Commentary | Open Access

Journal of Translational Genetics and Genomics

Xie et al. J Transl Genet Genom. 2026;10:545-50 DOI:10.20517/jtgg.2026.88

Precision reactivation of fetal hemoglobin: expanding the gene-editing landscape for β-thalassemia

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Citation: Xie S, Ye Y. Precision reactivation of fetal hemoglobin: expanding the gene-editing landscape for β-thalassemia. *J Transl Genet Genom.* 2026;10:545-50. https://dx.doi.org/10.20517/jtgg.2026.88

Received: 30 Jun 2026 **First Decision:** 4 Aug 2026 **Revised:** 28 Aug 2026 **Accepted:** 16 Sep 2026 **Published:** 28 Sep 2026

Academic Editor: Pier Paolo Piccaluga **Copy Editor:** Fangling Lan **Production Editor:** Fangling Lan

The recent clinical study by Frangoul *et al*. represents an important advance in genetic therapies for transfusion-dependent β-thalassemia[1]. Using an engineered CRISPR-Cas12a nuclease to disrupt BCL11A-binding motifs within the HBG1 and HBG2 promoters, the investigators recreated a hereditary persistence of fetal hemoglobin (HPFH)-like genetic state. All nine EdiThal participants became transfusion-free at last follow-up, and the six evaluable at 12 months or longer were transfusion-independent[1]. A companion sickle-cell study (RUBY) reported similar hematological improvements and fewer vaso-occlusive events[2]. These findings are encouraging, but both trials were relatively small and terminated early by the sponsor. They therefore provide proof-of-concept rather than definitive evidence of benefit. Conceptually, the work marks a shift in therapeutic genome editing: the goal is no longer merely to maximize cutting efficiency, but to choose edits that faithfully recapitulate protective natural variation.

TWO CLINICALLY RELEVANT TARGETS FOR HbF REACTIVATION: BCL11A ENHANCER *vs*. HBG PROMOTERS

BCL11A is a developmental stage-specific repressor of γ-globin expression, and its erythroid-specific enhancer has been a productive therapeutic target[3]. Exagamglogene autotemcel (exa-cel), which disrupts the +58 erythroid enhancer of BCL11A, has produced high rates of transfusion independence and has received regulatory approval for transfusion-dependent β-thalassemia and sickle cell disease[4,5]. Preclinical studies suggest that even erythroid-restricted enhancer disruption of BCL11A could subtly affect erythroid proliferation or differentiation[6-8], justifying continued surveillance.

Direct editing of the HBG1 and HBG2 promoters offers a more physiological alternative. Naturally occurring HPFH mutations have shown for decades that disruption of transcription-factor binding motifs within the γ-globin promoters can sustain high HbF levels without apparent adverse hematological consequences[9,10]. Promoter editing directly recreates protective HPFH variants, minimizing

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Table 1. Head-to-head comparison of BCL11A enhancer editing and HBG promoter editing

Feature	BCL11A enhancer editing (e.g. exa-cel)	HBG promoter editing (e.g., reni-cel)
Mechanism	Disrupt erythroid-specific +58 enhancer of BCL11A[3,8]	Disrupt BCL11A/ZBTB7A motifs in HBG1/HBG2 promoters[1,9,11,12]
HbF induction	High; reduces erythroid BCL11A and derepresses γ-globin[3,7,8]	High; directly derepresses HBG1/HBG2 transcription[1,11,12]
HPFH mimicry	Indirect: enhancer-deletion HPFH mechanism[9,10]	Direct: promoter HPFH point/indel variants[9,11,12]
Biological rationale	Reduced repressor derepresses γ-globin; global regulator remains intact outside erythroid cells[3,6-8]	Avoids altering a global transcription factor; targets a tissue-protective locus directly[1,11,12]
Off-target risk	Genome-wide Cas9 off-target cleavage; requires unbiased detection[14-16,28,29]	Cas12a PAM suits AT-rich promoters; HBG1/HBG2 paralogs can yield ~5 kb deletion[1,17-19]
Safety	Preclinical erythroid differentiation effects; long-term data accumulating[6-8]	Investigational phase 1-2; early termination; limited follow-up; one case of lymphopenia attributed to therapy[1,2]
Clinical evidence	Approved for TDT and SCD; longest edited-product follow-up[4,5]	Early-phase data show transfusion independence/VOC reduction; not yet approved[1,2]

interference with broader transcriptional networks[11,12]. In the EdiThal study, Cas12a editing generated the desired insertion-deletion patterns efficiently, but the report did not include a dedicated off-target analysis and follow-up remains short[1]. Whether promoter editing ultimately provides a biological advantage over enhancer-directed approaches will depend on long-term confirmation of preserved erythropoiesis and durable stem-cell function.

THE EVOLUTION OF GENOME-EDITING TECHNOLOGIES TOWARD GREATER PRECISION

First-generation CRISPR-Cas9 editing enabled efficient double-strand DNA cleavage[5,13]. However, double-strand breaks activate DNA-damage responses and may generate heterogeneous repair outcomes, chromosomal rearrangements, and p53 activation[14-16]. Cas12a offers a distinct PAM requirement, staggered DNA cleavage, simplified multiplex editing through a single CRISPR RNA, and high specificity in some contexts[17-19]. The indel spectrum generated by Cas12a at the HBG promoters closely resembles naturally occurring HPFH mutations[1,12], and engineered Cas12a variants may further improve flexibility[18].

Base-editing technologies permit single-nucleotide conversion without double-strand breaks, reducing genomic instability while precisely recreating protective variants[20,21]. HBG promoter base editing has entered early clinical evaluation[22,23]. Prime editing enables versatile sequence replacement without donor DNA or double-strand breaks, although efficiency in hematopoietic stem cells remains limiting[24]. In parallel, CRISPR interference, CRISPR activation, and epigenome editing seek to modulate gene expression without permanently altering genomic sequences, potentially providing reversible options[25,26]. As is summarized in Table 1, BCL11A enhancer editing and HBG promoter editing represent two distinct strategies for HbF induction, differing in their underlying mechanisms, biological rationale, off-target risks, safety profiles, and clinical evidence.

WHY Cas12a FOR THE HBG PROMOTERS?

Several mechanistic features make Cas12a well suited to HBG promoter editing. First, the TTTV PAM preferred by wild-type AsCas12a is abundant in the AT-rich γ-globin promoter region, expanding targetable sites compared with the NGG PAM required by commonly used SpCas9 variants[17,18]. Second, Cas12a produces staggered 5'-overhangs that yield a narrower, more predictable indel spectrum matching naturally occurring HPFH deletions[1,12]. Third, Cas12a processes its own CRISPR RNA from a single polycistronic transcript, simplifying multiplex editing of HBG1 and HBG2[17]. Fourth, because AsCas12a is derived from

Acidaminococcus spp., preexisting adaptive immunity may be less prevalent than against Staphylococcus aureus or Streptococcus pyogenes Cas9 proteins[27]. These features may make Cas12a particularly suitable for T-rich targets, multiplex editing, and potentially *in vivo* applications, although Cas9 may remain preferable in other settings.

SAFETY AND TRANSLATIONAL CONSIDERATIONS

Any double-strand-break-mediated editing strategy must be evaluated for genomic consequences. CRISPR-Cas9 editing can induce large deletions, complex rearrangements, and chromothripsis at on-target sites[14-16], and similar risks apply to Cas12a. Unbiased methods such as GUIDE-seq, targeted translocation assays (for example, CAST-Seq), and long-read sequencing are needed to detect both small off-target indels and large structural variants[28-30]. Because HBG1 and HBG2 are paralogous sequences separated by only about 5 kb, simultaneous editing can generate a recurrent intergenic deletion whose clonal consequences require monitoring[1,12].

Preexisting or induced immunity to Cas proteins is another translational consideration[27]. Myeloablative busulfan conditioning also contributes to long-term risks, including clonal expansion and secondary malignancies; a case of acute myeloid leukemia after lentiviral gene therapy underscores the importance of lifelong surveillance[31]. Non-genotoxic conditioning regimens, such as CD117-targeted antibody-drug conjugates, could reduce this burden[32]. Finally, manufacturing complexity, cost, and the need for specialized transplantation centers limit equitable access.

FUTURE DIRECTIONS

The next phase of β-hemoglobinopathy gene therapy will be shaped by greater precision in the edit, less invasive delivery and conditioning, and better characterization of edited cell products. Base and prime editing will continue to refine the installation of protective single-nucleotide variants without double-strand breaks[20-24], and epigenome editing may offer a reversible route to HbF induction[25,26]. *In vivo* hematopoietic stem-cell editing, exemplified by CD117-targeted lipid nanoparticles delivering mRNA-encoded editors, could eliminate *ex vivo* manipulation[33], and patient-specific *in vivo* editing has shown proof-of-concept in other genetic diseases[34].

Machine-learning models are increasingly used to predict guide activity and off-target potential across Cas systems[19]. Single-cell sequencing and long-read sequencing are moving from research tools toward quality-control assays that resolve clonal composition and structural variation[29,30]. Beyond therapeutics, CRISPR-based diagnostics, including Cas13a assays for nucleic acid detection in accessible samples, illustrate the expanding scope of CRISPR technologies in hematology and oncology[35,36], and gene editing is being integrated into cancer treatment strategies[37].

CONCLUSION

Taken together, these developments suggest that β-thalassemia gene therapy is entering an era of increasing precision rather than simply increasing editing efficiency. The field has progressed from lentiviral gene addition to enhancer editing, promoter editing, base editing, and potentially prime editing, with each generation aiming to maximize efficacy while minimizing unintended consequences[20-24,38-40]. Within this continuum, HBG promoter editing is promising because it emulates a naturally benign genetic condition. Nevertheless, the present study is limited by its small sample size, short follow-up, and early termination. Future head-to-head comparisons of the two editing strategies, together with long-term analyses of clonal stability, erythropoiesis, off-target effects, and stem-cell durability, will determine the optimal approach for HbF induction. The current data provide proof-of-concept that precise editing of the γ-globin promoters

may represent a promising therapeutic approach for β-thalassemia and other β-hemoglobinopathies. Taken together, these developments suggest that β-thalassemia gene therapy is entering an era of increasing precision rather than simply increasing editing efficiency. The field now encompasses lentiviral gene addition[38-40] alongside Cas9 enhancer editing[4,5], Cas12a promoter editing[1,2], base editing[20-23], and prime editing[24]; these strategies represent parallel and complementary approaches rather than sequential replacements, each aiming to maximize efficacy while minimizing unintended biological consequences. HBG promoter editing is particularly promising because it directly emulates a naturally benign genetic condition[9,11,12]. Nevertheless, the current evidence remains limited by small sample sizes, short follow-up, and early trial termination[1]. Future head-to-head comparisons of enhancer and promoter editing, together with long-term analyses of clonal stability, erythropoiesis, off-target effects, and stem-cell durability, will determine the optimal approach for HbF induction. The available data provide proof-of-concept that precise editing of the γ-globin promoters may represent a promising therapeutic strategy for β-thalassemia and other β-hemoglobinopathies[1].

DECLARATIONS

Authors' contributions

Designed the commentary, drafted the manuscript, and approved the final version: Xie S, Ye Y

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

During the preparation of this manuscript, the AI tool HY3 (version 3.0, released 2026-07-06) was used solely for language editing and reference formatting. The tool did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. The authors reviewed and approved all final content. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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