

Discrepant Results of JK Phenotypes in People of African Descent

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Abstract:

Antibodies in the Kidd blood group system are known to be clinically significant and difficult to detect. The JK null phenotype has predominantly been associated with people of Polynesian descent. However, with the increased availability of molecular genotyping, many new mutations have been identified in people of other ethnic backgrounds. Two mutations have been previously reported in African American people, *JK*01N.04* and *JK*01N.05*. To determine the prevalence of JK mutations in a regional African American population, a two part study was conducted. The first part reviewed the results of genotyping with previously recorded serological typings. The second part was an investigation into the occurrence rate of the *JK*01N.05* mutation. Although these mutations were not detected in the test population, two mutations previously undetected in African American people were identified. Though the use of SNP analysis for prediction of blood group antigens is becoming more prevalent, it is not without limitations.

Introduction:

The Kidd blood group system was first described in 1951 when anti-Jk^a was found to cause hemolytic disease of the fetus and newborn (HDFN).¹ Anti-Jk^b was reported in 1953, also as a case of HDFN² and in 1959, anti-Jk3 was described in a post-transfusion patient whose red blood cells typed Jk(a-b-).³ Since this time, many examples of anti-Jk^a and anti-Jk^b have been reported. Due to the rapid decrease in antibody titer, these clinically significant antibodies can be difficult to detect and are frequently associated with severe delayed hemolytic transfusion reactions. In fact, fatal delayed hemolytic transfusion reactions due to anti-Jk^a and anti-Jk^b remain among those most reported to the FDA.⁴

The Kidd glycoprotein is found on red blood cells as well as endothelial cells of the vasa recta, the vascular supply of the renal medulla. It is an integral protein of the red blood cell, spanning the membrane ten times. The glycoprotein functions as a urea transporter for the cells and helps maintain osmotic stability. Persons lacking the glycoprotein are known to have decreased urine concentration but have no known disease associations or red cell abnormalities. The Kidd glycoprotein is encoded by the *SLC14A1* gene located on chromosome 18 and consists of 389 amino acids. Eleven exons are distributed over 30 kb of DNA. As the first three exons are not translated, the mature protein is encoded by exons 4-11.⁵ The *SLC14A1* gene has two major co-dominant alleles, *JK*A* (*JK*01*) and *JK*B* (*JK*02*), which result from a single nucleotide polymorphism (SNP), 838A>G.

To date, 14 polymorphisms causing JK null phenotypes have been reported; five for *JK*A* and nine for *JK*B*. (Table 1). Of the known polymorphisms, only two have been associated with people of African American ethnicity (*JK*01N.04* and *JK*01N.05*).^{6, 7} However, each of these mutations were found in single individuals presenting with the Jk(a-b-) phenotype. To assess the occurrence of *JK* mutations in the Southern United States African American

population, a two part study was conducted. The first phase was an investigation into the concordance rate of genotyping with serological antigen typing of both Jk^a and Jk^b. In the second phase of the study, donors previously typed Jk(a+) by molecular typing were screened for the *JK*01N.05* mutation.

Materials and Methods – Sample Selection

After obtaining informed consent, samples from African American donors and patients from the tri-state service area (Louisiana, Texas and Arkansas) were obtained for testing. Genomic DNA was extracted and prepared using either manual or QIAcube automated methods (Qiagen, Valencia, California, USA). Red cell blood group genotyping was performed using the Human Erythrocyte Antigen (HEA) assay (BioArray version 1.2, Warren, New Jersey, USA) according to the manufacturer's directions.

Materials and Methods – Molecular Assays

Upon completion of genotype testing, results were compared with previous serological typing results for concordance. In addition, African American donors who genotyped as *JK*A* were screened for the Jk^a specific silencing mutation *JK*01N.05* (561C>A) by restriction fragment length polymorphism (RFLP) gel electrophoresis. Polymerase chain reaction of genomic DNA samples was performed with two primers, JK-16-F (5'tgttggcaggtgaaacaaag3') and JK-17-R (5'gccaccatagtctgagcat3'), (Roche Diagnostics, Germany). The amplification process consisted of an initial cycle of 94° for 2 minutes. The second cycle was 94°, 30 seconds; 56°, 30 seconds and 72°, 30 seconds and was repeated 29 times. The third cycle was a 72° hold for seven minutes followed by a 4° end hold. 5.0 µl of each amplified product was then digested with one

unit restriction enzyme *MaeIII*. The digested product was visualized on a 2% agarose gel containing ethidium bromide.⁸

Samples with discordant results were confirmed using the Red Cell EZ Type KDK assay (GTI Diagnostics, Wisconsin, USA) according to the manufacturer's directions. Samples that remained discordant were sent to Puget Sound Blood Center for genetic sequencing.

Results:

Data for the first phase was collected from January 1, 2009 through September 30, 2010. During the 21-month study period, 1,079 genotyped donors had previous Jk^a serological typing results and 1,235 genotyped donors had previous Jk^b serological results. Upon comparison with molecular typing results, five samples were found to be discordant. All samples were predicted to be Jk(a+b+) by genotyping, however one sample was Jk(a-), while four samples were Jk(b-) by serology.

Sequencing of all five samples resulted in identification of *JK* silencing alleles. The silenced *JK*A* allele was due to a promoter region mutation, while all four of the *JK*B* silencing alleles were the result of a 64R>Q substitution. This previously unpublished mutation has thus far only been detected in Asians. (Lakshmi Gaur, personal communication.)

A retrospective review of patient testing also yielded discrepancies. Of the 250 patient records reviewed, two were *JK*A/JK*B* by genotype and Jk^b negative by serology. Again, each sample was sequenced. One patient was confirmed to carry the 64R>Q substitution while the second patient appears to exhibit a currently undetermined mutation.

Of the 513 samples tested for the *JK*01N.05* silencing mutation, all were negative, suggesting that although this mutation may occur in populations of African descent, at this time it is not common in the Southern US Black population.

Discussion:

While no longer in its infancy, the role of molecular technologies in the blood bank environment is one that continues to evolve. With the advent of high-throughput molecular assays which are often more economical and efficient than routine serology, donor screening has been revolutionized.⁹ While some microarray assays have incorporated JK null detection, these are usually limited to the more common Polynesian and Finnish mutations, IVS5-1g>a and 871T>C respectively.¹⁰ Previously JK nulls have only rarely been found in African Americans.⁷ Indeed the *JK*01N.05* allele was not found in Southern Blacks. However, a *JK*B* null occurring in 1:308 donors was found. As shown by this study, antigen screening which uses only SNP analysis may not detect blood group mutations due to silencing mutations and may lead to erroneous testing results. This method of donor screening will not increase the risk to allo-immunized patients, as a unit would appear to be positive for the antigen and therefore not selected for transfusion to a patient with a known antibody.

However, there is a risk of mis-typing patients; and if a patient's genotype will be used for prophylactic antigen matching, as in the case of sickle cell disease or warm auto-immune hemolytic anemias, limitations of molecular assays must be considered. Given the prevalence of the 64R>Q mutation in the study population, a comparison of previously typed sickle cell disease patients was conducted and indeed JK mutations were detected. In addition, both of these patients have produced allo anti-Jk^b. This knowledge has led to a change in protocol, such that any African American patients typing *JK*B* will be confirmed serologically whenever possible with regard to previous transfusion at the time of sample collection.

African Americans are known to have other silencing mutations, eg. *FY* and *GYP*B* genes, and detection of them has been incorporated into existing microarrays. Perhaps as molecular assays continue to evolve, detection of additional mutations leading to JK nulls will be

incorporated as well. More data as to the occurrence rate of the 64R>Q mutation in African Americans of other geographic locations may be necessary. Additionally, given that anti-Jk^a and anti-Jk^b can be weakly reactive as well as show dosage, those donors used for reagent red cell panels should be confirmed as truly homozygous and not actually hemizygous. As with many other blood group systems, what once appeared to be a simple glycoprotein with two common antigens and one rare null type has increasingly become much more complex.

References

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Table 1, Draft, ISBT Transfusion Medicine Terminology

Phenotype	Allele Name	Nucleotide change	Intron/Exon	Amino acid change
JK:-3 or Jk(a-b-)	<i>JK*01N.01</i>	Exons 4 & 5 deleted	4 & 5	Initiation Met absent
JK:-3 or Jk(a-b-)	<i>JK*01N.02</i>	202C>T	5	Gln68Stop
JK:-3 or Jk(a-b-)	<i>JK*01N.03</i>	582C>G	7	Tyr194Stop
JK:-3 or Jk(a-b-)	<i>JK*01N.04</i>	956C>T	10	Thr319Met
JK:-3 or Jk(a-b-)	<i>JK*01N.05</i>	561C>A	7	Tyr187Stop
JK:-3 or Jk(a-b-)	<i>JK*02N.01</i>	IVS5-1g>a	Intron 5	Exon 6 skipped; in frame
JK:-3 or Jk(a-b-)	<i>JK*02N.02</i>	IVS5-1g>c	Intron 5	Exon 6 skipped; in frame
JK:-3 or Jk(a-b-)	<i>JK*02N.03</i>	222C>A	5	Asn74Lys
JK:-3 or Jk(a-b-)	<i>JK*02N.04</i>	IVS7+1g>t	Intron 7	Exon 7 skipped; framshift→Leu223Stop
JK:-3 or Jk(a-b-)	<i>JK*02N.05</i>	723delA	8	Frameshift→Ile262Stop
JK:-3 or Jk(a-b-)	<i>JK*02N.06</i>	871T>C	9	Ser291Pro
JK:-3 or Jk(a-b-)	<i>JK*02N.07</i>	896G>A	9	Gly299Glu
JK:-3 or Jk(a-b-)	<i>JK*02N.08</i>	956C>T	10	Thr319Met
JK:-3 or Jk(a-b-)	<i>JK*02N.09</i>	191G>A		Arg64Gln