



## GWAS to Therapy by Genome Edits?

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Finlay *et al.*'s work provides another example of such unintended consequences of solving only one of two linked environmental problems. Their analysis raises important questions about the spatial scale of nutrient management efforts. Phosphorus reductions have unquestionably improved conditions in many temperate lakes, but these improvements may exacerbate nitrogen pollution problems in sensitive coastal waters. Rising nitrate concentrations in fresh waters may also have implications for global climate, because nitrogen loading has been shown to enhance the

production of the potent greenhouse-warming and ozone-destroying gas nitrous oxide ( $N_2O$ ) in lakes (11, 12), wetlands, and streams. It is high time to consider the substantial environmental benefits at all scales that will be gained by addressing these tightly linked, essential, and overused nutrients—nitrogen and phosphorus—simultaneously (13).

#### References

1. S. Carpenter *et al.*, *Ecol. Appl.* **8**, 559–568 (1998).
2. E. Jeppesen *et al.*, *Freshw. Biol.* **50**, 1747–1771 (2005).
3. J. C. Finlay, G. E. Small, R. W. Sterner, *Science* **342**, 247 (2013).

4. C. A. Klausmeier, E. Litchman, T. Daufresne, S. A. Levin, *Nature* **429**, 171–174 (2004).
5. V. H. Smith, *Science* **221**, 669–671 (1983).
6. M. Jansson, R. Andersson, H. Berggren, L. Leonardson, *Ambio* **23**, 320 (1994).
7. D. A. Bruesewitz, D. P. Hamilton, L. Schipper, *Ecosystems* **14**, 341–352 (2011).
8. J. Harrison *et al.*, *Biogeochem.* **93**, 143–157 (2009).
9. M. Wild, H. Gilgen *et al.*, *Science* **308**, 847–850 (2005).
10. L. O. Hedin, G. E. Likens, *Sci. Am.* **275**, 88–92 (1996).
11. M. L. McCrackin, J. J. Elser, *Global Biogeochem. Cycles* **25**, GB4005 (2011).
12. M. L. McCrackin, J. J. Elser, *Ecology* **91**, 528–539 (2010).
13. D. J. Conley *et al.*, *Science* **323**, 1014–1015 (2009).

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## GENETICS

# GWAS to Therapy by Genome Edits?

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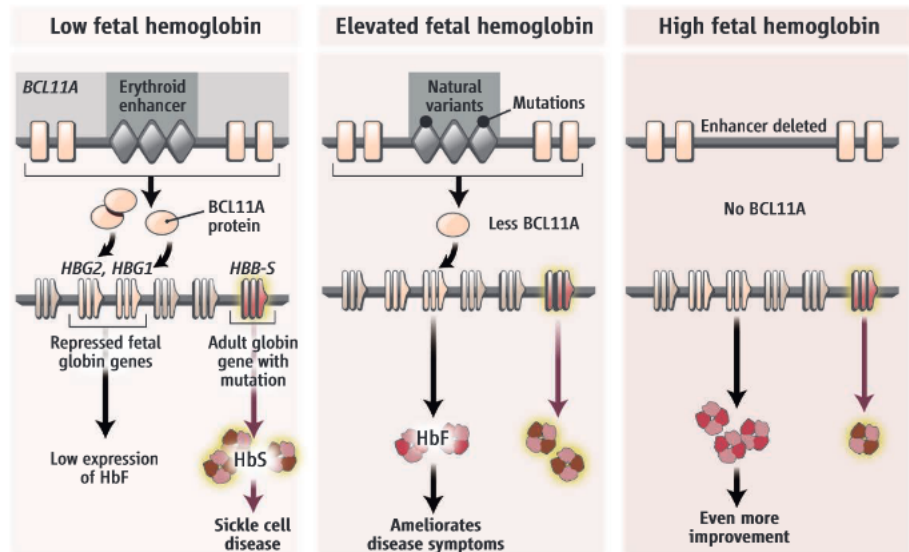
Disorders of hemoglobins are the most common monogenic diseases in the world, with substantial morbidity and mortality resulting from either defective function of the protein, such as in sickle cell anemia, or from insufficient protein production, such as the thalassemias (1). Genome-wide association studies (GWAS) have implicated two genes other than the globin genes as potential modulators of the pathology of these diseases by influencing the amounts of fetal hemoglobin (HbF). On page 253 in this issue, Bauer *et al.* (2) characterize common single-nucleotide polymorphisms (SNPs) in one of these genes, *BCL11A*. SNPs associated with mild increases in HbF amounts reside within a powerful tissue- and developmental stage-specific *BCL11A* enhancer (see the figure). Genome engineering reveals that this enhancer is essential for erythroid expression of *BCL11A*, and as a consequence, for globin gene expression. This exquisite specificity points to genome editing as a plausible approach to lasting corrective cell-specific therapy for certain hemoglobinopathies.

The first disease-linked mutation defined at the molecular level was the amino acid substitution in  $\beta$ -globin that leads to sickle cell anemia (3). Since then, genetic alterations have been identified as underlying causes for diverse congenital hemoglobinopathies. The search for therapies based on genetic

insights has driven investigation of the genetics and regulatory machinery of the globin genes. Most vertebrates produce different forms of hemoglobin during development, and humans produce a fetal stage-specific form called HbF. The concentration of HbF normally declines after birth, but the amount of HbF persisting in normal adults is a variable trait with strong heritability. Elevated HbF amounts can attenuate the symptoms in patients with sickle cell disease or thalassemias (4). GWAS initially identified two

Genetic and epigenetic studies of gene variants reveal a potential genomic target for treating hemoglobin disorders.

quantitative trait loci, not linked to the  $\beta$ -like globin genes, that determine the amount of HbF produced in normal populations (5, 6). One of these loci encodes *BCL11A*. Notably, ~15% of the variation in HbF in sickle cell anemia populations is accounted for by SNPs in *BCL11A*, which is high compared to most GWAS. *BCL11A* is a transcription factor that represses embryonic and fetal  $\beta$ -like globin transcription in human and mouse erythroid cells, and is a dominant regulator of developmental globin gene expression (7, 8). *Bcl11a*



**Exploiting nature's variants.** (Left) An erythroid enhancer promotes expression of *BCL11A*, which encodes a repressor that silences fetal globin genes *HBG1* and *HBG2* in adult human erythroid cells. The globin gene *HBB* is expressed in adult erythroid cells, but if mutated, disease can ensue, such as the production of sickle cell hemoglobin (HbS). (Middle) Natural variants in the enhancer reduce *BCL11A* production and boost fetal globin gene expression. Production of fetal hemoglobin (HbF) can ameliorate symptoms of some hemoglobinopathies. (Right) Removal of the enhancer is expected to reduce the amount of *BCL11A*, thereby allowing expression of the fetal globin genes and improving the pathologies from *HBB* mutations.

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loss ameliorates sickle cell anemia in mouse models of the disease (9).

Bauer et al. show that a region within an intron of the BCL11A gene has epigenetic signatures indicative of a transcriptional enhancer, as reflected in hypersensitivity to deoxyribonuclease, histone modifications associated with active enhancers, and physical juxtaposition with the BCL11A promoter regions through chromatin looping. SNPs with the most pronounced effects on HbF concentration fall at or near these critical regulatory sequences, and in one case impairs binding of erythroid transcription factors. In transgenic mice, this enhancer is sufficient to drive expression of a reporter gene predominantly in the fetal liver, the initial site of adult-type erythropoiesis in the developing mouse. Its deletion from the Bcl11a gene in a murine erythroid cell line, but not in a lymphocyte cell line, impaired BCL11A production, accompanied by an increase in embryonic globin expression, thus confirming the erythroid specificity of the enhancer.

More broadly, the results of Bauer et al. inform our understanding and expectations of the role of regulatory variants in complex traits, including disease susceptibility. Trait-associated variants discovered in GWAS are enriched in DNA segments carrying epigenetic hallmarks of regulatory regions (10, 11). However, most variants have modest effects on the phenotype, as is the case for the SNPs at the BCL11A locus. Yet, examination of the genomic context of these trait-associated variants has led to the identification of an

enhancer with a powerful effect on expression of BCL11A and, indirectly, the globin genes. Thus, the modest phenotypic effects of the common variants mapped in GWAS need not be interpreted as indicative of modest effects of the regulatory region or locus in which they reside. Rather, they should be considered as components of a regulatory complex that in total could have strong effects on phenotype.

Gene replacement therapy for the hemoglobinopathies has proved to be an extraordinarily difficult prospect (12). An alternative strategy to raise HbF concentrations would be to reduce BCL11A amounts or activity. However, BCL11A is widely expressed, and its loss is lethal due to nonerythroid effects (13). Those considerations and the generally undruggable nature of transcription factors led to the initial view of BCL11A as an implausible target for therapeutic intervention. The study of Bauer et al., however, raises the possibility of crippling the erythroid enhancer through genome editing in human hematopoietic stem or progenitor cells. Cells thus modified would be expected to display loss of BCL11A expression in erythroid cells while maintaining it in nonerythroid lineages. The potential benefits would be an increase in HbF production; a reciprocal decrease in expression of the defective adult globin in the case of sickle cell anemia; permanence of a one-time genetic deletion compared to gene replacement therapies that require long-term sustained expression of a transgene; and a selective growth advan-

tage of modified erythroid cells over unmodified counterparts.

Advances in genome editing (14, 15) are moving enhancer modifications into the arsenal of gene therapy approaches. However, additional work in animal models and primary human cells is needed to confirm and extend characterization of the enhancer, with emphasis on measuring effects on other tissues or genes. Further improvements in genome editing are essential to maximize efficiency and minimize off-target genomic alterations. Despite these challenges, the results of Bauer et al. suggest that genomic modification of an erythroid enhancer might ameliorate hemoglobinopathies, and the old dream of genetically guided therapies for this disease spectrum may become a reality.

#### References and Notes

1. T.N. Williams, D. J. Weatherall, *Cold Spring Harbor Perspect. Med.* 2, a011692 (2012).
2. D. E. Bauer et al., *Science* 342, 253 (2013).
3. V. M. Ingram, *Nature* 178, 792 (1956).
4. R. L. Nagel, *Semin. Hematol.* 28, 180 (1991).
5. S. Menzel et al., *Nat. Genet.* 39, 1197 (2007).
6. M. Uda et al., *Proc. Natl. Acad. Sci. U.S.A.* 105, 1620 (2008).
7. V. G. Sankaran et al., *Science* 322, 1839 (2008).
8. V. G. Sankaran et al., *Nature* 460, 1093 (2009).
9. J. Xu et al., *Science* 334, 993 (2011).
10. The ENCODE Project Consortium, *Nature* 489, 57 (2012).
11. M. T. Maurano et al., *Science* 337, 1190 (2012).
12. M. Cavazzana-Calvo et al., *Nature* 467, 318 (2010).
13. P. Liu et al., *Nat. Immunol.* 4, 525 (2003).
14. J. Boch et al., *Science* 326, 1509 (2009).
15. P. Mali et al., *Science* 339, 823 (2013).

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## MOLECULAR BIOLOGY

# RNAi, Antiviral After All

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**R**NA interference (RNAi) is an evolutionarily conserved pathway in cells for potent and specific silencing of gene expression. It is triggered by the accumulation of double-stranded RNA (dsRNA), which is subsequently processed into small interfering RNA (siRNA) and taken up into a protein complex that silences genes by cleaving their complementary RNAs (1). This mechanism has an important function in immunity against viruses in infected plants and invertebrates, but whether this is true

in mammals has been widely debated (2). On pages 235 and 231 of this issue, Maillard et al. (3) and Li et al. (4) provide evidence for the existence of a functional antiviral RNAi pathway in mammalian cells.

There has been a lack of compelling evidence for virus-derived small RNAs (vsRNAs) in mammalian cells (5). vsRNAs were detected in 41 human cell lines infected with six different RNA viruses, but it was unknown whether the vsRNAs were functional (6). It has been suggested that mammals have supplanted the RNA-based antiviral RNAi pathway with the protein-based, antiviral interferon (IFN) response (2, 7).

**No longer elusive, mammalian antiviral RNA interference is now confirmed.**

Maillard et al. noted that undifferentiated mouse embryonic stem cells (mESCs) lack an innate immune response that normally is stimulated in the presence of long dsRNA (2, 8). However, mESCs support the incorporation of siRNAs into functional RNA-induced silencing complexes (RISCs), demonstrating that mESCs contain an intact RNAi pathway (8–11). Thus, the authors reasoned that mESCs were a good experimental system to detect potential antiviral RNAi responses in the absence of innate IFN responses. Indeed, the authors observed that upon infection of mESCs with encephalomyocarditis virus (EMCV), vsRNAs were associated with the Argonaute 2 (the protein that loads RNA into

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