoietic stem cells (E) and transplanted (F) back into the patient after myelosuppression. iPS cell=induced pluripiotent stem cell.

treatment with blood transfusion and iron chelation that has nevertheless converted β-thalassaemia into a chronic survivable disease with a life expectancy of 50 years or more. Stem-cell transplantation will probably be available only to those with access to high-technology medicine, and not generally available to most patients with β thalassaemia.

Gene therapy for β thalassaemia

Thalassaemia was among the first genetic diseases for which gene therapy was proposed.[82] This disorder is to some extent a good target because defects in expression of globin genes affect only the haemopoietic system and specifically affect erythropoiesis. Stem-cell transplantation is well developed for the haemapoietic system; however, unlike many genetic diseases, very high levels of tissue-specific gene expression are necessary to correct the globin defect in β thalassaemia. In principle, the procedure is straightforward (figure 6). Haemopoietic stem cells from the patient are harvested; modified viruses containing the β-globin gene linked to its upstream regulatory elements are used to infect and insert the β-gene construct into the genome of HSCs. To reduce the disease burden and to create space in the bone-marrow niche to accept the modified HSCs, autologous stem cells are infused back into the patient after myelosuppression. Such a protocol overcomes the problems of donor availability and the immunological complications associated with allogeneic stem-cell transplantation.

Although seemingly simple, many challenges have been encountered in the development of a suitable protocol. Viruses can accommodate only small segments of DNA and therefore, in view of the complexity of globin gene expression, many years have been spent in the development of viral constructs that contain all of the sequences needed for adequate expression of the globin genes. Even when such constructs have been obtained, their expression might be positively or negatively affected by their position of integration in the genome. To overcome effects related to position, constructs now frequently contain the chromosomal boundary elements described above (figure 2). However, of great concern in some patients treated with retroviral constructs (for immune deficiency disorders), insertions of viral constructs next to crucial haemopoietic genes have led to the development of leukaemia.[83–85]

Despite these setbacks, considerable progress has been made in the past 10 years. Rather than use of retroviruses, safer lentiviral vectors have been developed, which have expressed long-term, therapeutic levels of β globin in preclinical trials, and have corrected the anaemia in a mouse model of thalassaemia.[86–90] Encouraged by these preclinical studies,[91] the first clinical trial using a lentiviral construct of β globin to treat patients with β thalassaemia was started in 2007.[92,93] Furthermore, at least two other groups[94,95] have announced plans to undertake similar trials. In the 2007 trial, two patients received gene therapy. A delayed haematological recovery in the first patient needed a rescue with back-up cells that had not been manipulated. The second patient with β^E-thalassaemia major (β^E/β^T) also had a delayed recovery, but eventually established 10% engraftment with modified HSCs. At present, this patient maintains a haemoglobin concentration of 90–100 g/L, partly because of the anticipated increase in β-globin expression, but also because of an unexplained increase in fetal haemaglobin. Consequently, this patient has remained well and transfusion independent for almost 2 years. However, a detailed analysis has shown that in a partially dominant haemopoietic clone, the viral vector has integrated into a proto-oncogene, which could be a harbinger of a leukaemic transformation as seen previously in gene-therapy protocols with retroviruses.[92]

Beyond gene therapy

In 2007, a landmark report[96] described how human somatic cells (eg, skin fibroblasts) could be

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reprogrammed to form multipotent cells resembling embryonic stem cells. These reprogrammed cells are called induced pluripotent stem (iPS) cells.[96,97] Generation of iPS cells involves the introduction and expression of four transcription factors (Oct4, Sox2, KLF4, and c-Myc) in somatic cells. These transcription factors are normally needed to establish and maintain pluripotency. iPS cells have enabled new research possibilities to be explored, establishing new models of human disease, providing new methods for drug screening, and offering the potential for new approaches to cell therapy.[76] A proof of principle for use of iPS cells was shown when these cells were generated from the somatic cells of a mouse model of sickle-cell disease.[98,99] The β^S mutation in the mouse iPS cells was corrected (with use of homologous recombination), and the cells were then differentiated into multipotent HSCs before being transplanted back into the irradiated mouse to regenerate a haemopoietic system in which the β-globin mutation was corrected. In a move towards the development of a similar strategy in human beings, skin fibroblasts from a patient with homozygous β thalassaemia were reprogrammed to form iPS cells; these cells could then be successfully differentiated to form red blood cells.[100]

Although all the necessary steps for cell therapy could be done with human cells (figure 7), some major challenges still need to be overcome before this approach can be considered for clinical use. The reagents for generation of iPS cells will need to be improved, as will the efficiency with which somatic cells are reprogrammed. Additionally, the generation of genuine HSCs from iPS cells is not yet possible. Finally, reprogrammed, genetically engineered iPS cells will need to be shown to have no potential to undergo subsequent malignant transformation.

Conclusions

Despite intensive clinical and scientific investigation of thalassaemia—a molecular disease that is perhaps better understood than any other—attempts to improve its management and to develop targeted drug therapy have not yielded a clear breakthrough. Stem-cell transplantation is an effective cure but still has a substantial risk of mortality and morbidity. Supportive results from cord-blood transplantation should encourage the development of cord-blood banking to address this issue. Gene therapy is now entering early-phase clinical trials, and cell therapy with iPS cells is an exciting prospect with many challenges to overcome. Even if these approaches are successful, such therapy might not be immediately applied to most patients in low-income countries. Developments in the understanding of the molecular circuitry involved in the transition from fetal to adult globin-gene expression might facilitate the development of new drugs to manipulate this switch. Even so, the cost of what would presumably be lifelong medication could rule out therapy for all but those who are supported by wealthy economies. The most important development for the next few years might be the use of high-throughput DNA analysis to provide cheap accurate genotyping and prenatal testing, which has been effective when applied systematically.

Contributors

Each author contributed equally to reviewing the published works and writing the Seminar. DRH had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflicts of interest

GS has received payment for board membership and holds stock for HemaQuest, and has received royalties from University of Washington. All other authors declare that they have no conflicts of interest.

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