POLYMERASE CHAIN REACTION ANALYSIS OF AN NcoI POLYMORPHISM OF THE HUMAN ERYTHROCYTE ANKYRIN GENE

To the Editor:

Ankyrin is the protein that binds the spectrin-actin based membrane skeleton to the anion transporter (band 3) in the erythrocyte plasma membrane. A great deal of evidence has been accumulated that implicates defects of ankyrin in many cases of hereditary spherocytosis (HS). This evidence comes from a variety of sources including karyotypic studies, biochemical analyses, and study of a murine model of hereditary spherocytosis.

Recently, linkage analysis has implicated a defect in the ankyrin gene of a large kindred with typical, autosomal dominant HS. Southern blots of genomic DNA digested with the enzyme NcoI identified a restriction fragment length polymorphism (RFLP) that showed linkage of HS to the ankyrin gene in this family with a lod score of 3.63.

Because of the ease and simplicity of polymerase chain reaction (PCR)-based assays, we have mapped the location of the NcoI polymorphism and designed a rapid PCR-based assay for its identification.

Overlapping human genomic DNA clones were isolated using ankyrin cDNA clones as probes. Restriction enzyme analysis and limited nucleotide sequencing of these genomic clones localized this polymorphism to an intron in the region of the ankyrin gene encoding the regulatory domain of the protein.

Genomic DNA in this region was amplified as previously described using the following oligomer primers: 5'-GC(GAATTC-C)GGGATGAATGAGClTGCGG-3' (sense, intronic) and 5'-GG(AAGCTT)GTGGACCGCGTGACCT'CCT'C-3' (anti-sense, exonic). Sequences in parentheses indicate synthetic restriction enzyme sites. According to the numbering of Lux et al, the

![Figure 1. Linkage of dominant hereditary spherocytosis to the ankyrin gene. The inheritance of an ankyrin NcoI RFLP in a large kindred was studied using a PCR-based assay. Semisolid symbols denote affected individuals. The symbols, 1,1 indicate homozygosity for allele 1 (740 bp); 1,2 double heterozygosity for alleles 1 and 2; and 2,2 homozygosity for allele 2 (550 bp and 190 bp).]
exonic primer corresponds to ankyrin cDNA positions 5251 through 5275. Amplification yielded a band of approximately 740 bp. Digestion of amplification products with NcoI identified allele 1 (740 bp) and allele 2 (550 + 190 bp).

Results of Southern blotting of the previously described kindred with ankyrin gene-linked HSIO were confirmed by this PCR-based assay (Fig 1). One hundred fifty-two alleles of unrelated individuals from diverse genetic backgrounds showed an allelic frequency of 0.58 (allele 1) and 0.42 (allele 2). Study of three informative, three-generation families showed that this polymorphism is inherited in a Mendelian fashion.

Another useful polymorphism, detectable by PCR-based assay, consists of variable numbers of dinucleotide repeats (VNDR) in the 3' noncoding region of the ankyrin gene.15

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PATRICK G. GALLAGHER
WILLIAM T. TSE
BERNARD G. FORGET
Departments of Pediatrics, Genetics, and Internal Medicine
Yale University School of Medicine
New Haven, CT

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PG Gallagher, WT Tse and BG Forget