The Jk(a–b–) phenotype results from alterations in the JK gene and is characterized by absence of the RBC urea transporter in the cell membrane. The frequency of Jk(a–b–) varies among populations, but this phenotype is most commonly found in people of Polynesian and Finnish descent. Although rare, Jk(a–b–) individuals present a clinical challenge because anti-Jk3 is produced readily in response to transfusion and pregnancy, and Jk(a–b–) blood is not routinely available. Identification of Jk(a–b–) patients and donors is most often performed serologically. However, ten JK*o alleles have been identified, and this information can be used in DNA-based typing. We selected five JK*o alleles that had been encountered by our reference laboratory in two or more samples from unrelated individuals and designed an allele-specific primer PCR assay for use as an initial screening tool. After in-house validation, we tested genomic DNA from a family: a mother and her two sons referred to us for genetic investigation of their Jk(a–b–) phenotypes. Two different nucleotide substitutions, −1g>a in intron 5 (IVS5) and 956C>T in exon 10, originally associated with Polynesian and Indian/African populations respectively, were identified in the family. The mother and one son were compound heterozygotes, and the second son was homozygous for IVS5−1g>a. We conclude that the effort to design and validate such a screening assay was cost-efficient when compared with DNA sequencing costs. Furthermore, selection of the more common JK*o mutations was a practical approach that resulted in rapid identification of the genetic bases behind the Jk(a–b–) phenotypes in this unusual family. Although an obvious target for eventual inclusion into high-throughput genotyping platforms for clinical diagnostic services, current systems are very limited. Our approach provides a simple and inexpensive method for the identification of these rare alleles.

**Key Words:** JK blood group system, null phenotypes, molecular basis, PCR-ASP

The antigens of the Kidd (JK; ISBT009) blood group system are carried on the RBC urea transporter (Kidd glycoprotein, SLC14A1), a multipass membrane-spanning glycoprotein. Individuals who lack the Kidd glycoprotein on their RBCs express the Jk(a–b–) phenotype, also known as JKnull. This phenotype is associated with a mild insufficiency in urine concentration (which goes largely undetected in normal conditions), but may often be identified by the presence of anti-Jk3 in the plasma of immunized individuals. The absence of the RBC urea transporter renders Jk(a–b–) RBCs resistant to lysis by 2 M urea, which is used as an efficient screening test in populations where the Jk(a–b–) phenotype is more prevalent.4–8

The Jkα and Jkβ antigens depend on the amino acid at position 280, aspartic acid for Jkα and asparagine for Jkβ.9 The Jk(a–b–) phenotype derives from homozygosity or compound heterozygosity for mutations in the JK gene (SLC14A1; synonyms: UT-B1, HUT11, RACH1), so-called JKnull or JK*o alleles. A rare dominant Jk(a–b–) phenotype named In(Jk) was reported in two Japanese individuals identified by 2 M urea lysis screening,10 although the molecular basis has not been identified.

The JK locus comprises 11 exons on chromosome 18, and several different genetic alterations have been described to cause the Jk(a–b–) phenotype. The two most frequent JK*o alleles are found in people of Polynesian (IVS5−1g>a) and Finnish (871T>C) descent,11–13 in which the incidence of the Jk(a–b–) phenotype was shown to be 0.27 percent and 0.03 percent, respectively.5,7 Other JK*o alleles have been only sporadically detected in other populations.14–19 In this report, we have named silencing JK alleles generally as JK*o; however, when the JK*01 or JK*02 backbone is known, this is indicated as JK*01N or JK*02N in line with current proposals under consideration by the ISBT committee on terminology for RBC surface antigens (http://ibgrl.blood.co.uk/). Although rare in the general population, Jk(a–b–) individuals present a clinical challenge because anti-Jk3 may be produced in response to transfusion and pregnancy, and Jk(a–b–) blood is not routinely available. Limited resources of anti-Jk3 make large-scale phenotype screening programs for Jk(a–b–) blood impractical.

Screening methods for blood group polymorphisms using genomic DNA are becoming widespread in many laboratories. In instances in which a rare null allele is found more commonly in one population than in others, it is practical and cost-efficient to test only for that mutation, and large numbers of samples can be tested at a time. We sought to investigate the usefulness of developing an allele-specific primer PCR (PCR-ASP) screen for those JK*o alleles that had been found in two or more unrelated individuals as a primary analysis for samples referred to the Nordic Reference Laboratory for Blood Group Genomic typing for identification or confirmation of their Jk(a–b–) phenotype. Our
algorithm was such that samples in which a causative polymorphism was identified could be reported directly. Exclusion by the assay of these five mutations would prompt DNA sequence analysis of exons 1 to 11 of the Jk\(^*\) gene, a much more time-consuming and labor-intensive undertaking.

**Materials and Methods**

Jk\(^*\)0 mutations that fulfilled our criteria for inclusion are listed as follows (shown in Fig. 1): deletion of exon 4 and 5 (Δexons 4+5),\(^{14,15}\) IVS5–1g>a,\(^{11,12,17}\) 582C>G,\(^{20}\) 871T>C,\(^{12,13}\) and 956C>T.\(^{18}\) Allele-specific primers (Table 1) were designed to detect both the normal and mutant alleles according to the HUT11A sequence (GenBank accession no. NM_015865) and were synthesized by DNA Technologies A/S (Aarhus, Denmark). A mismatch was introduced in two primers to enhance allele specificity (underlined in Table 1). We performed the new assay in parallel with our routine in-house PCR-ASP for Jk\(^*\)01/Jk\(^*\)02.\(^{21}\)

For the PCR reaction 100 ng of DNA was used in a total volume of 10 μL, using 0.2 mM dNTPs, 0.25 pmol of each primer, 0.25 pmol of control HGH primers, and 0.5 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ) in the buffer supplied. Amplification was performed in the GeneAmp PCR system 2700 or 2720 (Perkin Elmer Cetus, Norwalk, CT) as follows: 7 minutes at 97°C, then 35 cycles of 30 seconds at 94°C, 30 seconds at 62°C, 40 seconds at 72°C, and a final elongation of 2 minutes at 72°C. PCR products were separated by electrophoresis for 25 minutes at 150 V using 3 percent agarose gel (SeaKem. FMC BioProducts, Rockland, MA) containing ethidium bromide (0.5 μg/ml).

Once optimized, PCR reagent mixes for each mutation and for the corresponding consensus nucleotide were prepared to a final volume of 500 μL, and frozen at −20°C after quality control. Samples carrying the Jk\(^*\)0 mutations and samples of normal Jk type were from our in-house DNA collection.

Genomic DNA was prepared either by a salting-out method modified from Miller et al. and diluted in sterile water to 100 ng/μL,\(^{22}\) or by the Qiagen blood and body fluid protocol (Qiagen Inc., Valencia, CA).

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**Table 1. Primers used in the Jk\(^*\)0 assay**

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Primer name</th>
<th>Nucleotide sequence (5´→3´)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δexons 4+5</td>
<td>JK-i3-F2</td>
<td>AGTTCTTCTCAACTAGACTGAAC</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>JK-i3-R2</td>
<td>CCCTCCCTAAGGAAGCTGCGC</td>
<td></td>
</tr>
<tr>
<td>IVS5–1g</td>
<td>JK-i5-F2b</td>
<td>CGTTGTCCTCTTGGCCCAAA</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>JK-i5-R10</td>
<td>CAAAGCTATGGACATACCA</td>
<td></td>
</tr>
<tr>
<td>582G</td>
<td>JK-582G-F</td>
<td>CTTTCAGCCACAGGACATAGTA</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>JK-582G-R</td>
<td>GACCCCTATGCTCTGGAGTAG</td>
<td></td>
</tr>
<tr>
<td>871C</td>
<td>JK-870-F3</td>
<td>CATGCTGCCATAGGATCTGCG</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td>JK-871C-R</td>
<td>TGCCATGCGAGGCCAGGA</td>
<td></td>
</tr>
<tr>
<td>956T</td>
<td>JK-956-F</td>
<td>ACAGAGCCCATGAGGCTCC</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>JK-956-R2</td>
<td>CGGACTTCCAGATAGGCC</td>
<td></td>
</tr>
</tbody>
</table>

**A. Primers for detection of the mutations**

**B. Primers for the detection of consensus nucleotides**

**C. Control primers**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Nucleotide sequence (5´→3´)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGH-F</td>
<td>TGCCATTCAGCTCCACAATTCTTTCA</td>
<td>434</td>
</tr>
<tr>
<td>HGH-R</td>
<td>CCACTCAGGATTTTGTTGTT</td>
<td></td>
</tr>
</tbody>
</table>
The numbering of nucleotides in this paper starts with the A of the initiation codon in exon 4 as nucleotide number 1. The same codon encodes methionine at position number 1.

**Results**

After optimization of each PCR-ASP to the conditions listed above, the new screening tool readily detected both mutation and consensus single-nucleotide polymorphisms (SNPs) for each tested allele (Fig. 2), and homozygote and heterozygote controls gave the expected results. Once the assay had been established and the expected bands shown to be robust and specific, DNA analysis of the individuals included in the case report was performed.

**Case Report**

Samples from a woman, originating from Guam, and her two sons were received for serologic investigation. An earlier investigation of the woman’s plasma performed by the referring hospital demonstrated an anti-Jk<sup>+</sup> although she had not been transfused. The sample at the time of the current investigation demonstrated anti-Jk<sup>3</sup>. Her sons were tested together with other family members to locate potential compatible donors.

The RBCs from all three samples typed Jk(a–b–) by serology and showed no hemolysis by the urea hemolysis test. Genomic DNA from these samples was tested by the HEA BeadChip Kit (Immucor Inc., Norcross, GA), and all three were identified as JK*02 homozygotes. However, the screening assay results showed that the mother and one of her sons carried two different mutations and thus were compound heterozygotes for the IVS5–1g>a mutation and 956C>T in exon 10. The other son was homozygous for IVS5–1g>a (Fig. 3). The three individuals typed JK*02 by the in-house PCR-ASP.<sup>a</sup> The pedigree of this unusual family could be constructed to explain the phenotypic and genetic results (Fig. 3).

**Fig. 2** Representative PCRs showing the (A) specific JK*0 and (B) consensus bands for the five selected mutations. The φX174 HaeIII DNA ladder (Advanced Biotechnologies Ltd., UK) was used as a size marker. P indicates a positive reaction and N a negative. In every set a PCR reaction with H<sub>2</sub>O instead of DNA was run in parallel to ensure that the PCR reagents were not contaminated.

**Fig. 3** The family pedigree together with the results of the PCR-ASP performed on samples from a Jk(a–b–) mother and her two Jk(a–b–) sons. The ASP-PCR kit is arranged as follows: lane 1: exon 4/Δexon 4+5; lane 2: IVS5–1g/a; lane 3: 582C/G; lane 4: 871T/C; lane 5: 956C/T. Bands amplified by allele-specific primers corresponding to IVS5–1g/a and 956C/T are indicated by the arrows. An internal control is run in parallel in all PCR reactions, and the φX174 HaeIII DNA ladder (Advanced Biotechnologies Ltd., UK) was used as a size marker. The pedigree shows the IVS5–1a allele in black and the 956T allele in gray. White indicates that the allele is unknown. Samples from the boys’ father were not tested (n.t.), but one allele is presumed to carry the IVS5–1a mutation.

**Discussion**

With the more widespread use of genotyping for predicting blood group phenotypes, null alleles must be taken into consideration even if they are uncommon. Different approaches for DNA-based typing permit different levels of inclusiveness. In a more high-throughput automation setting, in which space on a chip or bead is not overly restricted, all known null mutations can be included. On the other hand, in a manual setting, cost-effectiveness of any assay lies in the relative frequency of a null mutation in a given population or in the expected cohort of referred samples. Based on this, the PCR-ASP described was designed to include JK*0 mutations that had occurred in two or more unrelated individuals and to be used as a primary screening tool in the investigation of cases with a suspected Jk(a–b–) phenotype. Thus, only samples that are unresolved by the screening assay will be subjected to costly and labor-intensive DNA sequencing analysis of all 11 exons in the JK gene.
Because these samples are uncommon even in a major reference laboratory, a simple one-mutation-in-one-PCR-tube approach was chosen, and no attempt to multiplex the assay was performed. In multiplex assays, competition for DNA (often of varying or unknown quality when referred from another laboratory) can lessen the robustness of the individual allele-specific PCR reactions. We have tried this approach previously but found that the reproducibility of the assay was poor when performed infrequently.21 However, our current approach is balanced by the advantage that novel SNPs of sufficient frequency are easily incorporated into the assay. As a matter of fact, the two CE-marked products approved for blood group diagnostic use in Europe include either no JK*0 markers (BAGene, BAG Health Care GmbH) or detection only of the Finnish and Polynesian variants (BLOODChip+, Progenika Biopharma SA). (The CE mark indicates that the product has met the relevant quality requirements from the appropriate European directive.) A third commercial blood group genotyping product (BeadChip, Immucor, Inc.), not yet approved for diagnostic use, also includes detection of JK*01/JK*02 only.

We were fortunate enough to be able to test the PCR-ASP assay by analyzing samples from an especially interesting family: a mother and two sons, all of whom carried the Jk(a–b–) phenotype. Somewhat expected from the ethnic background, we identified the Polynesian combination with a JK*02 allele in two samples only, which because this SNP has been described previously in combination with a JK*02 allele in two samples only, which originated from the Indian subcontinent.18 However, null genes are underdiagnosed in general and especially so in populations in which more sophisticated DNA techniques are not in common practice. We anticipate from our studies and from screening studies being performed in other laboratories (Connie Westhoff, personal communication) that the latter mutation is more common than we first thought. This highlights the importance of understanding the genetic variation within a regional population under study, and shows that it can be worthwhile to incorporate null mutations into any strategy for genotype and phenotype testing.

References


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