Sickle-cell disease 2

Fetal haemoglobin in sickle-cell disease: from genetic epidemiology to new therapeutic strategies

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This is the second in a Series of three papers about sickle-cell disease

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Sickle-cell disease affects millions of individuals worldwide, but the global incidence is concentrated in Africa. The burden of sickle-cell disease is expected to continue to rise over the coming decades, adding to stress on the health infrastructures of many countries. Although the molecular cause of sickle-cell disease has been known for more than half a century, treatment options remain greatly limited. Allogeneic haemopoietic stem-cell transplantation is the only existing cure but is limited to specialised clinical centres and remains inaccessible for most patients. Induction of fetal haemoglobin production is a promising strategy for the treatment of sickle-cell disease. In this Series paper, we review scientific breakthroughs in epidemiology, genetics, and molecular biology that have brought reactivation of fetal haemoglobin to the forefront of sickle-cell disease research. Improved knowledge of the regulation of fetal haemoglobin production in human beings and the development of genome editing technology now support the design of innovative therapies for sickle-cell disease that are based on fetal haemoglobin.

Introduction

Sickle-cell anaemia is a prototypical monogenic disorder caused by the autosomal recessive inheritance of an A→T transversion of the β-globin gene (β^s [*HBB*]). The entire complex pathophysiological cascade of sickle-cell anaemia, including haemolysis, anaemia, ischaemia, inflammation, susceptibility to infection, and organ injury, emanates from this single mutation. Although individuals homozygous for β^s have severe disease, prevalence of this allele has risen to extremely high levels in sub-Saharan Africa, the Arabian peninsula, and central India because people who are heterozygous for the allele are partly protected from malaria.[1] The mutant β^s globin peptide, carrying the characteristic Glu to Val aminoacid substitution, renders sickle haemoglobin susceptible to polymerisation when deoxygenated. This molecular alteration predisposes erythrocytes to adopt a sickled conformation, as first observed on blood films more than 100 years ago.[2] Sickle-cell disease refers to a set of sickling disorders that share an underlying β^s mutation, including homozygous β^s (sickle-cell anaemia) and various compound heterozygous disorders such as $\beta^s\beta^0$ and $\beta^s\beta^+$ (the sickle/β-thalassaemias) and $\beta^s\beta^C$ (haemoglobin SC sickle-cell disease). Despite the apparent straightforward aetiology of sickle-cell disease, its clinical manifestations are heterogeneous, with some individuals severely affected and others avoiding serious consequences—suggestive of a key role of disease modifiers. Chief among these modifying factors is the concentration of residual fetal haemoglobin.

Before consideration of the clinical, epidemiological, and molecular genetic observations indicative of the role of fetal haemoglobin in modulation of sickle-cell disease, we will discuss the various forms of haemoglobin (figure 1). Haemoglobin, the oxygen-carrying metalloprotein constituent of erythrocytes, is a tetramer of globins, each of which contains an iron-containing haem moiety. All functional haemoglobins are formed by a tetramer of two α-like and two β-like globins. Both α-globin and β-globin genes are encoded from a cluster of similar genes, the α-like and β-like globins on chromosome 16 and 11, respectively. The globin clusters undergo developmental regulation: during the latter two trimesters of gestation in human beings, fetal haemoglobin is the prevalent haemoglobin. Only after birth, in a process primarily driven by regulation of gene expression, is fetal haemoglobin replaced by adult haemoglobin. The β-globin gene cluster on chromosome 11 includes five β-like genes: ε-globin (expressed early in the first trimester), ^{G}γ-globin, ^{A}γ-globin (both of which are expressed throughout the remainder of gestation), δ-globin, and β-globin (the minor and major β-like globin genes expressed after birth). Whereas fetal haemoglobin contains two α and two γ-globins ($\alpha_2\gamma_2$), adult haemoglobin tetramer contains two α and two β-globins ($\alpha_2\beta_2$). A residual amount of fetal haemoglobin remains in the adult stage (typically <1% of total haemoglobin) along with the major form (about 97%) and minor form of adult haemoglobin, HbA_2 ($\alpha_2\delta_2$; about 2%). Residual fetal haemoglobin is typically not evenly distributed among erythrocytes but concentrated in a subset of cells known as F-cells, in which about 20% of the total haemoglobin content is fetal haemoglobin.

Search strategy and selection criteria

We searched ClinicalTrials.org registry and relevant articles with the terms "sickle cell" and "fetal haemoglobin". Meeting abstracts and reports were included only when they related directly to previously published work. We considered only articles published in English between 2000 and July 14, 2015.

Natural history and epidemiology

Scientific breakthroughs often result from series of apparently unrelated findings that ultimately lead to convergent conclusions. The recognition that fetal haemoglobin can ameliorate many of the symptoms in patients with sickle-cell disease is one clear example of this path to discovery. In 1948, Janet Watson, who was aware that newborn babies do not manifest sickle-cell disease complications until they reach about 6 months of age, made the seminal finding that erythrocytes of newborn babies with sickle-cell disease are relatively protected from sickling compared with adults or older infants.[3] She presciently hypothesised that this protection in infants was due to the high concentration of fetal haemoglobin in circulating erythrocytes. A few years later, anecdotal reports described asymptomatic patients with sickle-cell disease who co-inherited a hereditary persistence of fetal haemoglobin (HPFH) phenotype.[4] HPFH is typically caused by either large deletions of the β-globin gene cluster or point mutations in the promoters of the γ-globin genes and is characterised by high concentrations of fetal haemoglobin throughout life. Finally, some populations with sickle-cell disease, notably patients from Saudi Arabia and the Indian subcontinent, were noted for having higher concentrations of fetal haemoglobin and a milder form of sickle-cell disease than patients of African ancestry.[5,6] Although the view that sickle-cell disease is largely benign in patients of Arab-Indian descent has recently been challenged,[7] many studies have described the relatively low prevalence and delayed onset of many sickle-cell disease complications, attributed in part to high fetal haemoglobin production into adulthood, in patients from this part of the world.[5–9]

The modern techniques of epidemiology have supported fetal haemoglobin as a strong modifier of severity in sickle-cell disease. In the large prospective Cooperative Study of Sickle Cell Disease (CSSCD),[10] increased concentrations of fetal haemoglobin were independently associated with improved survival and decreased rates of painful crises,[11] acute chest syndrome,[12] and osteonecrosis.[13] The beneficial effect of fetal haemoglobin on several complications related to sickle-cell disease was also seen in toddlers and adolescents with the disease from the Jamaica sickle-cell disease birth cohort.[14,15] Certain complications, such as priapism and stroke, were originally thought to be independent of fetal haemoglobin concentrations,[16,17] although these negative results might simply represent a small number of clinical events and thus have limited statistical power to detect an effect. Surprisingly, although most patients with sickle-cell disease are born and live in Africa, few data exist on the possible role of fetal haemoglobin in ameliorating the severity of sickle-cell disease in African countries. This pronounced scarcity of information emphasises the need for a renewed academic interest in varying manifestations of sickle-cell disease in Africa where the worldwide disease burden predominates.

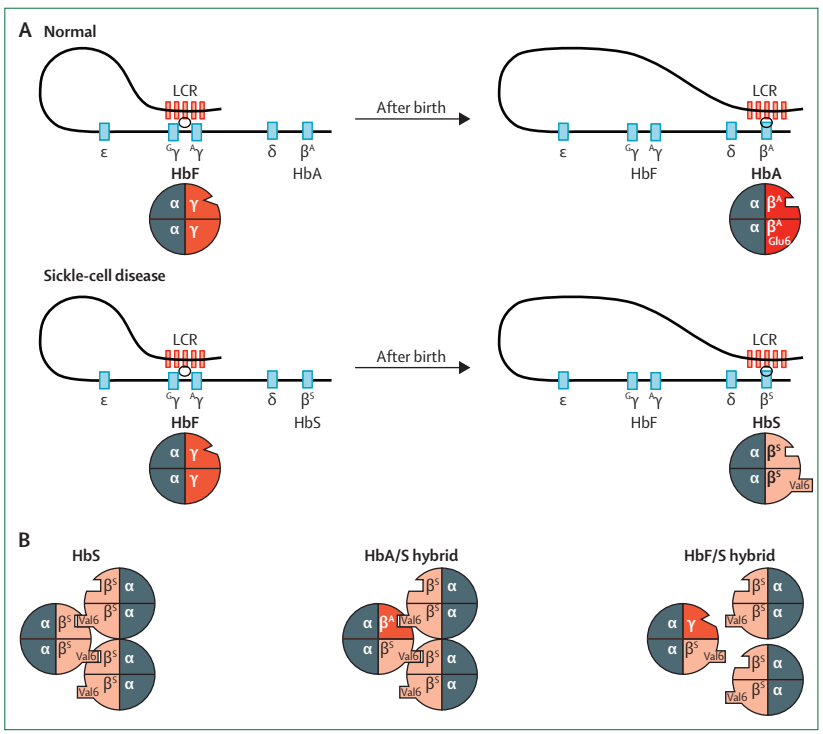

Figure 1: **Fetal haemoglobin and sickle-cell disease**
(A) The predominant haemoglobin before birth is fetal haemoglobin (HbF; $\alpha_2\gamma_2$) with a small contribution of adult haemoglobin (HbA; $\alpha_2\beta_2$). After birth, only a small residual production of γ-globin remains, such that HbF makes a small contribution and HbA predominates in the total amount of haemoglobin. Sickle-cell disease, due to its β^S mutation of β-globin, only manifests after birth once the contribution of HbF wanes. (B) Sickle haemoglobin (HbS) tetramers have a tendency to polymerise under deoxygenated conditions. The antisickling effect of HbF exceeds that of HbA since γ-globin has Gln rather than Thr at position 87 and thus a less hydrophobic patch for lateral interactions with Val 6 of the β^S peptide. In cells co-expressing γ-globin and β^S-globin, most of the γ-globin is incorporated into $\alpha_2\beta^S\gamma$ hybrid haemoglobin tetramers, which have an antisickling effect.

How does fetal haemoglobin inhibit sickling? The sickle haemoglobin polymer is a solid fibre consisting of seven pairs of strands of sickle haemoglobin tetramers stacked atop of one other. Except for a slight helical twist, each pair of strands is almost identical at atomic resolution to the structure of the deoxygenated form of sickle haemoglobin determined by x-ray crystallography.[18] The x-ray structure shows that the fibre is stabilised by the hydrophobic β 6 Val of sickle haemoglobin on one strand binding to a hydrophobic patch at β 85–88 on the adjacent strand. γ haemoglobin has a Gln rather than a Thr at position 87, which renders this hydrophobic interaction weaker.[19] Consequently, fetal haemoglobin tetramers containing γ-chains have a much lower probability of copolymerising with the sickle haemoglobin tetramers containing two β^S peptides (figure 1). This structural analysis is in full agreement with both kinetic and equilibrium studies,[20,21] the results of which show that mixtures of sickle haemoglobin and fetal haemoglobin form polymers much less readily than comparable mixtures of sickle haemoglobin and adult haemoglobin.

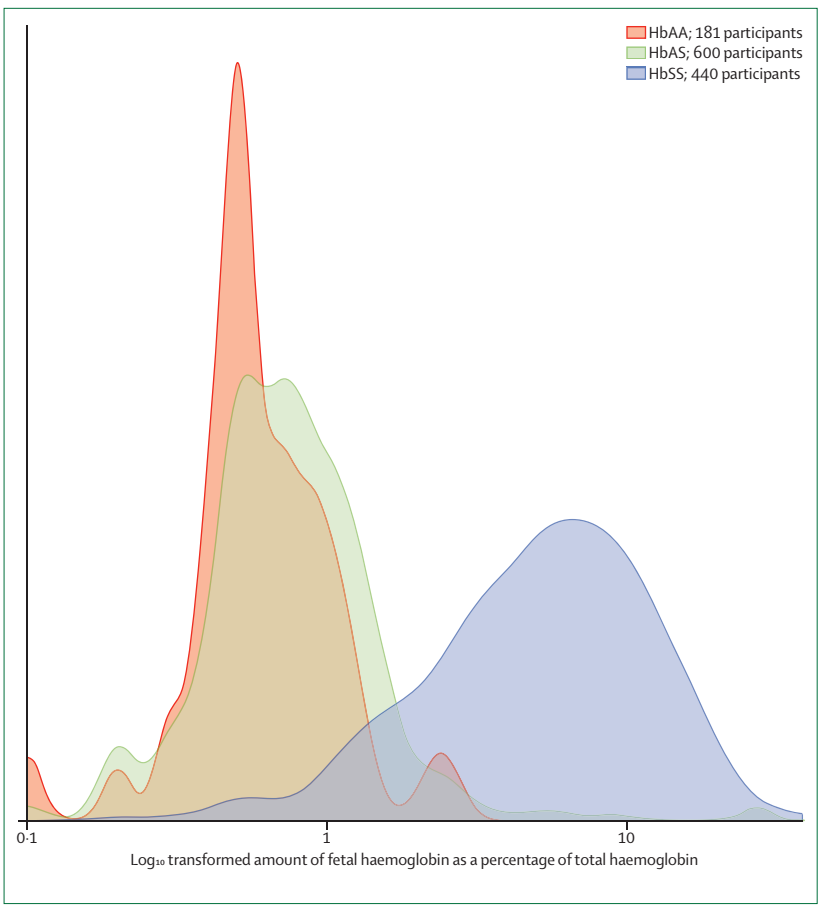

***Figure 2*: Distribution of fetal haemoglobin concentration in patients with sickle-cell disease**
181 participants assessed with GEN-MOD procedure carried no β^s allele (HbAA), 600 participants had one β^s allele (HbAS [sickle cell trait]), and 440 participants had two β^s alleles (HbSS [sickle-cell anaemia]). The mean HbF concentration is 0·8% (SD 2·0) in patients with HbAA, 0·9% (0·8) in patients with HbAS, and 6·8 (5·2) in patients with sickle haemoglobins.

Genetic regulation of fetal haemoglobin production

Although the switch from γ-globin to β-globin happens shortly after birth, most individuals continue to produce measurable amounts of fetal haemoglobin in adulthood. The concentrations of fetal haemoglobin are usually higher in patients with sickle-cell disease than in non-affected adult individuals, principally because of the increased survival of F-cells (figure 2). No studies have estimated the heritability of fetal haemoglobin in sickle-cell disease (panel), but the interindividual variation of the heritability is very high in the general population, with genetics explaining 60–90% of this variation.[22,23] The β-globin locus was the first chromosomal region that was shown to carry DNA sequence variants that affect the concentration of fetal haemoglobin, in the form of HPFH mutations (figure 3).[25] The comparison of large deletions within the β-globin locus associated with higher fetal haemoglobin concentrations (deletional HPFH) with smaller deletions associated more modest increases in fetal haemoglobin concentration ($\delta\beta^0$-thalassaemia) has implicated sequences between the ^{A}γ-globin and δ-globin genes as cis-acting determinants of γ-globin expression.[26] The association of small, naturally occurring deletions in the region between ^{A}γ-globin and δ-globin loci, such as the Corfu deletion, with high fetal haemoglobin concentrations further supports this hypothesis.[27] The fine-mapping of breakpoints of HPFH deletions identified a 3·5-kb genomic region within the β-globin gene cluster that seems to be important for γ-globin silencing (figure 3).[28] This minimal region also includes single nucleotide polymorphisms (SNPs) that are strongly associated with variations in fetal haemoglobin concentration in patients with sickle-cell disease.[29]

After the initial characterisation of HPFH alleles, it took another 20 years and improvements in genomic technology to identify genomic regions outside of the β-globin locus that control the variation in fetal haemoglobin concentration. The first hints came from linkage scans, which highlighted linkage peaks on chromosome 6p23, chromosome 8q, and chromosome Xp22.2.[30–32] Unlike the linkage signals at 8q and Xp22.2, which have not been confirmed independently, results for the 6p23 region were particularly convincing because multiple populations, including patients with sickle-cell disease, carried DNA sequence variants in that genomic region which were shown to have a strong effect on the variation of fetal haemoglobin concentration.[33–36] In a targeted genetic association study, the signal at chromosome 6p23 was fine-mapped to the intergenic sequence between *HBS1L* and *MYB*.[34] SNPs in this intergenic region interfere with the binding of key erythropoietic transcription factors (eg, GATA1, TAL1, KLF1) and modulate *MYB* expression (figure 4).[37] These results, together with naturally occurring rare variants in *MYB*[38] and functional results from primary human erythroid precursors in which MYB was inhibited,[39] strongly support MYB as a direct regulator of fetal haemoglobin in human beings.

Genome-wide association studies (GWAS) have revolutionised how geneticists tackle the difficult problem of associating common DNA sequence variants (eg, SNPs) with complex human diseases or traits. GWAS led to the identification of the *BCL11A* locus, first in healthy Europeans and those with β-thalassaemia,[36,40] then in patients with sickle-cell disease of African ancestry[33,41,42] and in patients of Asian ancestry with haemoglobin E/β-thalassaemia.[43] *BCL11A* encodes a zinc-finger transcription factor that had previously been investigated for a role in lymphocyte and neural development.[44,45] Results of knockdown experiments in erythroid cells validated BCL11A as a negative regulator of γ-globin gene expression[46] and showed that loss of *BCL11A* during embryogenesis prevented appropriate developmental silencing of γ-globin.[47] Targeted deletion of *BCL11A* in the erythroid lineage rescued the phenotype of a murine sickle-cell disease model.[48] The in-vivo role of BCL11A as a repressor of fetal haemoglobin production in human

beings was also confirmed through the characterisation of rare large genomic deletions of sequences that include this locus. These patients with *BCL11A* haploinsufficiency have an HPFH-like phenotype and neurodevelopmental alterations.[49,50] The specificity of the SNPs at *BCL11A* that are associated with fetal haemoglobin expression seems to be related to their location within an erythroid-specific enhancer (figure 4).[51,52] Although few examples have been experimentally validated like *BCL11A*, cell type-specific enhancer disruption by common genetic variation seems to be a prevalent mechanism of human disease susceptibility.[53]

By contrast with most SNPs identified in GWAS, which normally have a small effect on phenotypes, SNPs at the *BCL11A*, *HBS1L-MYB*, and β-globin gene cluster loci account for up to 50% of the heritable variation in fetal haemoglobin concentration in patients with sickle-cell disease.[38] The same loci are associated with both fetal haemoglobin concentration and the fraction of F-cells,[40,42] suggesting that the mechanisms controlling the quantity and frequency of fetal haemoglobin production are tightly linked. Because of the robust phenotypic effect and the strong causal relation between fetal haemoglobin concentration (or F-cell number) and sickle-cell disease-related complications, these SNPs are also associated with measures of the clinical severity of sickle-cell disease, including painful sickle-cell crises and hospitalisation rates.[33,54,55] Improvements in the ability to prognosticate clinical severity is one goal of precision medicine in the context of sickle-cell disease. To increase predictive power, the remaining genetic factors that affect fetal haemoglobin production need to be identified. One strategy is to increase the sample size of the GWAS of fetal haemoglobin through meta-analyses. So far, the largest GWAS for fetal haemoglobin in sickle-cell disease included fewer than 2000 patients, a very modest sample size in comparison with meta-analyses for other complex human phenotypes, which can include more than 100 000 participants.[56–58] Another possibility is to explore the contribution of rare genetic variants to the variation in fetal haemoglobin concentration. Because of their design, GWAS are not suitable study types for the investigation of this category of genetic variation. Next-generation DNA sequencing, including whole-exome and whole-genome sequencing, now provides a comprehensive approach to investigate whether (and how) rare genetic variants affect fetal haemoglobin concentration and production.[59] Targeted sequencing approaches have already established that rare genetic variants in *MYB* and *KLF1* might be associated with increased fetal haemoglobin concentrations in adults.[38,60–62] KLF1, a transcription factor with an expression profile that is restricted to the erythroid lineage, has a broad role in coordinating appropriate regulation of genes required for terminal erythroid differentiation. In Chinese patients with β-thalassaemia, rare *KLF1* missense mutations are associated with both increased fetal haemoglobin concentrations and a mild clinical course.[61]

***Panel*: Key concepts**

Heritability
Heritability is the fraction of the observable (phenotypic) variation that is explained by genetic factors. For instance, the heritability for fetal haemoglobin is 60–90%; the remaining variation is explained by other factors such as the environment. Single nucleotide polymorphisms (SNPs) at *BCL11A*, *HBS1L-MYB*, and β-globin (*HBB*) explain about 50% of the heritability for fetal haemoglobin. The remaining heritability—often termed missing or hidden—is explained by unknown genetic factors.

Complex human diseases
The risk of developing a complex human disease is affected by genetic and non-genetic factors. For instance, the risk of a heart attack depends on a combination of genetic variants and environmental factors (eg, diet and smoking). By contrast with complex human diseases, simple or mendelian diseases are caused by mutations in a single gene (eg, sickle-cell disease, which results from mutations in the β-globin gene).

Linkage study
Linkage scans measure the cosegregation of genetic markers and phenotypes in large families to identify chromosomal segments that affect disease risk. Linkage studies have had only limited success in the context of complex human diseases, in part because disease risk conferred by segregating common variants is usually small.

Association study
Association studies measure the correlation between alleles and phenotypes (diseases or quantitative traits). Genome-wide association studies (GWAS) test for hundreds of thousands (or even millions) of variants for association with a phenotype of interest. GWAS have identified more than 14 000 associations between SNPs and complex human phenotypes.

Genome editing
Genome editing refers to a set of techniques in which sequence-specific nucleases are used to alter genomic sequences at precise positions. DNA sequence specificity stems from an engineered protein domain (in the case of meganucleases, zinc finger nucleases, and transcription activator-like effector nucleases) or a complementary guide RNA (in the case of the clustered regularly interspaced short palindromic repeats [CRISPR]-associated 9 [Cas9] nuclease).

Therapeutic induction of fetal haemoglobin production to treat sickle-cell disease

The association between high fetal haemoglobin concentration and a mild sickle-cell disease course suggests the potential to treat patients by reactivating

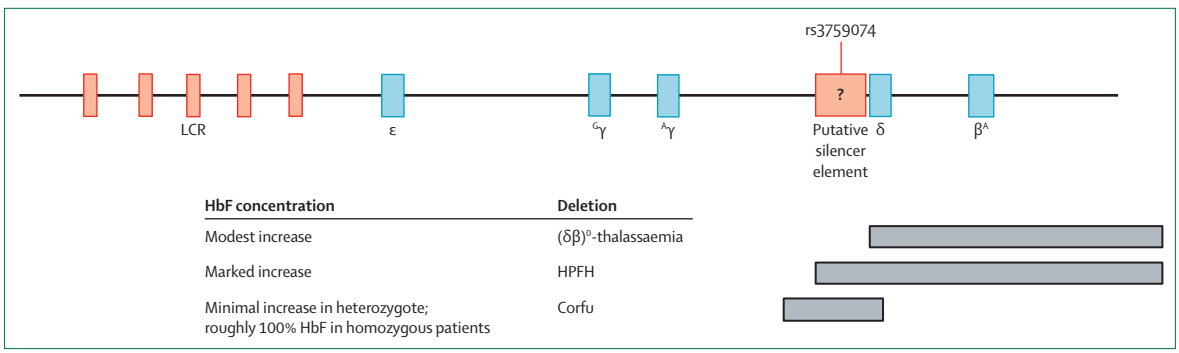

***Figure 3*: Rare, naturally occurring deletions at the β-globin gene cluster suggest the presence of a putative silencer element between ^{A}γ-globin and δ-globin**
Common genetic variants associated with high concentration of fetal haemoglobin (HbF) are located within these intergenic sequences. This figure was adapted from Sankaran and Orkin (2013).[24] LCR=locus control region.

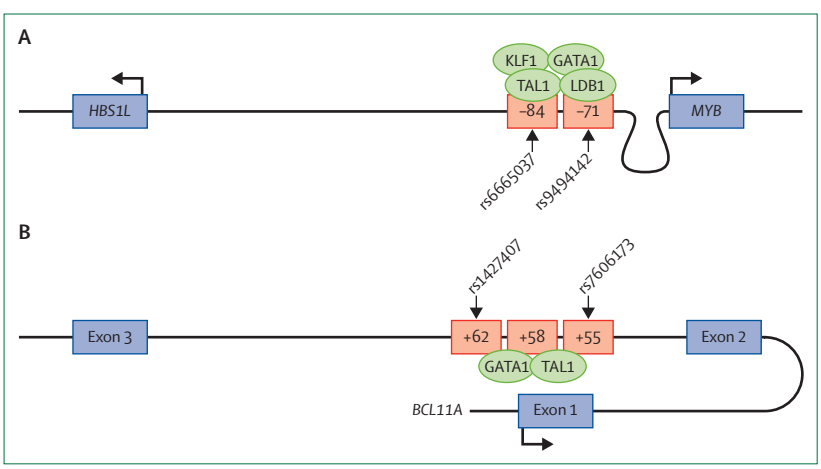

Figure 4*: Single nucleotide polymorphisms associated with fetal haemoglobin are located within erythroid enhancers and control the expression of *MYB* and *BCL11A
(A) Alleles associated with high concentration of fetal haemoglobin are found within intergenic regulatory sequences (–84 and –71) between *HBS1L* and *MYB* and interfere with the binding of transcription factors, affect enhancer activity and promoter–enhancer communication, and correlate with reduced *MYB* expression. (B) At the *BCL11A* locus, alleles associated with high concentration of fetal haemoglobin disrupt intronic erythroid-specific enhancers (+55 and +62), affect transcription factors binding, and associate with low *BCL11A* expression. Because BCL11A is a repressor of γ-globin expression, this results in an increased production of fetal haemoglobin.

fetal haemoglobin production. Clinical observations in the context of cancer therapy and bone marrow transplantation,[63,64] as well as experiments in anaemic baboons,[65] indicated that altered erythroid kinetics might result in increased expression of the γ-globin genes. Hydroxyurea, which blocks the cell cycle in S phase, has been shown to induce a robust production of fetal haemoglobin.[66] Although the specific mechanisms by which hydroxyurea modulates the haemopoietic system are still a topic of debate, results of clinical trials[67–69] have shown the efficacy of hydroxyurea in increasing fetal haemoglobin production, decreasing complications related to sickle-cell disease, and improving survival in children and adults with the disease. Hydroxyurea is generally well tolerated and shows reversible, dose-dependent myelosuppression and minimal non-haematological toxic effects. Findings of long-term studies of children and adults who received continuous treatment, with more than 15 years' follow-up, show excellent safety profiles.[70–72] Hydroxyurea is now the only drug approved by the US Food and Drug Administration and several European regulatory authorities to treat sickle-cell disease. Although patient-to-patient variation in response is seen, adults treated with hydroxyurea usually have a heterocellular increase in fetal haemoglobin concentration from about 5% at baseline to about 15%, and children can reach even higher concentrations of fetal haemoglobin, concordant with substantial clinical benefits. Nevertheless, this concentration of fetal haemoglobin is still lower than a threshold expected to completely prevent the complications of sickle-cell disease, as would be seen in patients with sickle haemoglobin/HPFH, for whom about 30% of total haemoglobin is fetal haemoglobin, which, with a pancellular distribution, is fully protective.[70,73] Identification of novel compounds to robustly induce fetal haemoglobin in the context of sickle-cell disease remains a pressing challenge. Although beyond the scope of this review, novel fetal haemoglobin-inducing therapeutic strategies might have a benefit not only in sickle-cell disease but also in β-thalassaemia, in which case γ-globin can substitute for absent β-globin.

Chromatin dynamics and fetal haemoglobin production

Advances in knowledge of the molecular mechanisms controlling globin gene expression raise hope for the development of novel pharmacotherapies to increase fetal haemoglobin concentration. Much of the understanding can be simplified into two predominant mechanisms of gene regulation: first, the effect of chromatin regulators; and second, the action of DNA-binding transcription factors. The developmental switch from fetal haemoglobin to adult haemoglobin, and thus from γ-globin to β-globin expression, is accompanied by biochemical changes at the globin gene cluster. These epigenetic changes include direct modification of the DNA itself and of histones, around which DNA is wrapped (ie, the chromatin

structure). For example, the γ-globin gene in adult erythroid cells, as compared with fetal erythroid cells, is associated with increased cytosine methylation, loss of surrounding active histone modifications (such as trimethylated lysine 36 on histone 3 [H3K36me3], acetylated lysine 9 on histone 3 [H3K9ac], and acetylated lysine 27 on histone 3 [H3K27ac]), and a decrease in chromatin accessibility.[74–77] These biochemical changes are in line with the finding that DNA methyltransferase inhibition, either through chemical inhibitors, knockdown of DNA methyltransferase 1 (DNMT1) in human erythroid precursors, or *Dnmt1* knockout in transgenic mice, results in derepression of fetal haemoglobin concentrations.[78–80] This developmental chromatin profile is also consistent with the understanding that histone deacetylase (HDAC) inhibitors, such as butyrate, can restore fetal-like histone acetylation around γ-globin and increase fetal haemoglobin concentration. The hypothesis that specific inhibitors of HDAC1 and HDAC2 or next-generation DNA methylation inhibitors could be used to adjust fetal haemoglobin concentration while avoiding excess adverse consequences is being tested in ongoing efforts.[81,82]

The nucleosome remodelling and deacetylase complex is a macromolecular protein structure that includes histone deacetylases, factors that recognise methylated DNA, such as MBD2, and chromatin remodelling factors, such as Mi2β. Inhibition of Mi2β, in particular, has potent effects on derepression of fetal haemoglobin production, consistent with an important role for the complex in maintaining γ-globin gene silencing.[83] Histone methylation has emerged as a novel pathway for the induction of fetal haemoglobin production. LSD1 is a demethylase that targets methylated lysine 4 of histone 3 (H3K4me; often associated with gene activation) and methylated lysine 9 of histone 3 (H3K9) (often associated with gene repression). Inhibition of LSD1 has been shown to increase fetal haemoglobin production in primary human erythroid precursors and transgenic mice.[80,84–86] However, the effects do not seem to be associated with a clear pattern of increased methylation at the globin genes, are of variable efficacy, and might be difficult to disentangle from inhibition of erythropoiesis or haemopoiesis. G9a is a histone methyltransferase that can deposit methyl groups onto H3K9, its inhibition has also been associated with increased expression of embryonic and fetal globin gene expression.[87–89] Several additional chromatin regulators are implicated in globin gene expression, including the arginine methyltransferase PRMT5 and the NCoR, SIN3, and SWI/SNF complexes.[80,90]

The challenge for pharmacological manipulation of any chromatin regulator is to achieve a desirable therapeutic index. Many of the chromatin regulators are prevalent mediators of gene regulation that orchestrate appropriate expression of many genes across diverse cellular contexts. The ultimate effect of a chromatin regulator on fetal haemoglobin production might result both from direct modulation of globin gene control and from indirect effects, such as erythroid stress. The difficulty is therefore not only to find a therapeutic perturbation that substantially increases fetal haemoglobin concentration, but to find one that does not adversely affect erythrocyte development or other functions within and beyond the haemopoietic system. A related challenge is that none of the model systems fully recapitulate in-vivo human erythropoiesis and globin gene regulation, so definitive results of the potency and specificity of candidate small molecules will be difficult to obtain outside careful clinical trials. Although clinical trials of some drugs are ongoing (table), none of the completed trials has released results (we highly recommend that investigators release results from clinical trials, even when they are negative) or identified a novel, tolerable drug that results in a robust increase in fetal haemoglobin concentration.

Transcriptional regulation: the basic theory of BCL11A

The identification of transcription factors that are important for γ-globin silencing offers promise for increased therapeutic specificity. BCL11A is the most potent repressor of fetal haemoglobin production identified to date. Findings from many experimental studies show that loss of BCL11A causes a large increase in fetal haemoglobin concentration with minimal effect on erythropoiesis.[46–48,94] The major potential drawback to the targeting of BCL11A would appear to be its key functions in non-erythroid lineages. BCL11A has important roles in neuron development, B-cell lymphopoiesis, and dendritic cell fate, perhaps also in haemopoietic stem cells and progenitor cells and pancreatic precursors.[95–100] A striking feature of BCL11A's repression of γ-globin is the exquisite dose-dependence of the effect. Mice carrying only one copy of *Bcl11a* show an intermediate effect in terms of γ-globin derepression compared with wild-type mice.[48] SNPs associated with variations in fetal haemoglobin concentration result in a modest decrease in *BCL11A* gene expression, preserving about 65% of normal concentration, and are associated with a roughly three-times increase in fetal haemoglobin concentration in patients with sickle-cell disease.[51] Individuals who are haploinsufficient for *BCL11A* show an increase in fetal haemoglobin concentration, which ranges from 4·8% to 29·7%, which is a remarkable increase in a non-haemoglobinopathy setting in which fetal haemoglobin concentration is typically less than 1% (figure 2).[49,50] No immunodeficiency or other haematological toxic effect has been recorded in patients with this haploinsufficiency. A small-molecule approach targeting BCL11A might be a plausible way to reduce BCL11A activity below a threshold needed for fetal haemoglobin repression but spare extra-erythroid functions. Additionally, although the details of all its molecular interactions are incompletely understood,

	Drug	Phase	Sample size	Results
Efficacy of vorinostat to induce fetal haemoglobin in sickle-cell disease (NCT01000155)	Vorinostat (HDAC inhibition)	1/2	5	Modest increase in HbF concentration[91]
Gum arabic as fetal haemoglobin agent in sickle-cell anaemia (NCT02467257)	Gum arabic (HDAC inhibition)	1/2	47	Completed in 2015, no results available
Study to determine the maximum tolerated dose, safety, and effectiveness of pomalidomide for patients with sickle-cell disease (NCT01522547)	Pomalidomide (anti-angiogenic, immunomodulator)	1	12	Completed in 2013, no results available
Evaluation of hydroxyurea plus L-arginine or sildenafil to treat sickle-cell anaemia (NCT00056433)	Hydroxyurea, L-arginine, sildenafil (nitric oxide production)	1	39	Sildenafil modestly increases HbF concentration[92]
Effect of broccoli sprouts homogenate on SS RBC (NCT01715480)	Broccoli sprouts homogenate (induction of sulforaphane by NRF2)	1	21	Completed in 2015, no results available
A phase 1/2 trial of recombinant-methionyl human stem cell factor (SCF) in adult patients with sickling disorders (NCT00005783)	Recombinant-methionyl human stem-cell factor (haemopoietic progenitor cells stimulation)	1/2	50	Completed in 2000, no results available
Study of panobinostat in patients with sickle-cell disease (NCT01245179)	Panobinostat (HDAC inhibition)	1	27	Expected completion in December, 2018
Effects of HQK-1001 in patients with sickle-cell disease (NCT01601340)	Sodium 2,2-dimethylbutyrate (HDAC inhibition)	2	77	No increase in HbF concentration[93]
Decitabine for high-risk sickle-cell disease (NCT01375608)	Decitabine (hypomethylating agent)	2	10	Completed in 2016, no results available
Study of decitabine and tetrahydrouridine in patients with sickle-cell disease (NCT01685515)	Decitabine and tetrahydrouridine (deaminase inhibitor)	1	25	Actively recruiting patients

HbF=fetal haemoglobin. HDAC=histone deacetylase.

***Table*: Drugs that have been or are being tested as inducers of fetal haemoglobin in patients with sickle-cell disease (other than hydroxyurea)**

BCL11A participates in multiprotein, corepressive complexes in erythroid cells. Unique aspects (eg, protein–protein interfaces) of the erythroid BCL11A-containing complex could possibly be targeted to achieve small-molecule specificity in erythroid precursors and avoid an effect on other cell types.

The basis of the specific beneficial role of *BCL11A* SNPs in the regulation of fetal haemoglobin production and amelioration of sickle-cell disease seems to stem from the functional disruption of erythroid-specific regulatory sequences by these intronic variants.[51] Indeed, these variants are found within an adult-stage-specific, erythroid-restricted enhancer element. The SNP that is most strongly associated with regulation of fetal haemoglobin concentration at the *BCL11A* locus disrupts a binding site of GATA1, an important transcription factor for gene expression in erythrocytes. Genome editing studies have clarified important *BCL11A* intronic sequences that are necessary for fetal haemoglobin repression and could be targeted in novel therapeutic strategies. Deletion of the *BCL11A* erythroid enhancer by genome editing results in loss of *BCL11A* expression in erythroid precursors but not in other lineages that depend on BCL11A such as neurons or B lymphocytes.[51,101] CRISPR-Cas9-directed disruption of the entire enhancer by a tiling series of individual cleavages has revealed that small mutations restricted to important minimal functional sequences are sufficient to substantially reduce *BCL11A* expression and concomitantly increase fetal haemoglobin production.[101] These results therefore suggest a novel genetic therapy for sickle-cell disease: isolation of haemopoietic stem cells from patients; delivery of genome editing technology, such as zinc-finger nucleases or CRISPR-Cas9, to disrupt the erythroid enhancer of *BCL11A*; and autologous reinfusion of modified cells (figure 5). Notably, inactivation of tissue-specific enhancers by genome editing might be an effective and widely applicable strategy to translate GWAS findings into therapeutic interventions for several complex human diseases. Although correction of the β^S mutation would be the definitive genome-editing approach to cure sickle-cell disease, this manoeuvre would require robust homology-directed repair in haematopoietic stem cells. Unfortunately, the molecular pathway for homology-directed repair is only active at exceedingly low levels in these quiescent cells.[103–105] By contrast, the error-prone non-homologous end-joining pathway, which might result in robust gene disruption,[106] is the predominant repair pathway in haemopoietic stem cells. In view of technology at present, a strategy that relies on genetic disruption, such as a single enhancer cleavage, is probably more feasible than gene repair.[101] Careful assessment of any off-target genomic effects of nucleases would need to precede any clinical implementation. An alternative approach to fetal haemoglobin induction is knockdown of *BCL11A* expression by RNA interference.[107] The development of novel vectors with the potential for erythroid-specific expression is a promising way to restrict the effects to the erythroid lineage.[108] With contemporary technology, such novel, autologous, cell-based approaches would be limited to highly sophisticated research centres that are capable of monitoring gene therapies in the context of haemopoietic stem-cell transplantation.

Geneticists have identified two additional transcription factors—*MYB* and *KLF1*—that play key parts in the regulation of fetal haemoglobin production in human beings and could serve as therapeutic targets. To target *MYB*, either by a small molecule or genetic approach, would be an alternative strategy for fetal haemoglobin reinduction. However, in view of the essential role MYB has in haemopoietic stem and progenitor cells, such an approach would need to be highly erythroid-specific to avoid toxic effects. KLF1 appears to regulate fetal haemoglobin in two different ways: by directly activating

β-globin expression and by promoting the expression of *BCL11A*, which in turn represses γ-globin.[60,109] To target *KLF1* might be challenging because of the widespread effect of KLF1 on erythroid gene regulation and profound clinical phenotypes of individuals with biallelic *KLF1* mutations. Indeed, whereas some *KLF1* variants are associated with mild phenotypes, such as increased fetal haemoglobin concentration, other mutations can lead to severe diseases such as congenital dyserythropoietic anaemia, transfusion-dependent haemolytic anaemia, and hydrops fetalis.[110–112]

The third of three loci identified by GWAS as associated with the regulation of fetal haemoglobin concentration is the β-globin gene cluster itself. Thorough investigation is necessary to determine whether modification or disruption of the β-globin gene cluster, either by mimicking naturally occurring, rare *HPFH* alleles or by producing novel changes associated with increased fetal haemoglobin concentration, could be a therapeutic option for patients with sickle-cell disease. The precise mechanism by which the common variants associated with increased fetal haemoglobin concentration achieve this effect remains incompletely understood. The potential for innovative therapeutics to reconfigure the β-globin gene cluster and increase fetal haemoglobin concentration has been established through work with synthetic transcriptional modulators.[113] Deng and colleagues[114] showed that a hybrid DNA-binding protein engineered to occupy the γ-globin promoter and interact with the major enhancer of the β-globin gene cluster (the locus control region) could support a long-range physical interaction between the locus control region and γ-globin at the expense of β-globin.

Other transcription factors and microRNAs that probably contribute to appropriate globin gene expression during development include SOX6, TR2/TR4, lin-28/let-7, and miR-15a/16-1,[39,85,115,116] but none have been validated or shown to have the necessary potency or specificity to justify their use as therapeutic targets. Transcription factors have traditionally been considered undruggable targets.[117] However, the recent success of novel, small-molecule approaches to the targeting of transcription factors gives renewed optimism about the possibility that the function of these key proteins can be chemically interrupted. For example, a modular, chemical approach, in which a protein-binding ligand could be appended to a phthalimide moiety, was shown to result in selective target degradation.[118] A set of reports provide evidence that thalidomide derivatives, such as lenalidomide, act by targeting specific transcription factors for proteasomal degradation, depending on cellular context. For example, in multiple myeloma cells, these drugs result in specific destruction of the IKZF1 and IKZF3 transcription factors, whereas in myelodysplastic syndrome del(5q) cells, the same drugs achieve their biological efficacy through selective destruction of casein kinase 1α.[119,120] Intriguingly, thalidomide derivatives have been reported to increase the concentration of fetal haemoglobin,[121,122] suggesting the selective degradation of factors that have yet to be discovered.

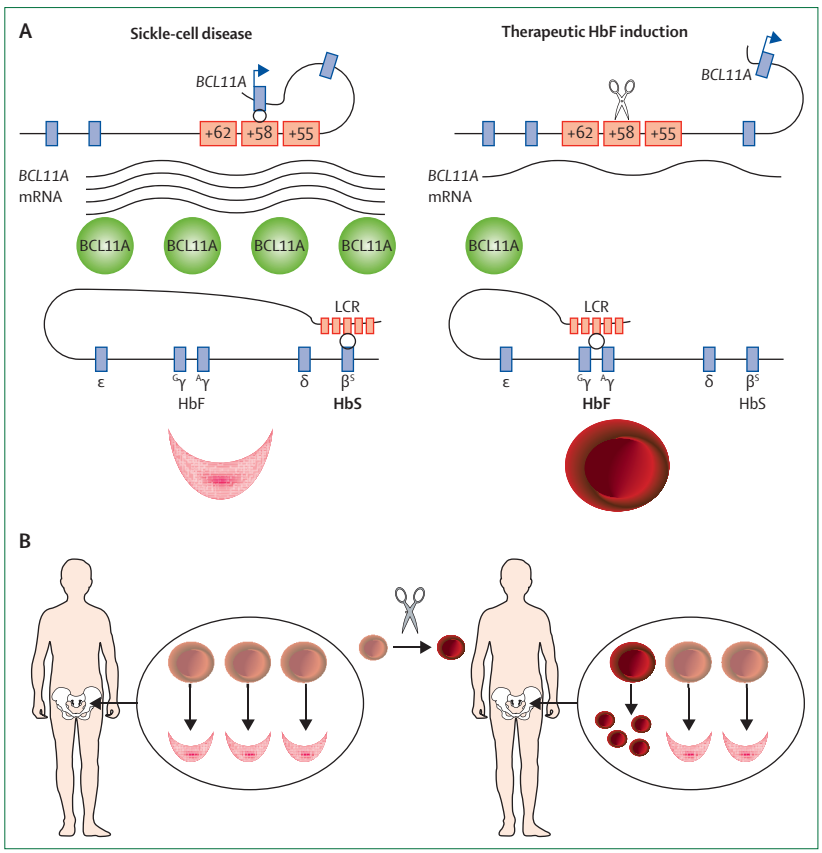

***Figure 5*: Therapeutic genome editing approach to induce fetal haemoglobin production in patients with sickle-cell disease**

(A) Programmable, targeted nuclease cleavage followed by error-prone non-homologous end-joining repair could disrupt critical sequences within the *BCL11A* erythroid enhancer; as a result, expression of *BCL11A* would be impaired in erythroid precursor cells, whereas other cells dependent on *BCL11A* expression (dependent on different enhancers) would be spared. The ultimate outcome would be reconfiguration of the β-globin gene cluster to favour high-level expression of γ-globin (and fetal haemoglobin [HbF]) at the expense of β^S-globin (and sickle haemoglobin [HbS]). (B) Envisaged genetic therapy approach would include isolation of haemopoietic stem cells from patients with sickle-cell disease, ex-vivo delivery of genome editing with targeted nucleases at a clinical scale, validation of the intended genomic modification by sequence analysis, preparative conditioning therapy, and autologous cellular reinfusion. Even in the setting of a mixed chimeric outcome (ie, the shared presence of modified and unmodified haemopoietic stem cells), the known survival advantage of HbF-expressing cells at the erythroblast and erythrocyte stages would favour clinical amelioration. LCR=locus control region. Adapted with permission from Bauer and Orkin.[102]

Conclusions

Comprehension of the genetic, developmental, and molecular events that control fetal haemoglobin production in human beings has substantially improved in the past decade. Although the most cost-effective public health strategy for sickle-cell disease remains prevention by education and prenatal diagnosis, this success has raised hope that knowledge could be harnessed to fine-tune the human genome and develop effective fetal haemoglobin-inducing therapies to treat sickle-cell disease. Although we embrace this progress, we also realise that delivery of these new treatments, even if

successful, would be very challenging to most patients worldwide. Small and affordable molecules that increase fetal haemoglobin production, such as hydroxyurea, are still the best short-term solution for most patients with sickle-cell disease in Africa and south Asia. In this respect, support is needed for clinical trials designed to show the safety and clinical efficacy of hydroxyurea in these countries.[123] Additionally, renewed interest from funding agencies will be essential to coordinate high-quality and large-scale studies of the natural history of sickle-cell disease and the disease response to interventions in countries where the global burden predominates. Such studies would also provide an infrastructure to collect biospecimens, including DNA, to foster genetic research of sickle-cell disease and genomic medicine in underserved parts of the world. African populations are characterised by extensive and often private genetic variation. Until more population diversity is included in the genetic search for novel fetal haemoglobin regulators, attractive new targets will probably be missed or newly developed therapies will be suboptimum.

Prospects have never been brighter for the strategy of fetal haemoglobin reinduction in patients with sickle-cell disease. With the sustained focus of scientists, doctors, public health officials, and philanthropists, we are optimistic that this excitement within the biomedical community could improve outcomes for patients around the world.

Contributors

GL and DEB contributed equally to this article.

Declaration of interests

We declare no competing interests.

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References

1 Allison AC. Protection afforded by sickle-cell trait against subtertian malareal infection. *BMJ* 1954; **1:** 290–94.

2 Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. 1910. *Yale J Biol Med* 2001; **74:** 179–84.

3 Watson J. The significance of the paucity of sickle cells in newborn Negro infants. *Am J Med Sci* 1948; **215:** 419–23.

4 Huisman TH. Sickle cell anemia as a syndrome: a review of diagnostic features. *Am J Hematol* 1979; **6:** 173–84.

5 Perrine RP, Brown MJ, Clegg JB, Weatherall DJ, May A. Benign sickle-cell anaemia. *Lancet* 1972; **2:** 1163–67.

6 Kar BC, Satapathy RK, Kulozik AE, et al. Sickle cell disease in Orissa State, India. *Lancet* 1986; **2:** 1198–201.

7 Alsultan A, Alabdulaali MK, Griffin PJ, et al. Sickle cell disease in Saudi Arabia: the phenotype in adults with the Arab-Indian haplotype is not benign. *Br J Haematol* 2014; **164:** 597–604.

8 Jastaniah W. Epidemiology of sickle cell disease in Saudi Arabia. *Ann Saudi Med* 2011; **31:** 289–93.

9 Serjeant GR. The natural history of sickle cell disease. *Cold Spring Harb Perspect Med* 2013; **3:** a011783.

10 Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med* 1994; **330:** 1639–44.

11 Platt OS, Thorington BD, Brambilla DJ, et al. Pain in sickle cell disease. Rates and risk factors. *N Engl J Med* 1991; **325:** 11–16.

12 Castro O, Brambilla DJ, Thorington B, et al. The acute chest syndrome in sickle cell disease: incidence and risk factors. The Cooperative Study of Sickle Cell Disease. *Blood* 1994; **84:** 643–49.

13 Milner PF, Kraus AP, Sebes JI, et al. Sickle cell disease as a cause of osteonecrosis of the femoral head. *N Engl J Med* 1991; **325:** 1476–81.

14 Stevens MC, Hayes RJ, Vaidya S, Serjeant GR. Fetal hemoglobin and clinical severity of homozygous sickle cell disease in early childhood. *J Pediatr* 1981; **98:** 37–41.

15 Bailey K, Morris JS, Thomas P, Serjeant GR. Fetal haemoglobin and early manifestations of homozygous sickle cell disease. *Arch Dis Child* 1992; **67:** 517–20.

16 Nolan VG, Wyszynski DF, Farrer LA, Steinberg MH. Hemolysis-associated priapism in sickle cell disease. *Blood* 2005; **106:** 3264–67.

17 Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. *Blood* 1998; **91:** 288–94.

18 Wishner BC, Ward KB, Lattman EE, Love WE. Crystal structure of sickle-cell deoxyhemoglobin at 5 A resolution. *J Mol Biol* 1975; **98:** 179–94.

19 Nagel RL, Bookchin RM, Johnson J, et al. Structural bases of the inhibitory effects of hemoglobin F and hemoglobin A2 on the polymerization of hemoglobin S. *Proc Natl Acad Sci USA* 1979; **76:** 670–72.

20 Goldberg MA, Husson MA, Bunn HF. Participation of hemoglobins A and F in polymerization of sickle hemoglobin. *J Biol Chem* 1977; **252:** 3414–21.

21 Sunshine HR, Hofrichter J, Eaton WA. Requirement for therapeutic inhibition of sickle haemoglobin gelation. *Nature* 1978; **275:** 238–40.

22 Garner C, Tatu T, Reittie JE, et al. Genetic influences on F cells and other hematologic variables: a twin heritability study. *Blood* 2000; **95:** 342–46.

23 Pilia G, Chen WM, Scuteri A, et al. Heritability of cardiovascular and personality traits in 6,148 Sardinians. *PLoS Genet* 2006; **2:** e132.

24 Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. *Cold Spring Harb Perspect Med* 2013; **3:** a011643.

25 Labie D, Dunda-Belkhodja O, Rouabhi F, Pagnier J, Ragusa A, Nagel RL. The -158 site 5' to the G gamma gene and G gamma expression. *Blood* 1985; **66:** 1463–65.

26 Bank A. Regulation of human fetal hemoglobin: new players, new complexities. *Blood* 2006; **107:** 435–43.

27 Chakalova L, Osborne CS, Dai YF, et al. The Corfu deltabeta thalassemia deletion disrupts gamma-globin gene silencing and reveals post-transcriptional regulation of HbF expression. *Blood* 2005; **105:** 2154–60.

28 Sankaran VG, Xu J, Byron R, et al. A functional element necessary for fetal hemoglobin silencing. *N Engl J Med* 2011; **365:** 807–14.

29 Lessard S, Beaudoin M, Benkirane K, Lettre G. Comparison of DNA methylation profiles in human fetal and adult red blood cell progenitors. *Genome Med* 2015; **7:** 1.

30 Garner C, Mitchell J, Hatzis T, Reittie J, Farrall M, Thein SL. Haplotype mapping of a major quantitative-trait locus for fetal hemoglobin production, on chromosome 6q23. *Am J Hum Genet* 1998; **62:** 1468–74.

31 Garner C, Silver N, Best S, et al. Quantitative trait locus on chromosome 8q influences the switch from fetal to adult hemoglobin. *Blood* 2004; **104:** 2184–86.

32 Dover GJ, Smith KD, Chang YC, et al. Fetal hemoglobin levels in sickle cell disease and normal individuals are partially controlled by an X-linked gene located at Xp22.2. *Blood* 1992; **80:** 816–24.

33 Lettre G, Sankaran VG, Bezerra MA, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc Natl Acad Sci USA* 2008; **105:** 11869–74.

34 Thein SL, Menzel S, Peng X, et al. Intergenic variants of HBS1L-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. *Proc Natl Acad Sci USA* 2007; **104:** 11346–51.

35 Close J, Game L, Clark B, Bergounioux J, Gerovassili A, Thein SL. Genome annotation of a 1.5 Mb region of human chromosome 6q23 encompassing a quantitative trait locus for fetal hemoglobin expression in adults. *BMC Genomics* 2004; **5**: 33.

36 Uda M, Galanello R, Sanna S, et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. *Proc Natl Acad Sci USA* 2008; **105:** 1620–25.

37 Stadhouders R, Aktuna S, Thongjuea S, et al. HBS1L-MYB intergenic variants modulate fetal hemoglobin via long-range MYB enhancers. *J Clin Invest* 2014; **124:** 1699–710.

38 Galarneau G, Palmer CD, Sankaran VG, Orkin SH, Hirschhorn JN, Lettre G. Fine-mapping at three loci known to affect fetal hemoglobin levels explains additional genetic variation. *Nat Genet* 2010; **42:** 1049–51.

39 Sankaran VG, Menne TF, Scepanovic D, et al. MicroRNA-15a and -16-1 act via MYB to elevate fetal hemoglobin expression in human trisomy 13. *Proc Natl Acad Sci USA* 2011; **108:** 1519–24.

40 Menzel S, Garner C, Gut I, et al. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. *Nat Genet* 2007; **39:** 1197–99.

41 Mtatiro SN, Singh T, Rooks H, et al. Genome wide association study of fetal hemoglobin in sickle cell anemia in Tanzania. *PLoS One* 2014; **9:** e111464.

42 Bhatnagar P, Purvis S, Barron-Casella E, et al. Genome-wide association study identifies genetic variants influencing F-cell levels in sickle-cell patients. *J Hum Genet* 2011; **56:** 316–23.

43 Nuinoon M, Makarasara W, Mushiroda T, et al. A genome-wide association identified the common genetic variants influence disease severity in beta(0)-thalassemia/hemoglobin E. *Hum Genet* 2010; **127:** 303–14.

44 Medina KL, Singh H. Genetic networks that regulate B lymphopoiesis. *Curr Opin Hematol* 2005; **12:** 203–09.

45 Leid M, Ishmael JE, Avram D, Shepherd D, Fraulob V, Dollé P. CTIP1 and CTIP2 are differentially expressed during mouse embryogenesis. *Gene Expr Patterns* 2004; **4**: 733–39.

46 Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science* 2008; **322:** 1839–42.

47 Sankaran VG, Xu J, Ragoczy T, et al. Developmental and species-divergent globin switching are driven by BCL11A. *Nature* 2009; **460:** 1093–97.

48 Xu J, Peng C, Sankaran VG, et al. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. *Science* 2011; **334:** 993–96.

49 Basak A, Hancarova M, Ulirsch JC, et al. BCL11A deletions result in fetal hemoglobin persistence and neurodevelopmental alterations. *J Clin Invest* 2015; **125:** 2363–68.

50 Funnell AP, Prontera P, Ottaviani V, et al. 2p15-p16.1 microdeletions encompassing and proximal to BCL11A are associated with elevated HbF in addition to neurologic impairment. *Blood* 2015; **126:** 89–93.

51 Bauer DE, Kamran SC, Lessard S, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science* 2013; **342:** 253–57.

52 Canver MC, Smith EC, Sher F, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature* 2015; **527:** 192–97.

53 Maurano MT, Humbert R, Rynes E, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012; **337:** 1190–95.

54 Wonkam A, Ngo Bitoungui VJ, Vorster AA, et al. Association of variants at BCL11A and HBS1L-MYB with hemoglobin F and hospitalization rates among sickle cell patients in Cameroon. *PLoS One* 2014; **9:** e92506.

55 Bhatnagar P, Keefer JR, Casella JF, et al. Association between baseline fetal hemoglobin levels and incidence of severe vaso-occlusive pain episodes in children with sickle cell anemia. *Pediatr Blood Cancer* 2013; **60:** E125–27.

56 Wood AR, Esko T, Yang J, et al. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet* 2014; **46**: 1173–86.

57 Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015; **518:** 197–206.

58 Shungin D, Winkler TW, Croteau-Chonka DC, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015; **518:** 187–96.

59 Auer PL, Lettre G. Rare variant association studies: considerations, challenges and opportunities. *Genome Med* 2015; **7:** 16.

60 Borg J, Papadopoulos P, Georgitsi M, et al. Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. *Nat Genet* 2010; **42:** 801–05.

61 Liu D, Zhang X, Yu L, et al. KLF1 mutations are relatively more common in a thalassemia endemic region and ameliorate the severity of beta-thalassemia. *Blood* 2014; **124:** 803–11.

62 Huang J, Zhang X, Liu D, et al. Compound heterozygosity for KLF1 mutations is associated with microcytic hypochromic anemia and increased fetal hemoglobin. *Eur J Hum Genet* 2015; **23:** 1341–48.

63 Sheridan BL, Weatherall DJ, Clegg JB, et al. The patterns of fetal haemoglobin production in leukaemia. *Br J Haematol* 1976; **32:** 487–506.

64 Alter BP, Rappeport JM, Huisman TH, Schroeder WA, Nathan DG. Fetal erythropoiesis following bone marrow transplantation. *Blood* 1976; **48:** 843–53.

65 DeSimone J, Heller P, Hall L, Zwiers D. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. *Proc Natl Acad Sci USA* 1982; **79:** 4428–31.

66 Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. *J Clin Invest* 1984; **74:** 652–56.

67 Kinney TR, Helms RW, O'Branski EE, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Pediatric Hydroxyurea Group. *Blood* 1999; **94:** 1550–54.

68 Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. *N Engl J Med* 1995; **332:** 1317–22.

69 Thornburg CD, Files BA, Luo Z, et al. Impact of hydroxyurea on clinical events in the BABY HUG trial. *Blood* 2012; **120:** 4304–10.

70 Hankins JS, Aygun B, Nottage K, et al. From infancy to adolescence: fifteen years of continuous treatment with hydroxyurea in sickle cell anemia. *Medicine (Baltimore)* 2014; **93:** e215.

71 Steinberg MH, McCarthy WF, Castro O, et al. The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: a 17·5 year follow-up. *Am J Hematol* 2010; **85:** 403–08.

72 Voskaridou E, Christoulas D, Bilalis A, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: results of a 17-year, single-center trial (LaSHS). *Blood* 2010; **115:** 2354–63. 73 Charache S, Dover GJ, Moore RD, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. *Blood* 1992; **79:** 2555–65.

74 Xu J, Shao Z, Glass K, et al. Combinatorial assembly of developmental stage-specific enhancers controls gene expression programs during human erythropoiesis. *Dev Cell* 2012; **23:** 796–811.

75 van der Ploeg LH, Flavell RA. DNA methylation in the human gamma delta beta-globin locus in erythroid and nonerythroid tissues. *Cell* 1980; **19:** 947–58.

76 Forsberg EC, Downs KM, Christensen HM, Im H, Nuzzi PA, Bresnick EH. Developmentally dynamic histone acetylation pattern of a tissue-specific chromatin domain. *Proc Natl Acad Sci USA* 2000; **97:** 14494–509.

77 Mabaera R, Richardson CA, Johnson K, Hsu M, Fiering S, Lowrey CH. Developmental- and differentiation-specific patterns of human gamma- and beta-globin promoter DNA methylation. *Blood* 2007; **110:** 1343–52.

78 Ley TJ, DeSimone J, Anagnou NP, et al. 5-azacytidine selectively increases gamma-globin synthesis in a patient with beta+ thalassemia. *N Engl J Med* 1982; **307:** 1469–75.

79 Charache S, Dover G, Smith K, Talbot CC Jr, Moyer M, Boyer S. Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the gamma-delta-beta-globin gene complex. *Proc Natl Acad Sci USA* 1983; **80:** 4842–46.

80 Xu J, Bauer DE, Kerenyi MA, et al. Corepressor-dependent silencing of fetal hemoglobin expression by BCL11A. *Proc Natl Acad Sci USA* 2013; **110:** 6518–23.

81 Bradner JE, Mak R, Tanguturi SK, et al. Chemical genetic strategy identifies histone deacetylase 1 (HDAC1) and HDAC2 as therapeutic targets in sickle cell disease. *Proc Natl Acad Sci USA* 2010; **107**: 12617–22.

82 Ruiz MA, Rivers A, Ibanez V, et al. Hydroxymethylcytosine and demethylation of the gamma-globin gene promoter during erythroid differentiation. *Epigenetics* 2015; **10:** 397–407.

83 Amaya M, Desai M, Gnanapragasam MN, et al. Mi2β-mediated silencing of the fetal γ-globin gene in adult erythroid cells. *Blood* 2013; **121:** 3493–501.

84 Shi L, Cui S, Engel JD, Tanabe O. Lysine-specific demethylase 1 is a therapeutic target for fetal hemoglobin induction. *Nat Med* 2013: **19:** 291–94.

85 Cui S, Lim KC, Shi L, et al. The LSD1 inhibitor RN-1 induces fetal hemoglobin synthesis and reduces disease pathology in sickle cell mice. *Blood* 2015; **126:** 386–96.

86 Rivers A, Vaitkus K, Ruiz MA, et al. RN-1, a potent and selective lysine-specific demethylase 1 inhibitor, increases gamma-globin expression, F reticulocytes, and F cells in a sickle cell disease mouse model. *Exp Hematol* 2015; **43:** 546–53.

87 Chaturvedi CP, Hosey AM, Palii C, et al. Dual role for the methyltransferase G9a in the maintenance of beta-globin gene transcription in adult erythroid cells. *Proc Natl Acad Sci USA* 2009; **106:** 18303–08.

88 Krivega I, Byrnes C, de Vasconcellos JF, et al. Inhibition of G9a methyltransferase stimulates fetal hemoglobin production by facilitating LCR/gamma-globin looping. *Blood* 2015; **126:** 665–72.

89 Renneville A, Van Galen P, Canver MC, et al. EHMT1 and EHMT2 inhibition induces fetal hemoglobin expression. *Blood* 2015; **126**: 1930–39.

90 Rank G, Cerruti L, Simpson RJ, Moritz RL, Jane SM, Zhao Q. Identification of a PRMT5-dependent repressor complex linked to silencing of human fetal globin gene expression. *Blood* 2010; **116:** 1585–92.

91 Okam MM, Esrick EB, Mandell E, Campigotto F, Neuberg DS, Ebert BL. Phase 1/2 trial of vorinostat in patients with sickle cell disease who have not benefitted from hydroxyurea. *Blood* 2015; **125:** 3668–69.

92 Little JA, Hauser KP, Martyr SE, et al. Hematologic, biochemical, and cardiopulmonary effects of L-arginine supplementation or phosphodiesterase 5 inhibition in patients with sickle cell disease who are on hydroxyurea therapy. *Eur J Haematol* 2009; **82:** 315–21.

93 Reid ME, El Beshlawy A, Inati A, et al. A double-blind, placebo-controlled phase II study of the efficacy and safety of 2,2-dimethylbutyrate (HQK-1001), an oral fetal globin inducer, in sickle cell disease. *Am J Hematol* 2014; **89:** 709–13.

94 Esteghamat F, Gillemans N, Bilic I, et al. Erythropoiesis and globin switching in compound Klf1::Bcl11a mutant mice. *Blood* 2013; **121:** 2553.

95 John A, Brylka H, Wiegreffe C, et al. Bcl11a is required for neuronal morphogenesis and sensory circuit formation in dorsal spinal cord development. *Development* 2012; **139**: 1831–41.

96 Wiegreffe C, Simon R, Peschkes K, et al. Bcl11a (Ctip1) controls migration of cortical projection neurons through regulation of Sema3c. *Neuron* 2015; **87:** 311–25.

97 Liu P, Keller JR, Ortiz M, et al. Bcl11a is essential for normal lymphoid development. *Nature Immunol* 2003; **4:** 525–32.

98 Yu Y, Wang J, Khaled W, et al. Bcl11a is essential for lymphoid development and negatively regulates p53. *J Exp Med*2012; **209:** 2467–83.

99 Ippolito GC, Dekker JD, Wang YH, et al. Dendritic cell fate is determined by BCL11A. *Proc Natl Acad Sci USA* 2014; **111:** E998–1006.

100 Benitez CM, Qu K, Sugiyama T, et al. An integrated cell purification and genomics strategy reveals multiple regulators of pancreas development. *PLoS Genet* 2014; **10:** e1004645.

101 Canver MC, Smith EC, Sher F, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature* 2015; **527:** 192–97.

102 Bauer DE, Orkin SH. Hemoglobin switching's surprise: the versatile transcription factor BCL11A is a master repressor of fetal hemoglobin. *Curr Opin Genet Dev* 2015; **33:** 62–70.

103 Hoban MD, Cost GJ, Mendel MC, et al. Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. *Blood* 2015; **125:** 2597–604.

104 Genovese P, Schiroli G, Escobar G, et al. Targeted genome editing in human repopulating haematopoietic stem cells. *Nature* 2014; **510:** 235–40.

105 Mohrin M, Bourke E, Alexander D, et al. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. *Cell Stem Cell* 2010; **7:** 174–85.

106 Tebas P, Stein D, Tang WW, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med* 2014; **370:** 901–10.

107 Wilber A, Hargrove PW, Kim Y-S, et al. Therapeutic levels of fetal hemoglobin in erythroid progeny of β-thalassemic CD34+ cells after lentiviral vector-mediated gene transfer. *Blood* 2011; **117**: 2817–26.

108 Guda S, Brendel C, Renella R, et al. miRNA-embedded shRNAs for lineage-specific BCL11A knockdown and hemoglobin F induction. *Mol Ther* 2015; **23:** 1465–74.

109 Zhou D, Liu K, Sun CW, Pawlik KM, Townes TM. KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching. *Nat Genet* 2010; **42:** 742–44.

110 Arnaud L, Saison C, Helias V, et al. A dominant mutation in the gene encoding the erythroid transcription factor KLF1 causes a congenital dyserythropoietic anemia. *Am J Hum Genet* 2010; **87:** 721–27.

111 Viprakasit V, Ekwattanakit S, Riolueang S, et al. Mutations in Kruppel-like factor 1 cause transfusion-dependent hemolytic anemia and persistence of embryonic globin gene expression. *Blood* 2014; **123:** 1586–95.

112 Magor GW, Tallack MR, Gillinder KR, et al. KLF1-null neonates display hydrops fetalis and a deranged erythroid transcriptome. *Blood* 2015; **125:** 2405–17.

113 Deng W, Lee J, Wang H, et al. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. *Cell* 2012; **149:** 1233–44.

114 Deng W, Rupon JW, Krivega I, et al. Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell* 2014; **158:** 849–60.

115 Xu J, Sankaran VG, Ni M, et al. Transcriptional silencing of {gamma}-globin by BCL11A involves long-range interactions and cooperation with SOX6. *Genes Dev* 2010; **24:** 783–98.

116 Lee YT, de Vasconcellos JF, Yuan J, et al. LIN28B-mediated expression of fetal hemoglobin and production of fetal-like erythrocytes from adult human erythroblasts ex vivo. *Blood* 2013; **122:** 1034–41.

117 Hennemann H, Wirths S, Carl C. Cell-based peptide screening to access the undruggable target space. *Eur J Med Chem* 2015; **94:** 489–96.

118 Winter GE, Buckley DL, Paulk J, et al. Drug Development. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* 2015; **348:** 1376–81.

119 Kronke J, Fink EC, Hollenbach PW, et al. Lenalidomide induces ubiquitination and degradation of CK1alpha in del(5q) MDS. *Nature* 2015; **523:** 183–88.

120 Kronke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science* 2014; **343:** 301–05.

121 Meiler SE, Wade M, Kutlar F, et al. Pomalidomide augments fetal hemoglobin production without the myelosuppressive effects of hydroxyurea in transgenic sickle cell mice. *Blood* 2011; **118:** 1109–12.

122 Moutouh-De Parseval La, Verhelle D, Glezer E, et al. Pomalidomide and lenalidomide regulate erythropoiesis and fetal hemoglobin production in human CD34+ cells. *J Clin Invest* 2008; **118:** 248–58.

123 Obaro SK. Hydroxyurea for sickle-cell anaemia in Africa: mind the gap. *Lancet Glob Health* 2015; **3:** e124–25.