Case Report

Development of anti-Jk3 associated with silenced Kidd antigen expression and a novel single nucleotide variant of the *JK* gene

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Anti-Jk3 is a rare alloantibody to a high-prevalence antigen primarily seen in individuals of Polynesian descent and is associated with a handful of well-established variants of the *SLC14A1* gene. We report a case of the Jk_{null} phenotype, associated with formation of anti-Jk3, in a patient of non-Polynesian descent. This patient, a 51-year-old woman self-described as of Jamaican and Scottish ancestry, presented to our hospital for oncologic care. The patient's blood sample typed as blood group A, D+. All screening and panel reagent red blood cells showed reactivity, ranging from 2 to 4+; autocontrol and direct antiglobulin test were both negative. Antigen phenotyping revealed Jk(a–b–), leading to suspicion for anti-Jk3, which was subsequently confirmed by our immunohematology reference laboratory. Given her reported familial background, testing of the *SLC14A1* gene was performed, revealing that the patient was heterozygous for the single nucleotide variant (SNV) at c.838G>A in exon 8 and therefore carries both *JK*01* and *JK*02* alleles that encode Jk^a and Jk^b, respectively. However, the patient was found to be heterozygous for several additional SNVs: c.28G>A in exon 3; c.191G>A, c.226G>A, and c.303G>A in exon 4; and c.757T>C in exon 7. The patient's Jk(b–) phenotype can be explained by coinheritance of c.838A with c.191G>A, which defines null allele *JK*02N.09*. Coinheritance of SNVs c.28G>A and c.838G with rare SNV c.757C that is predicted to cause a non-conservative amino acid change (p.S253P) likely accounts for the complete serologic absence of Jk^a and the ability to form anti-Jk3 in this case. This finding would represent a new *JK*01* null allele. This evaluation illustrates the importance of genetic analysis in identifying the factors preventing a high-prevalence antigen from being expressed, particularly when discovered outside of an expected racial or ethnic group. ***Immunohematology* 2021;37:109–112. DOI: 10.21307/immunohematology-2021-015.**

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Alloimmunization to antigens within the Kidd blood group system is highly clinically relevant, potentially associated with hemolysis in the setting of incompatible red blood cell (RBC) transfusion and hemolytic disease of the fetus and newborn in the setting of pregnancy.[1,2] The *SLC14A1* gene encodes the Kidd antigens, of which Jk^a and Jk^b are antithetical and determined by a common single nucleotide variant (SNV) in exon 8. While anti-Jk^a and -Jk^b are the most common alloantibodies encountered within this system in clinical practice,[3] infrequently, patients will manifest anti-Jk3, reflecting alloimmunization against the high-prevalence Jk3 antigen.[2] From a patient/individual standpoint, although rare, such antibodies are most frequently encountered in those of Polynesian ancestry, but are also seen in other populations including those of Finnish ancestry, and are associated with a number of well-defined genetic variants in the *JK* alleles.[4] We report a case of a Jk_{null} phenotype with a novel SNV, associated with formation of anti-Jk3 in a patient of non-Polynesian descent.

Case Report

A 51-year-old G3P2 woman of self-reported Jamaican and Scottish (non-Polynesian) ancestry presented with abdominal pain and fullness for several months. Her symptoms were associated with weight loss, fever, and abdominal distension. Colonoscopy and barium enema showed an external stricture in the sigmoid colon and follow-up computed tomography scan showed a 17.0-cm complex, cystic pelvic mass likely of ovarian origin in the setting of elevated CA125 (454 U/mL, normal range 0–35 U/mL). A biopsy of this pelvic mass was sent to pathology for review and was consistent with widely metastatic adenocarcinoma of intestinal origin. The patient was not a candidate for surgery and was treated with neoadjuvant chemotherapy with paclitaxel/carboplatin and FOLFOX. During treatment, she experienced recurrent malignant ascites causing chronic pain and abdominal distension. The patient was scheduled for a palliative tunneled peritoneal drainage catheter placement by interventional radiology. A type and screen request was submitted to the blood bank pre-procedurally. The patient had reported only a single previous RBC transfusion given many years before presentation and her pregnancy history, as noted earlier.

Materials and Methods

Initial testing for Kidd antigens (Jk^a and Jk^b) was performed by serologic methods using monoclonal antibodies (Immucor, Norcross, GA). Antibody screening and identification were performed using gel (Ortho Diagnostics, Raritan, NJ) as well as solid-phase (Immucor) platforms. Direct antiglobulin test (DAT) was performed by the tube method using polyspecific antiglobulin reagent (Immucor). Genetic testing was carried out by PreciseType HEA Molecular BeadChip using BASIS 4G (Immucor BioArray, Warren, NJ) with subsequent *SLC14A1* gene analysis done via Sanger sequencing (American Red Cross National Molecular Laboratory, Philadelphia, PA, and GeneWiz, South Plainfield, NJ) and alignment to reference sequence NM_015865 using Sequencher software, v. 5.4.6 (GeneCodes, Ann Arbor, MI).

Results

As reflected in Table 1, the patient's plasma demonstrated panreactivity with antibody screening and identification reagent RBCs, with 2–4+ reactivity seen in all testing with a negative autocontrol and a negative DAT. This workup led to suspicion of an alloantibody to a high-prevalence antigen. All other clinically significant alloantibodies were excluded, and we speculate that the variability in reactivity is due to differential antigen density expression on donor panel RBCs; notably, homozygous Jk(a+b–) RBCs did appear to react more strongly than heterozygous Jk(a+b+) RBCs. Table 2 highlights the *SCL14A1* sequencing results correlating with a lack of expression of both Jk^a and Jk^b; thus, alloimmunization to the high-prevalence Jk3 was highly probable and ultimately confirmed by our reference laboratory. Notably, our reference laboratory also demonstrated reactivity consistent with anti-Jk^a in parallel. No evidence for anti-Jk^b was found.

Table 1. Initial immunohematologic results including antibody screen and serologic typings

Test type	Result
ABO and D typings	Group A, D+
Antibody detection	Positive (all three cells reactive)
Antibody identification panel	Positive (all cells reactive; 2–4+ agglutination)
Autocontrol	Negative
Direct antiglobulin test	Negative
Jk^a typing	Negative
Jk^b typing	Negative

Table 2. *SLC14A1* gene sequencing results

Exon	Result: nucleotides detected*	Interpretation: predicted amino acid
3	28G>A	V10M
4	191G>A 226G>A 303G>A	R64Q V76I Silent
5	No changes	No changes
6	No changes	No changes
7	757T>C	S253P
8	838G>A	D280N
9	No changes	No changes

*Only nucleotides different from consensus are listed.

Given our patient interview and her self-reporting of non-Polynesian ancestry, we further speculated that the patient may harbor a novel genetic variant giving rise to her Kidd phenotyping and alloimmunization. As such, testing of the *SLC14A1* gene was requested via our reference laboratory. These studies (Table 2) revealed that the patient was heterozygous for the c.838G>A SNV and therefore carries both *JK*01* and *JK*02* alleles. However, and notably, several other SNVs of importance were also discovered (Table 3). The allele carrying c.191A and c.838A represents *JK*02N.09,* a known null allele associated with the Jk(b–) phenotype. The other allele is novel, carrying a rare SNV in exon 7 (c.757T>C, rs371769347) and two additional SNVs (c.28A and c.226A) separately associated with weakened expression of Jk^a.[5] The c.757C has a reported allele frequency of 0.0004 and is predicted to encode a Jk antigen with a non-conservative amino acid change (p.S253P) that is likely responsible for the complete loss of Jk^a in this patient.[6,7]

Discussion

We report here a rare SNV in exon 7 of the *SLC14A1* gene, which we believe results in deleterious amino acid substitution and loss of Jk^a, coinherited with a known *JK*02* allele associated with a Jk^b null phenotype in a patient of non-Polynesian ancestry with anti-Jk3. The patient's self-reported Jamaican and Scottish background, as well as our initial evaluation and confirmation of the anti-Jk3, raised suspicion that she carried a rare variant and suggested cause to pursue genetic analysis in this individual.

Table 3. Summary of single nucleotide variants and their relationships to the alleles and phenotypes discussed

Phenotype	28G>A V10M	191G>A R64Q	226G>A V76I	303A V101V synonymous	588A>G P196P synonymous	757T>C S253P	838G>A D280N	ISBT/Source	Ethnicity/population
Jk(a+^{w})	28A							*JK*01W.03*	African American
Jk(a+^{w})			226A					*JK*01W.04*	African American
Jk(a+)	28A		226A					*JK*01W.11*	NA
Jk(a) NA	28A		226A					Wheeler AABB 2018	NA
Jk(a–b–)	28A		226A	303A	588G			*JK*01N.20*	African
Jk(a) NA	28A		226A	303A	588G			Keller AABB 2016	African American
Jk NA						757C		Dinardo 2020	Patients with sickle cell disease
Jk(a–b–)	28A		226A	303A		757C	838G>A	This report	Jamaican-Scottish
Jk(a–b–)		191A						*JK*02N.09*	African American, Bosnian

ISBT = International Society of Blood Transfusion; w = weak; NA = not available.

Genetic compound heterozygosity involving non-synonymous SNVs in the *SLC14A1* gene is known to affect antigen phenotype and has been reported to be associated with the Jk_{null} phenotype in patients expressing *JK*01* or *JK*02* alleles.[8] Though phasing of the SNVs cannot be definitively determined using Sanger sequencing and would require long-range polymerase chain reaction or cDNA analysis, based on the comparative commonality of the *JK*02N.09* allele[7] and the patient's Jk(b–) phenotype, this allele was presumed most likely in this patient, leaving the other SNVs for the other allele. Alleles carrying c.28A and c.838G or c.226A and c.838G are associated with *JK*01W.03* and *JK*01W.04,* respectively,[7] whereas the allele carrying c.28A, c.226A, and c.838G is associated with *JK*01W.11,* and the allele carrying c.28A, c.226A, c.303A, and c.588G is associated with *JK*01N.20*.[7] Our patient does not carry c.588G but was found to carry a rare SNV in exon 7 not previously reported with a Jk phenotype.[6] This SNV is predicted to cause a non-conservative amino acid change (p.S253P) and, combined with the other SNVs that are associated with antigen weakening, likely results in complete serologic absence of Jk^{a}. Unfortunately, adsorption/elution studies were not performed to definitively confirm. Nonetheless, with the co-inheritance of two distinct null alleles, this patient appeared to demonstrate a serologic Jk(a–b–) phenotype and formation of anti-Jk3, likely stimulated by previous transfusion or pregnancy.

In anticipation of possible RBC transfusion needs during surgery, we were able to allocate a select number of compatible, Jk3– RBC units via our regional donor center and the rare donor registry. The units were not needed for the planned surgery and were subsequently frozen near their expiration date after approval, should the patient require them at a later date. Given the patient's unique genetic findings, familial studies were recommended for her children to determine their Kidd antigen phenotype and genotype. In addition, genetic testing of the children could be used to deduce the phase of the multiple SNVs in the patient. As of this writing, extended familial analysis has not been performed.

In summary, we report a novel SNV in exon 7 of the *SLC14A1* gene found in a patient of non-Polynesian ancestry with a Jk_{null} phenotype and anti-Jk3 alloimmunization. This case illustrates the practical utility of genetic analysis in unveiling the basis of an antibody against a high-prevalence antigen, particularly when detected outside of an expected racial or ethnic group. Familial studies will also be useful to help predict needs for offspring, should RBC transfusion be needed at some point for those individuals.

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