Variation of Fetal Hemoglobin and F-Cell Number With the LCR-HS2 Polymorphism in Nonanemic Individuals

To the Editor:

In normal adults, synthesis of fetal hemoglobin (HbF) persists at a very low level (<1%) and is restricted to a subpopulation of erythrocytes termed F cells. The number of F cells in an individual remains constant and seems to be under genetic control. Available data suggest that at least two types of genetic determinants might be involved in the level of HbF expression: one linked to the β-globin gene cluster and the other(s) unlinked to this region on chromosome 11.

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Fig 1. Polymorphic configurations of the 5' LCR-HS2 region observed in 48 AS individuals.

*Type A configuration was found exclusively on β¹ chromosome. All other configurations are associated with β⁸ chromosome.
Among the markers linked to the β-globin gene cluster, a common C→T variation at position −158 upstream of the gγ gene (detected as Xmn I restriction site polymorphism) is consistently associated with high gγ expression, but inconsistently with elevated HbF expression.6 It also has a modest effect on F cell number in hematologically normal individuals.3 This variation, in combination with other sequences of the β-globin gene cluster, has been associated with elevated HbF output in thalassemias to levels that are clinically beneficial.9

Another marker for HbF expression has been mapped to the 5′ hypersensitive site 2 (HS2) of the β-globin locus control region (LCR).10,11 Studies of patients with sickle cell anemia and β thalassemia show that specific sequence variations in the HS2 region are associated with unusually high levels of HbF. It was postulated that factors produced under conditions of hematopoietic stress interact with the HS2-associated genetic determinants and result in increased HbF expression. However, the influence of the LCR-HS2 polymorphism on HbF expression in nonanemic conditions is unknown and needs to be explored to validate this hypothesis.

We evaluated the relationship of LCR-HS2 sequence polymorphism with HbF level and F-cell number in a group of 48 unrelated healthy AS heterozygotes from South East Sicily (23 men and 25 women; mean age, 44 years; age range, 25 to 70 years). Hematologic indices were obtained for all individuals. Both the HbF level and the Hbs/HbA ratio were determined by a sensitive high-performance liquid chromatography (HPLC) method,12 and the F cells were assayed by a microscopic immunofluorescent method13 using a monoclonal antibody raised against human γ globin chain. The nucleotide sequence of the LCR-HS2 region was determined as previously described,1,14 and the sequence data were further confirmed by an allele-specific sequencing procedure described below. Because the studied AS heterozygotes were also heterozygotes for the Afl III polymorphism in the LCR-HS2 region (the recognition site for Afl III is exclusively present on β′ chromosome), the β′ allele was sequenced after polymerase chain reaction (PCR) amplification and allele separation of the Afl III-digested PCR product by electrophoresis on a 3% NuSieve agarose gel.

Hematologic indices and the proportion of Hbs indicated that none of these individuals was anemic or carried a-thalassemic genes. Family studies including homozygous sickle cell patients confirmed that, in all cases, the β′ chromosomes were of the restriction fragment length polymorphism (RFLP) defined “Benin” β′ haplotype and carried the specific LCR-HS2 sequence characteristic of this haplotype. Thus, we studied AS individuals who had an identical β′ chromosome and a β′ chromosome with varying sequence configurations in the LCR-HS2 region (Fig 1). Consequently, we were able to examine correlations between sequence variations in the β′ chromosomes and the expression status of HbF, with the other β′ chromosome being identical in all the studied individuals.

Given our sample size, it is unlikely that factors such as sex and age influence the findings of HbF expression. Indeed, no correlation between F-cell number and either age or sex was detected. Pairwise comparisons of different nucleotide sequences of the LCR-HS2 show that only configuration D exhibits a statistically significant association with increased HbF and F cell levels (Table 1). This nucleotide sequence is identical to the one that has been associated previously with elevated HbF expression in response to erythropoietic stress in sickle cell anemia and β-thalassemia.10,11

Our data indicate that the effect of this variant on HbF expression can be evidenced even in the absence of erythropoietic stress. The simultaneous increase in HbF level and F-cell number could reflect the fact that increments in HbF synthesis drive more cells across the F-cell detection threshold. These sequence variations affect known protein binding sites within the LCR-HS2 region.11 However, we cannot totally exclude the possibility that other sequence variations in the β cluster in linkage disequilibrium with the LCR-HS2 type D sequence contribute to HbF expression.

Our data do not refute the previous observations that F-cell production could be influenced by additional autosomal or X-linked genes12,16 but highlight the contribution of the β globin gene cluster, in particular the LCR-HS2 region, in F-cell expression.

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REFERENCES


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