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Investigation of JK Typing Discrepancies Reveals Three Novel Alleles

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INTRODUCTION

- RBC genotyping supports antigen-negative donor databases and patient typing, alone or with serology.
- Discrepancies between genotyping and serology can lead to discovery of variant alleles.
- There are currently over 80 reported *JK* alleles, many encoding weak or null phenotypes.
- In this study, we investigated three samples with discrepant JK typing.

MATERIALS AND METHODS

Serology Testing
- Serologic testing was performed by standard tube methods.
- JK typing was performed using monoclonal anti-Jk^a and –Jk^b (Werfen or Alba Bioscience).
- Elution was done using Gamma ELU-KIT II (Werfen).
- Antibody identification was performed by PEG IAT.

Molecular Testing
- Genomic DNA was isolated from leukocytes (Qiagen).
- Genotyping methods included PreciseType HEA (Werfen), Sanger sequencing of *JK* exons 3-10 including flanking intronic regions, long-range allele-specific PCR (LR-AS PCR) and next-generation sequencing (NGS) (Illumina) of various *JK* exons for variant phasing.

CASE STUDIES

Sample	Serology	PreciseType HEA
#1: 24 y/o female donor	Jk(a–b+)	*JK*01/02*
#2: 48 y/o female patient	Jk(a–b–)	*JK*02/02*
#3: 73 y/o female patient	Anti-Jk^a in plasma	*JK*01/02*

Table 1. Samples referred for JK investigation

RESULTS

Sample #1
Serology:
- RBCs were non-reactive with anti-Jk^a
- RBCs were strongly reactive with anti-Jk^b

PreciseType HEA Beadchip:
- c.838G>A (heterozygous) predicted Jk(a+b+)

Sanger Sequencing:
- c.838G>A (heterozygous) [p.(Asp280Asn)]
- c.588A>G (homozygous) [p.(Pro196=)]
- c.130G>A (heterozygous) [p.(Glu44Lys)]
- c.276G>A (heterozygous) [p.(Trp92Ter)]

Long-range allele-specific PCR:
- Phasing confirmed c.838G, c.276A, c.130A were in cis

JK genotype: *JK*01(130A,276A)/JK*02*

RESULTS

Sample #1 Sequencing

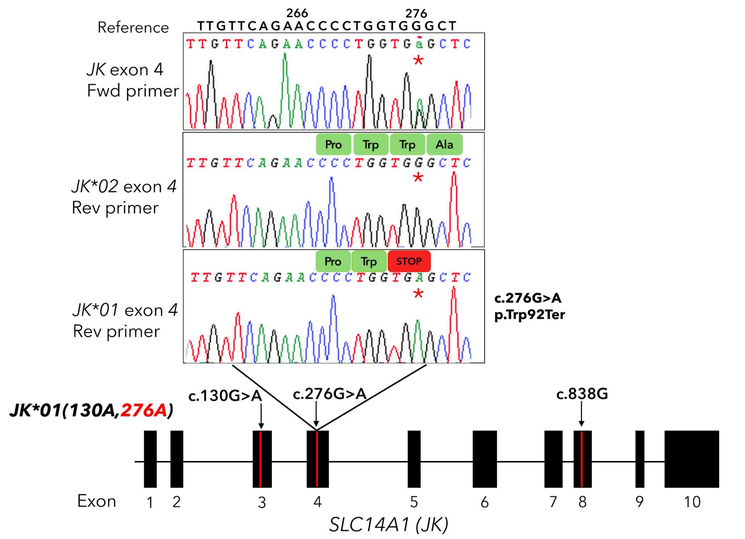


Figure 1. Sanger sequencing electropherogram of *JK* exon 4

Sample #2
Serology:
- Historical records on a pretransfusion sample were reported by the referring hospital as Jk(a–b–)
- Antigen typing could not be confirmed on the current sample because of recent transfusion

PreciseType HEA Beadchip:
- c.838G>A (homozygous) predicted Jk(a–b+)

Sanger Sequencing:
- c.838G>A (homozygous) [p.(Asp280Asn)]
- c.588A>G (homozygous) [p.(Pro196=)]
- c.956C>T (heterozygous) [p.(Thr319Met)]
- c.989T>C (heterozygous) [p.(Met330Thr)]

Next-Generation Sequencing:
- Phasing confirmed c.956T and c.989C were in trans

JK genotype: *JK*02N.08(588G)/JK*02(588G,989C)*

Sample #2 Sequencing

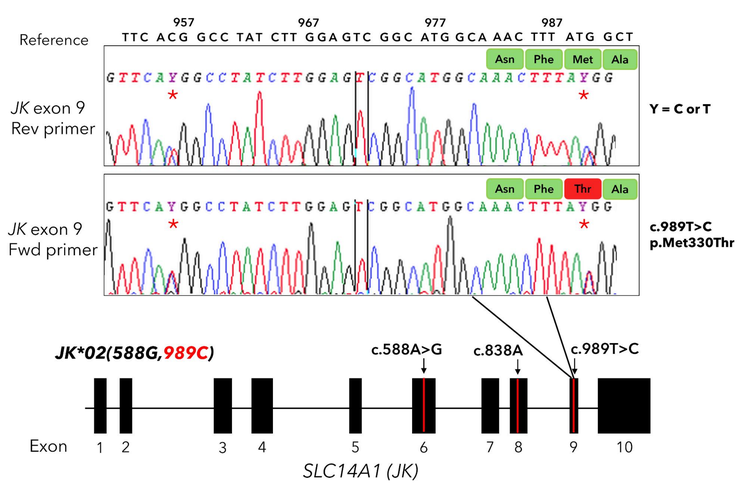


Figure 2. Sanger sequencing electropherogram of *JK* exon 9

RESULTS

Sample #3
Serology:
- Anti-Jk^a identified in plasma and eluate by PEG IAT
- Antigen typing could not be confirmed because of recent transfusion

PreciseType HEA Beadchip:
- c.838G>A (heterozygous) predicted Jk(a+b+)

Sanger Sequencing:
- c.838G>A (heterozygous) [p.(Asp280Asn)]
- c.588A>G (heterozygous) [p.(Pro196=)]
- c.491-500delTGAATTCCAT (heterozygous) [p.(Leu164CysfsTer40)]

JK genotype: *JK*01(491-500del)/JK*02(588G)*

Sample #3 Sequencing

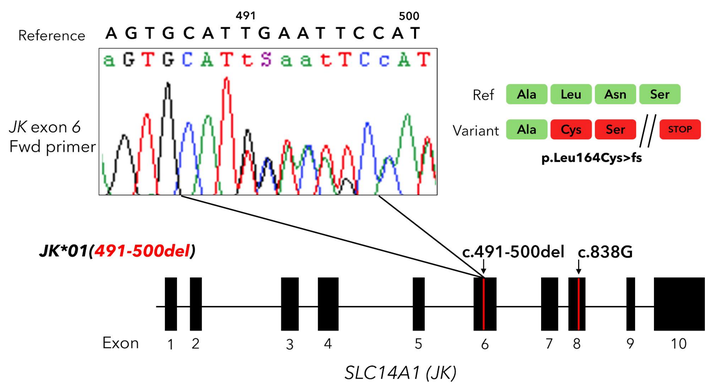


Figure 3. Sanger sequencing electropherogram of *JK* exon 6 and corresponding amino acid change

CONCLUSIONS

We report three novel *JK* alleles predicted to produce null or severely weakened phenotypes through nonsense, missense, and frameshift mechanisms:

***JK*01(130A,276A)* [p.(Trp92Ter)]**
***JK*02(588G,989C)* [p.(Met330Thr)]**
***JK*01(491-500del)* [p.(Leu164CysfsTer40)]**

- c.276G>A is a nonsense variant that encodes a premature stop codon and is predicted to result in a null Jk^a phenotype. The variant is present in gnomAD; frequency 0.000001239 (rs763262711)
- c.989T>C is a missense variant that results in p.(Met330Thr) (hydrophobic Met to hydrophilic Thr). Additional testing is required to distinguish a true null phenotype from weak Jk^b expression
- c.491-500delTGAATTCCAT is a 10-bp deletion that causes a frameshift and introduces a premature stop codon; it is predicted to result in a null Jk^a phenotype, which is supported by the patient having made anti-Jk^a

None of the three novel *JK* alleles were detectable by SNV-based Beadchip genotyping, and two required phasing to resolve.