Brief report

Partial deletion in the JK locus causing a Jk\textit{null} phenotype

Nicole Lucien, Jacques Chiaroni, Jean-Pierre Cartron, and Pascal Bailly

A new alteration of the blood group JK*A allele was identified in a Jk\textit{null} patient from Tunisia with an allo-anti-Jk3 in her serum. Southern blot and exon mapping analyses revealed an internal deletion within the Kidd (JK) locus encompassing exons 4 and 5. Sequence analysis of the Jk transcript showed that exons 4 and 5 were missing but were replaced by a 136-base-pair (bp) intron 3 sequence located 315 bp and 179 bp upstream from exon 4. This sequence is flanked by typical donor–acceptor cryptic splice sites used in the mutant but not in the normal JK gene. Because the translation initiation codon is located in exon 4, the Jk protein is not produced. (Blood. 2002;99: 1079-1081)


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Introduction

The urea transporter of human erythrocytes (hUT-B1) is encoded by the Kidd (JK) locus, which spans 30 kb of DNA on chromosome 18q12-q21 and is organized into 11 exons.\(^1,2\) The JK*A/JK*B polymorphism arises from an Asp280Asn substitution on the JK/hUT-B1 polypeptide.\(^3\) Red blood cells (RBCs) lacking Kidd antigens define a rare phenotype called Jk(a−b−) or Jk\textit{null}, the frequency of which is increased in some populations.\(^4\) This phenotype may result from homozygous inheritance of a silent allele at the JK locus or, less often, from a dominant inhibitor gene not linked to the JK locus.\(^5\) Persons with the Jk\textit{null} phenotype should be detected because anti-Jk3 antibody can develop after immunization by transfusion or pregnancy, and this antibody may cause immediate and delayed hemolytic transfusion reactions.

Jk\textit{null} RBCs have reduced urea permeability,\(^5\) but the Jk deficiency is not associated with any obvious clinical syndrome except for a urine concentration defect\(^6\) that probably results from the absence of Jk/hUT-B1 protein expressed on endothelial cells of the vasa recta of kidney.\(^7,8\) The silent-type Jk\textit{null} may arise by at least 3 distinct mechanisms: (1) splice-site mutations in JK*B alleles, causing the skipping of either exon 6\(^9,10\) or exon 7\(^11\); (2) missense mutation in the JK*B allele resulting in a Ser291P substitution\(^9,10\); and (3) nonsense mutation in a JK*A allele resulting in a Tyr194Stop substitution.\(^12\) We now report a fourth mechanism, a deletion removing exons 4 and 5 of a JK*A allele.

Study design

Reagents

Expand High Fidelity, Expand Long Template PCR, and Titan One tube RT-PCR systems were from Boehringer-Mannheim/Roche Diagnostics (Mannheim, Germany). Nucleotide sequences were determined with ThermoSequenase sequencing kit from Amersham Pharmacia Biotech (Bucks, United Kingdom) using 5’(Cy5)-primers (Genset, Paris, France). Affinity-purified rabbit antibodies directed against the N-terminal and C-terminal of the Kidd/hUT-B1 protein were as described.\(^1,3\)

Amplification by reverse transcription coupled with polymerase chain reaction

For primer designation, position +1 refers to the first nucleotide of the initiation codon of the JK gene (GenBank accession number Y19039). Total blood RNA extracted by the acid-phenol-quinoline method\(^1,3\) was used for the first PCR in Titan One tube reverse transcription–polymerase chain reaction (RT-PCR) (50°C for 30 minutes [1 cycle], 94°C for 2 minutes [1 cycle], 94°C for 30 seconds, 64°C for 30 seconds, 68°C for 2 minutes [30 cycles], 68°C for 7 minutes [1 cycle]) between primers SP-1 (positions –41 to –22, exon 3) and AS-2 (position 1260-1237) according to the manufacturer’s instructions. The second PCR was performed with one twentieth-fifth of the first reaction in the same conditions with primers SP-1 and AS-3 (position 1234-1211, exon 11) and Expand High Fidelity system.

Genomic DNA analysis

PCR reactions (v = 50 μL) contained 500 ng leukocyte DNA extracted with the Wizard Genomic DNA Purification kit from Promega (Madison, WI). A first PCR between primers SP-4 (5’-ggttagcattacagacactgatggc-3’, position 207-184 upstream exon 4) and AS-5 (position 470-446) encompassing the internal deletion was performed under stringent conditions (93°C for 2 minutes [1 cycle], 93°C for 10 seconds, 66°C for 30 seconds, 68°C for 5 minutes [10 cycles], 93°C for 10 seconds, 66°C for 30 seconds, 68°C for 5 minutes plus 20 sec/cycle [25 cycles], 68°C for 7 minutes [1 cycle]) using Expand Long Template PCR. The second PCR was performed with one fiftieth of the first reaction using primers SP-4 and AS-6 (position 445-421, exon 6) under the same conditions except for the annealing temperature (62°C). PCR products were subcloned and sequenced.

Results and discussion

The patient with a Jk\textit{null} phenotype identified at the EFS Alpes-Provence (Marseille, France) was typed Jk(a−b−) with routine reagents (not shown). Western blot analysis with affinity-purified antibodies directed against the N-terminal and C-terminal of the Jk/hUT-B1 polypeptide showed that RBCs from the propositus

From INSERM-U76, Institut National de la Transfusion Sanguine, Paris, France, and EFS Alpes-Provence, Marseille, France.

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Reprints: Jean-Pierre Cartron, INSERM-U76, Institut National de la Transfusion Sanguine, 6 rue Alexandre Cabanel, 75015-Paris, France; e-mail: cartron@idf.inserm.fr.

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lacked the Jk membrane protein of 45 to 69 kd (Figure 1A), and genotyping indicated homozygosity for a JK*A allele (not shown). To characterize the molecular defect occurring in the silent JK*A allele, genomic DNA from the propositus was digested with SacI and was analyzed by Southern blot. Hybridization with a cDNA probe encoding the Jk/hUT-B1 protein revealed the lack of a 7-kb fragment that normally contains exons 4 and 5 (Figure 1B). To confirm this finding, total RNA from the Jknull blood was used as a template to amplify the Jk-cDNA by heminested PCR using primer pairs located in exons 3 and 11 (see “Study design”). A 1049-kb fragment (vs a 1275-kb fragment for Jk(a+/b+/H11001b/H11001b) controls) was obtained, and sequence analysis confirmed that exons 4 and 5 were missing (Jk[Δ4,5] mutant) but were replaced by a 136-bp sequence called E* on Figure 1C. Sequence comparison indicated that the 136-bp sequence was identical to an intronic sequence located 315 bp and 179 bp upstream from exon 4. This sequence is flanked by typical donor–acceptor cryptic splice sites used in the mutant but not in the normal JK gene. In vitro transcription–translation assays showed that no protein could be produced from the Jk(Δ4,5) cDNA, as expected from the loss of the translation initiation codon normally present in exon 4 (not shown).

To better characterize the internal deletion and to locate the breakpoints, a genomic fragment was PCR-amplified using primer pairs located in E* and exon 6 (Figure 2). The size difference of the PCR-1 products amplified from the Jk(a+/b+/H11001b/H11001b) sample (4.3 kb) and the Jknull sample (2.7 kb) suggested a deletion of approximately 1.6 kb (Figure 2). After sequence analysis, the 5’ and 3’ breakpoints were localized 131 base pair (bp) upstream from exon 4 and 575 bp downstream from exon 5, respectively. A PCR spanning the breakpoint may be used to discriminate this novel silent JK*A allele from other Jk-deficient alleles, including that recently found in an English family.

The mechanism responsible for the deletion is unknown, and there are no typical sequence motifs around the deletion breakpoint. However, the deletion breakpoint is flanked by small direct repeats (Figure 2), suggesting, as found in mitochondrial DNA, that recombination or slipped mispairing may cause the deletion.
References

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