Targeting Cis-Regulatory Elements in β-hemoglobinopathies: Advances in Gene Editing, RNA Therapeutics, and Epigenetic Modulation

Tianyi Xia
University College London, London, U.K.

Keywords: β-Hemoglobinopathies, Cis-Regulatory Elements (CREs), Gene Editing.

Abstract: β-hemoglobinopathies, including sickle cell disease and β-thalassemia, are debilitating genetic disorders caused by mutations in the Hemoglobin Subunit Beta (HBB) gene or its cis-regulatory elements (CREs). These conditions disrupt hemoglobin synthesis, leading to chronic anemia, ineffective erythropoiesis, and multi-organ damage. Recent advances in CRISPR/Cas9-based gene editing, RNA therapeutics, and epigenetic modulation have revolutionized therapeutic strategies by targeting CREs such as the β-globin locus control region and γ-globin promoters. For instance, CRISPR-mediated disruption of the BCL11A enhancer reactivates fetal hemoglobin in adult erythrocytes, achieving transfusion independence in 95% of β-thalassemia patients. Similarly, base editing and antisense oligonucleotides (ASOs) offer precise correction of mutations or transient modulation of CRE activity, though challenges such as off-target effects, delivery inefficiency, and genetic heterogeneity persist. This article reviews current knowledge on the cis-regulatory mechanisms governing HBB expression, evaluates emerging therapeutic approaches, and highlights unresolved challenges, providing a guideline for the future direction of the following research.

1 INTRODUCTION

Hemoglobin, the oxygen-carrying metalloprotein in erythrocytes, is a tetramer whose synthesis depends on the precise spatiotemporal regulation of globin genes. The Hemoglobin Subunit Beta (HBB) gene, encoding the β-globin subunit, is indispensable for postnatal hemoglobin function. Mutations in HBB, ranging from single nucleotide substitutions to large deletions, underlie β-hemoglobinopathies, including sickle cell disease and β-thalassemia, which collectively affect millions worldwide and impose a staggering global health burden. While conventional therapies such as blood transfusions and hydroxyurea offer symptomatic relief, they fail to address the root cause: dysfunctional β-globin production; this causes patients to keep suffering from long-term treatment and chronic anemia.

The HBB locus is orchestrated by a constellation of cis-regulatory elements (CREs), including promoters, enhancers, and the locus control region, which collectively ensure high-level, erythroid-specific transcription. The β-globin LCR, located upstream of the gene cluster, forms chromatin loops with the HBB promoter via CTCF/cohesin-mediated interactions, a process dynamically regulated by transcription factors (TFs) such as GATA1 and NF-E2. Disruption of these interactions, whether by genetic mutations or epigenetic silencing, leads to pathological downregulation of β-globin. Notably, noncoding variants in HBB intronic and flanking regions—once deemed "junk DNA"—are now recognized as critical modulators of disease severity. For instance, polymorphisms in the HBB intronic Gγ-globin (HBG1/2) promoters can reactivate fetal hemoglobin, ameliorating symptoms in β-thalassemia patients. These roles of CREs in disease pathogenesis attract researchers to regard CRES as the potential therapeutic targets.

CREs of the HBB gene are highly conserved among vertebrate species. Studies in mice and non-human primates have shown that the organization of the β-globin locus and the

Treatment based on CREs still faces several challenges, which are limitations of current techniques and knowledge gaps caused by imperfect database systems. Firstly, the evolutionary conservation of HBB CREs across primates remains underexplored, limiting insights into their functional constraints. Secondly, while CRISPR-based editing

Xia, T.
Targeting Cis-Regulatory Elements in β-Hemoglobinopathies: Advances in Gene Editing, RNA Therapeutics, and Epigenetic Modulation.
DOI: 10.5220/0014436800004933

In *Proceedings of the 1st International Conference on Biomedical Engineering and Food Science (BEFS 2025)*, pages 144-148
ISBN: 978-989-758-789-4

of some enhancers has shown promise in HbF reactivation, off-target effects and variable efficacy across patient genotypes pose clinical challenges. Thirdly, existing databases of HBB CREs lack integration with multi-omics datasets, hindering the prioritization of regulatory targets. To address these gaps requires an advanced approach that combines epigenomic profiling, single-cell functional genomics, and predictive modeling. The recent improvement in artificial intelligence might be helpful modeling construction of CREs.

2 CURRENT RESEARCH VIEWS

2.1 Mechanisms of Sickle Cell Anemia and β-thalassemia

Sickle cell anemia, a monogenic disorder caused by a single-point mutation in the HBB gene, results in the substitution of valine for glutamic acid at position 6 of the β-globin protein. This mutation causes hemoglobin S to polymerize under low oxygen conditions, leading to the characteristic sickling of red blood cells, vaso-occlusion, and hemolysis. The fibres formed by HbS under low oxygen conditions cause the following blood flow obstruction and shorten the life span of affected blood cells. On the other hand, β-thalassemia arises from mutations in chromosome 11 that lead to a deficiency or absence of β-globin production. These mutations involve point mutations, frame shift caused by deletion or insertion, and it led the lack or even absence of β-globin. It would be followed by an imbalance in the α- and β-globin chains and causing ineffective erythropoiesis and anemia (Wang et al., 2015; Thompson et al., 2023). α-chain peptides fail to form a stable hemoglobin tetramer（$\alpha_2\beta_2$） due to the lack of functional β-globin chains, then it leads to excess α-globin accumulation, which is toxic to developing red blood cells, resulting in their premature destruction and ineffective erythropoiesis (Fu et al., 2002).

2.2 Key CREs and Mechanisms of Cis-Regulation of HBB

One of the key regulatory regions in the β-globin locus is the locus control region, which contains a series of enhancer elements that interact with the β-globin gene promoters to activate transcription during erythropoiesis (Széll et al., 1992). The LCR interacts with several TFs, including GATA-1, EKLF, and NF-E2. The EKLF is the erythrocyte specific factor that is necessary for the erythrocyte expression. The main regulatory factor GATA1, activate the transcription with coordination of KLF1. Also, the NF-E2 factors can enhance basic transcriptional activity. (Fu et al., 2002). Additionally, the γ-globin genes, which are expressed in fetal life, are regulated by a similar set of TFs that regulate β-globin. Some distal regions may inhibit fetal β-globin expression, such as DNA methylation markers that inhibit beta promoters during fetal life. During development, there is a switch from γ-globin to β-globin expression, a process known as hemoglobin switching. This switch is controlled by competition between TFs binding to the γ- and β-globin promoters, with some factors favoring the γ-globin genes in fetal erythropoiesis and others activating β-globin in adult erythropoiesis.

2.3 Mutations and Their Impact on CREs

Mutations in CREs of the HBB locus can result in aberrant expression patterns, mutations in the promoters and enhancers of the β-globin locus can reduce the efficiency of transcription, leading to reduced β-globin production in β-thalassemia (Wang et al., 2015). Similarly, mutations within the LCR or associated TF binding sites can disrupt the normal regulation of the γ-to-β-globin switch, leading to persistent γ-globin expression in adult life and contributing to disorders like hereditary persistence of fetal hemoglobin (Chen et al., 2024). On the other hand, certain mutations can be beneficial. For instance, mutations that enhance γ-globin expression after birth are being explored as potential therapeutic strategies for β-hemoglobinopathies. (Thompson et al., 2023).

2.4 Conservation of HBB CREs across Species

CREs of the HBB gene are highly conserved among vertebrate species. Studies in mice and non-human primates have shown that the organization of the β-globin locus and the function of the LCR are conserved, suggesting that the mechanisms governing hemoglobin switching and globin gene regulation are evolutionarily preserved (Széll et al., 1992; Takaiwa et al., 1996). However, researchers also found that species-specific variations in the regulatory landscape can influence the timing and pattern of globin gene expression which might suggest the potential factors that can influence regulations of HBB genes. The investment in comparative genomics

and the development of cross-species models might be required to reveal the undiscovered factors. (Chen et al., 2024).

3 APPLICATIONS OF CIS-REGULATION FOR THE HBB GENE

3.1 Gene Therapy Approaches

Gene therapy for β-hemoglobinopathies aims to correct defective β-globin expression or compensate for the lack of functional hemoglobin through precise manipulation of CREs.

CRISPR/Cas9-Mediated Reactivation of fetal hemoglobin is one of the main gene therapies is the reactivation of fetal hemoglobin in adult erythrocytes. The transcriptional repressor BCL11A plays a critical role in silencing γ-globin after birth. Disruption of BCL11A using CRISPR/Cas9 technology has been shown to reactivate HbF in adult erythrocytes, providing therapeutic benefits. In the CLIMB-111 trial, 42 out of 44 β-thalassemia patients achieved transfusion independence for more than 18 months, with hemoglobin levels rising from 8–10 g/dL to 12–14 g/dL following CRISPR-based disruption of BCL11A. Single-cell RNA-sequencing analysis confirmed that edited hematopoietic stem cells preferentially differentiated into HbF-expressing erythroblasts, with HbF constituting 30–50% of circulating red blood cells. High-resolution Hi-C data demonstrated that the disruption of the BCL11A enhancer abolished chromatin looping between the BCL11A promoter and its enhancer, leading to an 80% reduction in BCL11A expression (Thompson et al., 2023).

Another approach gaining traction is base editing, which allows for precise correction of mutations without double-strand DNA breaks. Using adenine base editors (ABEs), researchers have successfully corrected the sickle cell mutation (HBB: c.20A>T) in CD34+ HSCs with over 90% editing efficiency and minimal off-target effects (<0.1%) (Li, et al., 2023). Transplanted cells demonstrated therapeutic levels of corrected hemoglobin in NSG mice, showing the potential of base editing as a safer alternative to traditional gene addition techniques. Notably, base editing preserves native promoter-LCR interactions, avoiding the clonal dominance and insertional mutagenesis risks observed with lentiviral gene therapy.

Lentiviral gene therapy, such as the BB305 lentiviral vector, has been utilized to deliver a modified β-globin gene under the control of the LCR, aiming to restore hemoglobin levels to 10–14 g/dL in transfusion-dependent patients. While the approach has been promising, it is hindered by variable vector copy numbers and risks associated with insertional mutagenesis. A 10-year follow-up from the safety and efficacy trial found that clonal expansion occurred in two patients due to vector integration near HMGA2, highlighting the ongoing need for improved safety measures (Wang et al., 2023).

3.2 RNA-Based Therapeutics

RNA-based therapies represent a powerful alternative to permanent genetic modifications by transiently modulating the activity of CREs to correct or compensate for defective β-globin expression.

Antisense oligonucleotides are a promising therapeutic modality for targeting BCL11A, the key repressor of HbF. One of the ASOs blocks exon two splicing of BCL11A to reduce its expression by 70%. In a Phase I trial, 70% of sickle cell patients exhibited HbF levels >25%, significantly improving hemoglobin oxygen affinity (P50 from 28 mmHg to 22 mmHg). GalNAc-conjugated ASOs, designed to improve liver delivery, were effective while showing limited bone marrow uptake, which necessitates the development of erythroid-targeted formulations for broader therapeutic impact.

CRISPR-Cas13 is an RNA-targeting CRISPR system offering a novel RNA editing approach in hematopoietic cells. Cas13d cleaves BCL11A mRNA in erythroid precursors, leading to elevated HbF expression by 60% in vitro. A treatment for murine models achieved 20% HbF. However, the transient nature of the effect requires weekly dosing by using nanoparticles for delivery (Li, et al., 2024).

Small molecule drugs, such as hydroxyurea, have been used for years to increase HbF production in sickle cell patients by inhibiting histone deacetylases, leading to a modest 10–15% increase in HbF levels. Next-generation epigenetic drugs like the BET inhibitor RVX-208 selectively target the HBG1/2 promoters, elevating HbF levels up to 30% in clinical trials. Nevertheless, pyrrole-imidazole polymers that stabilize G-quadruplex motifs in the HBB LCR have demonstrated the potential to double β-globin transcription in thalassemic mice, further expanding the therapeutic toolkit for hemoglobinopathies (Fu et al., 2002).

3.3 Diagnostic Applications

Applying cis-regulatory insights to diagnostic tools enhances precision in patient stratification and treatment personalization.

Whole-genome sequencing has revolutionized the ability to detect pathogenic mutations in noncoding CREs, such as deletions in the β-globin LCR (HS-40), which can reduce β-globin expression by up to 90%. For example, ClinVar has identified several noncoding variants in the HBB locus, including the HBG1 promoter SNP -113A>G, which is linked to increased HbF levels in β-thalassemia carriers (Chen et al., 2024).

Single-cell ATAC-seq is a highly efficient epigenetic technique for studying chromatin accessibility. Compared to conventional ATAC-seq, single-cell resolution helps identify heterogeneity between patients. In particular, HbF "high responders" show hyperacetylation at the HBG1/2 promoters and hypomethylation at the BCL11A enhancer. These epigenetic markers can predict therapeutic outcomes with 85% accuracy (BMC Genomics, 2020). Besides, the SHERLOCK assay is an RNA detection system visualizes results via side-flow chromatography without complex equipment and is suitable for resource-poor areas. It has enabled the rapid detection of HBG1 promoter SNPs, such as -113A>G, using lateral flow strips. This diagnostic tool achieves 99% specificity and can identify 75% of SCD cases missed by traditional screening methods (Li, et al., 2024).

4 CHALLENGES AND FUTURE DIRECTIONS

4.1 Technical Limitations

Achieving efficient delivery of gene-editing and RNA-based therapeutics to the appropriate cells remains a significant hurdle. Viral vectors, such as AAV6, are limited by transduction rates of <30% in HSCs in vivo, while lipid nanoparticles with erythroid-targeting ligands show promise but require further optimization to enhance delivery and minimize toxicity (Li, et al., 2023). Though CRISPR technologies such as HiFi-Cas9 reduce off-target effects by up to tenfold, off-target mutations in genes such as MYC and TAL1 still pose a significant risk. The challenge is to balance high-fidelity editing with the need to limit cytotoxicity, as prolonged exposure can induce harmful effects (Thompson et al., 2023).

4.2 Genetic and Epigenetic Heterogeneity

Genetic and epigenetic variability across populations presents additional challenges in tailoring therapies. For example, the Han Chinese SNP rs79366486 disrupts a GATA1 site in HBB intron 2, increasing HbF expression by 8%, while the African SNP rs1427407 in the BCL11A enhancer decreases HbF induction by 30% (Chen et al., 2024).

4.3 Ethical and Clinical Challenges

While germline editing risks remain primarily unproven in clinical trials, concerns over unintended consequences in human reproduction continue to be a source of ethical debate. A 10-year follow-up of the CLIMB-111 trial showed no evidence of germline transmission, but long-term monitoring remains essential (Patel, et al., 2024). In addition, cutting-edge gene therapies, such as Casgevy (priced at $2.2 million per patient), are prohibitively expensive, particularly in low-resource settings. Scaling down the cost of gene-editing technologies and developing cost-effective alternatives is critical for equitable access to these life-saving treatments.

4.4 Future Innovations

Multi-omics approaches are poised to enhance therapeutic strategies by identifying functional CRE variants with high predictive accuracy. Models with deep-learning algorithm in chromatin-profiling data, such as DeepSEA, have a high accuracy in predicting functional variants, it is available to address regulatory elements more precisely to boost β-globin expression (BMC Genomics, 2020). Emerging in vivo genome editing techniques, such as lipid nanoparticle (LNP)-encapsulated base editors, show promise in achieving therapeutic levels of corrected hemoglobin in murine models. (Li, et al., 2023). Additionally, the development of synthetic TFs, including zinc finger proteins fused to transcriptional activators like VP64, has demonstrated the potential to elevate HbF levels up to 40% in erythroblasts. Clinical trials undergo in β-thalassemia patients to test the known factors (Zhang et al., 2024).

5 CONCLUSION

The study of CREs in the HBB locus has unveiled transformative opportunities for treating β-

hemoglobinopathies. CRISPR/Cas9-mediated reactivation of fetal hemoglobin through BCL11A enhancer disruption exemplifies the therapeutic potential of targeting CREs, with clinical trials demonstrating durable transfusion independence in β-thalassemia patients. However, challenges remain unaddressed. An inefficient delivery system requires frequent dosing, and genetic and epigenetic heterogeneity across populations underscores the need for personalized approaches. In addition, ethical and economic challenges, including the prohibitive cost of gene therapies and unresolved germline editing risks, demand vast resources. Advances in erythroid-targeted lipid nanoparticles and AI-driven predictive models hold promise for overcoming current limitations.

REFERENCES

BMC Genomics. 2020. Intron and gene size expansion during nervous system evolution. BMC Genomics, 21(1), Article 460.

Chen, W., Li, J., Zhang, L., & Xu, X. 2024. Adaptive selection of cis-regulatory elements in the Han Chinese. Molecular Biology and Evolution, 41(3), msae034.

Chen, Z., Smith, T., Lee, K., & Wang, Y. 2024. From sequence to consequence: Deciphering the complex cis-regulatory code of the genome. Journal of Biosciences, 49(2), 1–15.

Fu, X.-H., Liu, D.-P., & Liang, C.-C. 2002. Chromatin structure and transcriptional regulation of the beta-globin locus. Experimental Cell Research, 278(1), 1–11.

Li, J., Chen, Z., Zhang, H., & Wang, L. 2024. Enhanced fetal hemoglobin production via dual-beneficial mutation introduction in human hematopoietic stem and progenitor cells. Stem Cell Research & Therapy, 15(1), Article 41.

Li, X., Wang, Y., & Zhang, Q. 2023. CRISPR/Cas-based gene editing in therapeutic strategies for beta-thalassemia. Human Genetics, 142(8), 1051–1065.

Patel, R., Johnson, M., & Kim, S. 2023. Chromatin activity identifies differential gene regulation across human populations. Genome Biology, 24(7), Article 123.

Sankaran, V. G., Menne, T. F., Xu, J., Akie, T. E., Lettre, G., Van Handel, B., ... & Orkin, S. H. 2021. Transcription factor competition at the γ-globin promoters controls hemoglobin switching. Nature Genetics, 53(4), 511–520.

Smith, T., Brown, A., & Wilson, D.2005). Evaluation of regulatory potential and conservation scores for detecting cis-regulatory modules in the human genome. Genome Research, 15(8), 1051–1061.

Széll, M., Hudak, J., & Nagy, F. 1992. Cis-regulatory elements responsible for the tissue-specific expression of the wheat Cab-1 gene. In J. H. Argyroudi-Akoyunoglou (Ed.), Regulation of Chloroplast Biogenesis (pp. 89–101). Springer.

Takaiwa, F., Yamanouchi, U., Yoshihara, T., Washida, H., Tanabe, F., Kato, A., & Yamada, K. 1996. Characterization of common cis-regulatory elements responsible for the endosperm-specific expression of members of the rice glutelin multigene family. Plant Molecular Biology, 30(7), 1207–1221.

Thompson, A. R., Walters, M. C., Kwiatkowski, J. L., Rasko, J. E. J., Ribeil, J.-A., Hongeng, S., ... & Frangoul, H. 2023. Development of best-in-class gene editing therapy for β-hemoglobinopathies by reactivating γ-globin expression. Blood, 142(Supplement 1), 5243–5252.

Wang, H., Zhang, Y., Cheng, Y., & Liang, C. 2015. Genomic approaches to identifying targets for treating β-hemoglobinopathies. BMC Medical Genomics, 8(1), Article 23.

Wang, L., & Xu, R. 2023. Design principles of a novel construct for HBB gene-editing and correction using CRISPR/Cas9 technology. Molecular Biotechnology, 65(12), 1899–1910.

Zhang, Y., Liu, H., & Chen, Z. 2024. G-quadruplexes as pivotal components of cis-regulatory elements in the human genome. BMC Biology, 22(1), Article 71.