A New *Trans*-Acting Modulator of Fetal Hemoglobin?

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Polypeptides of the human hemoglobin (Hb) tetramer are encoded in the β -globin gene cluster on chromosome 11p15.5 and the α -globin gene cluster on chromosome 16p16 ter. From embryonic development, through fetal life, and into adulthood, switches in gene expression in these clusters specify the synthesis of 6 different hemoglobin tetramers, each functionally suited for its developmental stage. Many mutations affect these genes and consequently the structure and function of their protein products. Among them are the β -globin gene (*HBB*) sickle Hb (E7V; HbS) mutation and the many mutations accounting for β -thalassemia. Together, sickle cell anemia (HbS homozygosity; $\alpha_2\beta^S_2$) and β -thalassemia are mankind's most common Mendelian diseases. Within the β -globin gene cluster are the γ -globin genes (*HBG2* and *HBG1*). Increasing their expression is a potential "cure" for these disorders. Fetal Hb (HbF), a tetramer of α - and γ -globin chains ($\alpha_2\gamma_2$), can prevent the polymerization of HbS, the proximate cause of sickle cell anemia, and HbF also compensates for the lack of β -globin and normal HbA ($\alpha_2\beta_2$) in β -thalassemia. A detailed understanding of the regulation of HbF gene expression could suggest therapeutic approaches to inducing increased HbF gene expression.

As the HbS gene spread throughout Africa, the Middle East and India, following its possible origin during the Wet Sahara era about 7,000 years ago, its regional expansion became associated with 5 different genetic backgrounds, or haplotypes [1]. Each HbS gene haplotype has a characteristic level of HbF [2]. A loose association is present between the level of the HbF characteristic of a haplotype and the clinical severity of patients with this haplotype. Individuals with the Central African Republic or Bantu haplotype typically have the lowest HbF of all haplotypes (approx. 5%) and the most severe disease; patients with the Arab Indian (AI) haplotype have the highest HbF (approx. 20%) and have the mildest disease. Nevertheless, within any haplotype group, there is a broad dispersion of HbF levels and clinical features suggesting that regulatory elements that are not linked to the HbS gene or its haplotype have key roles as modulators of *HBG* expression.

Three major quantitation trait loci (QTL) are associated with HbF gene expression. On chromosome 2p16 is the *BCL11A* gene. *BCL11A* is a repressor of *HBG* expression. Polymorphisms, or SNPs within an erythroid-specific enhancer of *BCL11A* affect the transcript level and occupancy of its binding domains in the promoters of the

HbF genes. A bit less is known about actions of the second *trans*-acting HbF repressor, *MYB*, on chromosome 6q23. A 3-base pair deletion (rs66650371) in the *HBSIL-MYB* intergenic region is likely to be the functional motif with enhancer-like activity and binding sites for erythroid transcription factors. The third QTL is *cis*-acting, marked by the SNP rs7482144 that is 158 base pairs upstream of the *HBG2* promoter, and affects the expression of this gene only. Together, these QTL accounted for 10–50% of HbF variance among different populations [3].

HbF levels in the AI haplotype of sickle cell anemia are 2- to 4-fold higher than in other haplotypes. This might be partially explained by a haplotype effect that appears to be unique to Saudi Arabs. Both the African Senegal haplotype, where HbF levels average about 10%, and the AI haplotype, with HbF levels near 20%, contain rs7482144. However, homozygosity for minor alleles at rs16912979, rs7119428, and rs7482144 (T/A/T), which are in the major regulatory region of the *HBB* gene cluster, was present exclusively in the AI haplotype and might represent a functional *cis*-acting domain modulating *HBG2* expression [4]. *BCL11A* and *MYB*, the 2 known *trans*-acting QTL accounted for only 8.8% of HbF variance in the AI haplotype population, suggesting the likelihood of other *trans*-acting elements whose polymorphisms could account for the varying levels of HbF [5]. To further examine this possibility, whole-genome sequencing was carried out in 14 highly selected adults with the AI haplotype, 7 with HbF of 8.2% and 7 with HbF of 23.5%. Intronic SNPs (rs4527237, rs35685045; $D' = 1$) in the gene *ANTXR1* on chromosome 2p13, an anthrax toxin receptor, were found to be associated with HbF [6].

These observations were replicated in 2 cohorts of unselected Saudi AI haplotype homozygotes but not in other sickle cell anemia populations. *ANTXR1* variants explained approximately 10% of HbF variability compared with 8% for *BCL11A*; these 2 genes had independent additive effects on HbF, together accounting for 15% of its variance. Studies on CD34+ erythroid progenitors and erythroid cells derived from induced pluripotent stem cells suggested that this gene acted as a repressor of HbF expression.

In the last issue of *Acta Haematologica*, Al-Ali et al. [7] replicated the original observations in 630 new AI haplotype homozygotes and used different methods of genotyping and analysis, thereby lending further credence to this association. Unfortunately, association provides no information about causation. If *ANTXR1* represses HbF, what is its mechanism? Although *BCL11A* and *ANTXR1* had independent effects on HbF and there was no linkage disequilibrium between SNPs in *ANTXR1* and *BCL11A* [6]; both genes are on chromosome 2p, albeit in different chromosomal bands. Can there be some long-range interactions between these loci? Is this variant limited to Saudi Arabs? Indian sickle cell anemia patients with the AI haplotype did not show an association of *ANTXR1* with HbF; however, only 44 individuals were studied, raising the possibility of a false-negative result. Finally, more than three-quarters of the HbF variation in the AI haplotype sickle cell anemia remains unexplained and awaits discovery. Increasing HbF to levels that prevent HbS polymerization in most sickle erythrocytes will “cure” this disease. Is the *ANTXR1* locus another clue to achieving, therapeutically, this goal?

References

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