Spectrin St Louis and the $\alpha_{LELY}$ Allele

To the Editor:

A protein polymorphism of the erythrocyte membrane protein $\alpha$ spectrin, $\alpha_{VAL}$, identified by increased proteolysis of the $\alpha_{ELV}$ domain junction has been described. This polymorphism is associated with low expression of the affected $\alpha$-spectrin chain.\(^1\) When coinherited in trans with a structural variant of $\alpha$ spectrin associated with inherited disorders of red blood cell shape such as hereditary elliptocytosis (HE), the polymorphic $\alpha_{VAL}$ allele results in a more severe phenotype than when a normal $\alpha$-spectrin gene is present in trans with the gene for the same structural variant. Recently, the genetic basis of this polymorphism was determined to be a nucleotide substitution in codon 1857 of the $\alpha$-spectrin gene (CTA to GTA, changing Leu to Val) and the allele was named $\alpha_{LELY}$ (low expression, Lyon).\(^2\)

Dalla Venezia et al\(^1\) described the presence in two kindreds of the $\alpha_{LELY}$ polymorphism in cis with a structural variant of $\alpha$ spectrin, L207P. This structural variant, which we have retrospectively named spectrin St Louis, is associated with HE or hereditary pyropoikilocytosis (HPP) in patients of African ancestry.\(^3\) Dalla Venezia et al\(^1\) hypothesized that the combination of spectrin St Louis and $\alpha_{LELY}$ in cis is responsible for the very low level of expression of the abnormal allele in the heterozygous state. We developed a rapid, polymerase chain reaction (PCR)-based assay for detection of the $\alpha_{LELY}$ polymorphism and used this assay to analyze DNA from six unrelated individuals with the spectrin St Louis mutation, including one of the individuals studied by Dalla Venezia et al.\(^1\)

The $\alpha_{ELV}$ polymorphism is located in exon 40 of the $\alpha$-spectrin gene.\(^2\) The polymorphism abolishes two overlapping Mwo I restriction enzyme recognition sites present in this region (Fig 1A). The following oligonucleotide primers that flank the $\alpha_{LELY}$ polymorphic site were synthesized for use in the PCR: 5'-CGTGAGTCTGATTAGCG-3' (sense, intron 39) and 5'-GGAATTCCTGAACTAGCTACC-3' (antisense, intron 40). Genomic DNA was isolated and amplified using the polymerase chain reaction as previously described.\(^5\) Amplification yielded a product of 193 bp. Digestion of amplification products with Mwo I followed by agarose gel electrophoresis (Fig 1B) allows differentiation of the wild-type allele with two major fragments of 107 and 77 bp and a minor fragment of 9 bp from the digestion-refractory $\alpha_{LELY}$ allele (a 193-bp fragment).

Analysis of DNA from patients with spectrin St Louis is shown in Fig 1C. Three HPP patients are homozygous for the $\alpha_{ELV}$ polymorphism (lanes 1, 5, and 6); two of these patients are homozygous for the spectrin St Louis genes (lanes 1 and 5) and one is heterozygous for the mutation (lane 6). Three patients are heterozygous for both the $\alpha_{ELV}$ polymorphism and the spectrin St Louis gene (lanes 2 through 4). Two of these patients have an HPP phenotype (lanes 2 and 3) and one has asymptomatic HE (lane 4). DNA from other family members was not available for study to allow unequivocal demonstration that the spectrin St Louis and $\alpha_{ELV}$ base changes were on the same $\alpha$-spectrin allele in these three heterozygous individuals.

These results provide additional confirmatory evidence for the hypothesis that the spectrin St Louis allele occurs in cis to the $\alpha_{ELV}$ allele. However, other factors must modify the phenotype, as patients who carry the spectrin St Louis + $\alpha_{ELV}$ allele in the heterozygous state vary in their clinical phenotype. Coinheritance in trans of thalassemia-like defects of $\alpha$-spectrin mRNA synthesis and/or accumulation may contribute to the HPP phenotype observed in some of
these patients. In the two studied patients with spectrin St Louis and HPP who are heterozygous for the αLELY allele (Fig 1C, lanes 2 and 3), we assume that the αLELY and spectrin St Louis base changes are in the same α-spectrin allele. Therefore, the allele in trans associated with markedly decreased accumulation of α spectrin in these two unrelated patients does not carry the αLELY polymorphism.

The PCR-based assay described here will be valuable in studies of the coinheritance of the αLELY allele in patients with other α-spectrin structural variants and the characterization of its role as a modifier of the clinical phenotype of α-spectrin gene disorders.

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REFERENCES


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