Screening of six racial groups for the intron 5 G→A 3´ splice acceptor mutation responsible for the Polynesian Kidd (a–b–) phenotype: the null mutation is not always associated with the *JKB* allele

The Kidd blood group is a major antigenic system in human RBCs and is defined by two alternate codominant antithetical specificities, Jk^a and Jk^b. The Kidd antigens are localized on a 43-kDa RBC integral membrane protein that functions as a urea transporter.[1] The *JKA* and *JKB* allele frequencies in whites are 0.51 and 0.49, respectively, and they give rise to the three common phenotypes Jk(a+b–), Jk(a–b+), and Jk(a+b+). The Jk(a–b–) phenotype, which is very rare in whites, is observed in 0.1 to 1.4 percent of Polynesians. The *KIDD*/urea transporter gene (*HUT11*) is located on chromosome 18 (q12-q21) and encodes a peptide of 391 amino acid residues.[1] The Jk^a and Jk^b epitopes arise through a single G→A transition at nucleotide position 838, which results in the incorporation of aspartic acid or asparagine, respectively, at amino acid residue 280.[2]

We previously reported the development and validation of a *Kidd* allele-specific PCR (ASPCR) genotyping assay that defined *JKA* and *JKB* by detecting the G→A transition within codon 280.[3] During development of this assay, we typed 10 Polynesians possessing the recessive silent Jk(a–b–) phenotype. All 10 samples genotyped as *JKB* homozygotes, which indicated that the null allele responsible for this phenotype was derived from *JKB*, and this allele would generate false *JKB*-positive results in our genotyping assay. Recently, molecular mechanisms responsible for the Jk(a–b–) phenotype have been described.[4,5] In Polynesian Jk(a–b–) persons, a G→A 3´ splice-acceptor-site mutation was observed within intron 5, which results in skipping of exon 6, while in Finnish Jk(a–b–) persons, another point mutation within exon 9 (T871C) predicted the loss of a potential glycosylation site.[5] To determine the potential impact of the more common Polynesian null *JK* allele on our prenatal genotyping service, we developed an ASPCR assay for detection of the intron 5 G→A 3´ splice-acceptor-site mutation and screened 753 unrelated subjects of six different racial or ethnic groups.

Peripheral blood was collected from racially self-identified random donors in sodium citrate (3.2%) or EDTA for DNA isolation in accordance with institutional ethical guidelines. Samples from African Americans (blacks) (n = 204) were collected in the Midwest, Southwest, and Pacific Northwest regions of the United States. Samples from whites (n = 180) were drawn in the metropolitan Milwaukee, WI, area. Samples from Hispanics (n = 92) and Native Americans, primarily Navajo persons (n = 86), were collected in the Southwest region of the United States. Samples from Koreans (n = 100) were drawn in Seoul, South Korea. Samples from Asian Indians (n = 91) were collected from Asian Indians living in the greater Milwaukee area and Bangalore, India. DNA was isolated from 250 to 500 µL of blood by the use of a kit (QiAmp Blood Kit, QIAGEN, Chatsworth, CA) according to the manufacturer's instructions. Analysis was accomplished by subjecting samples of genomic DNA from each subject to normal and null-specific PCR reactions (Fig. 1) utilizing forward primers that targeted the last 25 nucleotides of intron 5. Both reactions included an exon 6-specific reverse consensus primer (clone HUT11 nt 466-488; GenBank Accession Number L36121) that directed the amplification of a 154-bp fragment when paired with either of the allele-specific primers.

The intron 5 G→A 3´ splice-acceptor-site mutation responsible for the Polynesian Kidd (a–b–) phenotype was not

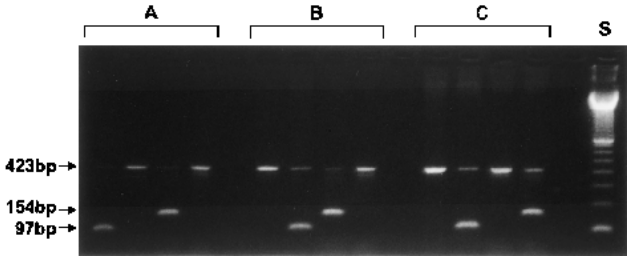

Fig. 1. Kidd genotyping by ASPCR. Products of each analysis are run in sets of four, ordered as follows: the *JKA* (nt 838G) reaction, the *JKB* (nt 838A) reaction, the Kidd normal (exon 6 nt-1G) reaction, and the Kidd null (exon 6 nt-1A) reaction. Allele-specific amplification of either *JKA* or *JKB* generates a 97-bp product as described previously,[3] and allele-specific amplification of the intron 5 product generates a 154-bp product. A 423-bp human growth hormone gene is coamplified in each reaction possessing DNA template. A) Analysis of a white *JKA/JKA* homozygote; B) analysis of a white *JKB/JKB* homozygote; C) analysis of a Polynesian Jk(a–b–) person. Forward intron 5-specific primers, based upon sequences provided by J.-P. Cartron [normal: 5´CCGTGCTCTGTCTTCTTGCCCCACtG3´; null: 5´CCGTGCTCTGTCTTCTTGCCCCACtA3´], and a common reverse consensus primer targeting exon 6 [5´CAAGTGATGGACATAGCACATAC3´] were designed. Primer specificity was enhanced by introducing intentional mismatches at the penultimate position (lowercase letter). ASPCR was performed with 125 ng of genomic DNA in 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* dNTPs, 0.5 µ*M* Kidd forward and reverse primers, 0.2 µ*M* human growth hormone forward and reverse primers, and 1 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, CT) in a total reaction volume of 25 µL. Thirty cycles were performed, consisting of 30 seconds at 94°C, 30 seconds at 63°C, and 30 seconds at 72°C in a thermal cycler (9600, Perkin-Elmer Cetus). Fifteen to 20 µL of each amplified PCR product was analyzed by electrophoresis through 2-percent agarose gels and stained with ethidium bromide.

observed in 204 blacks, 180 whites, 92 Hispanics, 86 Native Americans, and 100 Koreans screened, while one heterozygous carrier was observed among 91 Asian Indians. Genotyping the heterozygous individual for the Jk^a/Jk^b dimorphism at nt 838 revealed only the presence of "G," indicating a single normal *JKA* allele and a single null *JKA* allele. Unfortunately, fresh blood for serologic analysis of this Asian Indian individual is not available.

Ten Polynesian Jk(a–b–) persons typed in this study were homozygous for a silent *JKB* allele possessing the intron 5 G→A 3´ splice-acceptor-site mutation. Irshaid et al.[5] recently reported the absence of any mutations associated with silent *JK* alleles among 64 Swedish whites and the detection of eight intron 5 heterozygotes among 46 Polynesians.[5] Together, these limited results indicate that the silent Polynesian *JK* allele, as judged by its low frequency, is unlikely to cause false-positive results in prenatal genotyping of persons from non-Polynesian groups, but the risk of potential misinterpretation of genotyping results must be considered, especially as this mutation has been observed as the molecular basis of the Jk(a–b–) phenotype in a single Chinese American.[4] The existence of this rare allele again highlights the importance of establishing concordance between the parental genotypes and serotypes before predicting the fetal serotype on the basis of genotyping results.

Gail C. Ekman, MS
Martin J. Hessner, PhD
The Diagnostic Laboratories of The Blood Center
638 North 18th Street
Milwaukee, WI 53201-2178
e-mail: MJHessner@bcsew.edu

REFERENCES

1. Olives B, Mattei MG, Huet M, et al. Kidd blood group and urea transport of human erythrocytes are carried by the same protein. J Biol Chem 1995;270:15607-10.
2. Olives B, Merriman M, Bailly P, et al. The molecular basis of the Kidd blood group polymorphism and its lack of association with type 1 diabetes susceptibility. Hum Mol Genet 1997;6:1017-20.
3. Hessner MJ, Pircon RA, Johnson ST, Luhm RA. Prenatal genotyping of Jk^a and Jk^b of the human Kidd blood group system by allele-specific polymerase chain reaction. Prenat Diagn 1998;18:1225-31.
4. Lucien N, Sidoux-Walter F, Olives B, et al. Characterization of the gene encoding the human Kidd blood group/urea transporter protein. Evidence for splice site mutations in Jk_{null} individuals. J Biol Chem 1998;273:12973-80.
5. Irshaid NM, Henry SM, Olsson ML. Genomic characterization of the Kidd blood group gene: different molecular basis of the Jk(a–b–) phenotype in Polynesians and Finns. Transfusion 2000;40:69-74.