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Another example of a KEL1 variant red cell phenotype due to a threonine to serine change a position 193 of Kell gp

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Abstract

Background—Healthy subjects whose red blood cells (RBCs) react variably with anti-KEL1, but strongly express other Kell blood group antigens have been described and called KEL1 variant. We identified a 53-year-old Caucasian blood donor whose RBCs were reactive with 3 monoclonal and 2 polyclonal anti-KEL1 and nonreactive with 2 monoclonal and 1 polyclonal anti-KEL1. The molecular basis of this phenotype was investigated.

Methods—Genomic leukocyte DNA was analyzed for *KEL**1/2 genotype by utilizing sequence specific primers (SSP) and PCR. In addition, the region of the *KEL**1/2 polymorphism at position 578 of *KEL* was analyzed by DNA sequencing.

Results—Genotyping of the donor with the KEL1 variant phenotype revealed that he was *KEL**2 homozygous. Sequencing revealed one typical *KEL**2 allele and a *KEL**2 allele with an adenosine (A) to thymidine (T) substitution at position 577 that predicted a threonine to serine change at position 193.

Conclusion—It is not known if this phenotype is clinically relevant, but for, at least, some genotyping applications probes that identify this polymorphism should be used and anti-KEL1 should be tested for reactivity to this allele.

Introduction

Approximately 30% of highly transfused patients develop antibodies to red blood cell (RBC) antigens in addition to anti-A and anti-B. One of the most immunogenic blood group antigens is KEL1 (K1, K, Kell). Antibodies to KEL1 can cause hemolytic transfusion reactions and hemolytic disease of the fetus and newborn.

An unusual KEL1 blood group antigen phenotype has been described, KEL1 variant.^{1,2} This antigen is detected by some, but not all, sera containing anti-KEL1. The expression of other Kell blood group system antigens is normal on variant RBCs,^{1,2} however, in subjects with *K*_{mod}, McLeod, and K:-13 phenotypes the expression of all Kell blood system antigens is reduced.^{3–6} In addition the K:3,4 phenotype has a cis modifying effect on KEL group antigens that are cis to K:3 (*Kp*^a).⁷

KEL1 and its antithetical antigen, KEL2, are located on the 93 kD single pass Kell glycoprotein (gp).^{8–11} The *KEL* gene codes for the Kell gp, is located at 7q33, and contains 19 exons.⁹

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¹¹ *KEL*1* and *KEL*2* differ at one nucleotide in exon 6 at position 578. ¹² *KEL*1* has a thymine (T) at position 578 and *KEL*2* a cytosine (C) which results in an amino acid change in the Kell gp at position 193. The *KEL1* form of the Kell gp has a methionine (Met) at amino acid 193 while the *KEL2* form has a threonine (Thr) at 193. ¹² This change affects the glycosylation of Kell gp. The *KEL2* form has an N-glycosylation site at asparagine (Asn) at position 191, but the *KEL1* form does not. The *KEL2* form of the Kell gp contains the N-glycosylation consensus sequence for Asn 191; Asn-Arg-Thr 193, but the *KEL1* contains Asn-Arg-Met 193 which is not an N-glycosylation consensus sequence.

The *KEL1* variant phenotype in three related subjects has been found to be due to an adenosine (A) to thymidine (T) substitution at position 577 that results in a threonine to serine change at amino acid 193 which was called *KEL*1-Ser193*. ¹³ A second single nucleotide polymorphism has been preliminarily reported to cause a *KEL1* variant phenotype. This single nucleotide substitution involves a C to guanine (G) change at position 578 that results in a threonine to arginine substitution at amino acid 193. ¹⁴ We identified a donor with a *K1* variant phenotype and analyzed the DNA sequence of *KEL* in the region of the *KEL*1/2* polymorphism to determine the molecular basis of the atypical phenotype. This donor was found to have *KEL*1-Ser193*.

Case Report

The RBCs from a 53-year-old male Caucasian blood donor were phenotyped upon his initial donation. His phenotype was D-C-E-c+, K:-1, Fy(a-b+), Jk(a+b-) S+s-. Upon subsequent donations, the donor was matched to a sickle cell patient who was being transfused with antigen matched RBCs in an attempt to prevent alloimmunization to RBC antigens. Prior to their transfusion, RBCs from each component were confirmed to be *KEL1* negative with a commercial reagent. At the time of the fifth donation the donor's RBCs were weakly reactive with an IgM human monoclonal anti-*KEL1* (Table 1).

Methods

DNA isolation

DNA was isolated from EDTA whole blood samples using spin columns (QIAamp DNA Blood Mini Kit, Qiagen, Valencia, CA) according to manufacturer's instructions and stored at 2°C to 6°C until use.

Serology

KEL1 Phenotyping was performed using commercial monoclonal anti-*KEL1* reagents (Immucor, Norcross, GA) at direct agglutination according to manufacturers' instructions. A murine monoclonal anti-*KEL1* reagent, MIMA-22 from the New York Blood Center (New York, NY) was used to type the donor RBCs. In addition, the donors RBCs were typed for *KEL1*, *KEL2*, *KEL3* and *KEL4* using commercial polyclonal source antisera (Ortho-Clinical Diagnostics, Inc, Raritan, NJ) and tested by the indirect antiglobulin test. In-house reagents were also used to test for *KEL1*, *KEL2*, *KEL3*, *KEL4* and Ge:3 by the polyethyleneglycol (PeG) indirect antiglobulin test and the indirect antiglobulin technique using the Ortho IgG gel card (Ortho-Clinical Diagnostics).

To screening blood donors for *KEL1* variant phenotype all donors with tested with an anti-*KEL1*, MIMA 22 (New York Blood Center, New York, NY), that reacted strongly with *KEL1* variant RBCs from our donor. RBCs that reacted with MIMA-22 were tested with a anti-*KEL1* that reacted weakly with our *KEL1* variant donors RBCs (Immucor 6M7522 and 7C7523).

RBCs that reacted strongly with MIMA-22 and weakly with Immuncor anti-KEL1 were considered to be KEL1 variant.

KEL *1 and KEL *2 Genotyping

Genotyping for *KEL**1 and *KEL**2 was performed using sequence specific primers (SSP) and the polymerase chain reaction (PCR) (KKD-Type, BAGene, Biologische Analysensystem GmbH, Lich, Germany). The PCR conditions included an initial denaturation step for 5 minutes at 96°C, followed by 5 cycles of 10 seconds at 96°C, and 60 seconds at 70°C. The next 10 cycles were 10 seconds at 96°C, 50 seconds at 65°C, and 45 seconds at 72°C. The final 15 cycles were 10 seconds at 96°C, 50 seconds at 61°C, and 45 seconds at 72°C, and were followed by a final extension step of 5 minutes at 72°C. The amplicons were analyzed by gel electrophoresis on a 2% analytical gel prepared with SeaKem GTG agarose (BMA, Rockland, ME) and 1× buffer (Cambrex Bio Sciences Rockland, Inc, Rockland, ME). The samples (10 µL) were ready-to-load following PCR and electrophoresed at a constant 100v for 75 min in a Gibco-BRL 11.14 Horizontal gel apparatus. The bromophenol blue dye front ran approximately 4 cm. To determine the size of the final amplicons 7 µL of Ready-to-Load 100bp Plus DNA ladder (Qiagen) was loaded in a separate lane.

Sequenced-based *KEL* *1/2 genotyping

KEL gene sequences from GenBank, accession number AY228336, were used to design universal forward (K1K2LPA) and reverse (K1K2RPA) primers (Table 2) for the primary amplification of a 743 bp product which spanned intron 4 through intron 6. The PCR reaction mixture had a total volume of 20µL and included genomic DNA (40–80 ng). AmpliTaq Gold DNA Polymerase (0.5 U) (Applied Biosystems, Foster City, CA), 4 pmole of each forward and reverse primer in Gene Amp PCR Gold Buffer (final concentration: 15 mM Tris-HCl pH=8.0, 50 mM KCl) (Applied Biosystems), 2.5 mM MgCl₂, and 200 µM dNTP (Applied Biosystems).

Conditions for amplification (GeneAmp PCR System 9700, Applied Biosystems) were 10 minutes of denaturation at 95°C followed by ten cycles of 15 seconds at 95°C, 20 seconds at 63°C, and 2 minutes at 72°C. The next 30 cycles were: 15 seconds at 95°C, 30 seconds at 58°C, and 2 minutes at 72°C. The PCR product was purified using ExoSAP-IT (Amersham Life Science) to remove excess dNTP and primers according to the supplier's standard protocol.

The sequencing reaction was performed using the standard protocol BigDye® Terminator v1.1 Sequencing Kit (Applied Biosystems), with a 1:4 dilution of reaction premix. Each template was sequenced in the forward and reverse orientation using forward and reverse sequencing primers indicated in Table 2 and the conditions were: 1 minute temperature at 96°C, followed by 35 cycles of 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C.

Excess dye terminators were removed from the sequencing products utilizing an ethanol precipitation method. The product was precipitated using NaOAc/EDTA buffer and absolute ethanol, followed by two 70% ethanol wash.

The reaction products were reconstituted with 10 µl of Hi-Di™ Formamide (Applied Biosystems) and were loaded into a capillary electrophoresis sequencer (ABI PRISM® 3730XL DNA Analyzer, Applied Biosystems) using POP7™ polymer (Applied Biosystems). Analysis of sequencing results was performed with the computer programs Sequencing Analysis v5.2 (Applied Biosystems) and MT Navigator PPC v.1.0.2b3 (Applied Biosystems) by comparing sequences to the standard reference sequence AY228336.x5–6.seq. The nucleotide numbers used were in reference to human Kell blood group protein mRNA.

Results

Serological Testing

The donors RBCs were initially phenotyped as KEL1 negative upon the first four donations, but discrepancies in the KEL1 typing were observed on the fifth donation. (Table 1). Notably, the lot number of the anti-KEL1 reagent used to test RBCs from the fifth donation was different than the two lots used to type the previous 4 donations. RBCs from the fifth donation were typed with 4 additional anti-KEL1 reagents and reacted weakly as compared to the positive control with 3 of the 4 anti-KEL1 reagents (Table 3). These results were confirmed when he donated a sixth unit of blood.

The donor's RBCs reacted strongly with an anti-KEL2 and anti-KEL4 IgG polyclonal commercial reagents ruling out the possibility of a McLeod phenotype.^{3,4} In addition, the donor RBCs were strongly reactive with a polyclonal anti-Ge3 in house reagent ruling out the possibility of a Ge:-2,-3 phenotype causing the depression of the KEL1 antigen.^{15,16}

This donor's RBC components were transfused on 3 separate occasions to the same sickle cell patient without incident. The patient was KEL2 homozygous and has not produced anti-KEL1.

KEL *1 and KEL *2 genotyping results

The *KEL*1* and *KEL*2* genotype of 23 blood donors was analyzed by SSP. In all donors the genotype was the same as the phenotype; 19 donors were *KEL*2* homozygous and 4 were *KEL*1*, *KEL*2*. Genotyping the donors with the K1 variant phenotype revealed that he was apparently *KEL*2* homozygous (Figure 1). These results suggest that this donor had a typical *KEL*2* allele and a *KEL* gene that was atypical near the area of the *KEL*1/2* polymorphism, position 578.

DNA Sequencing of *KEL*

Sequencing of *KEL* in the region of position 578 of 3 *KEL*2* homozygous and 3 *KEL*1/2* donors found that all 6 had the expected polymorphism at bp 578. Sequencing of the donor revealed one typical *KEL*2* allele and a *KEL*2* allele with an A to T substitution at position 577. This change predicts a Thr to Ser substitution at amino acid 193. This polymorphism has been described by Poole and colleagues and called *KEL*1-Ser193*.¹³

Screening Blood Donors for KEL1 Variant

300 blood donors were tested for KEL1 variant phenotype by testing with anti-KEL1 MIMA-22 which reacts strongly with KEL1 and the antigen encoded by *KEL*1-Ser193* in our donor and those identified by Poole and colleagues.¹³ RBCs reactive with anti KEL1 MIMA-22 were tested anti-KEL1 Immuncor which reacts strongly with KEL1 but only weakly or not at all with the antigen encoded by *KEL*1-Ser193*. Among the 300 donors 254 were Caucasian, 18 were African American, 13 Asian, 5 Hispanic, 2 Native American, and 8 were of mixed or unknown ethnicity. None of the donors expressed KEL1 variant antigen as demonstrated by reactivity with anti-KEL1 MIMA 22, but weak or no reactivity with anti-KEL1 Immuncor.

Discussion

A blood donor was identified whose RBC phenotyped as KEL1 positive with 4 monoclonal and 2 polyclonal anti-KEL1 and KEL1 negative with 2 monoclonal and 1 polyclonal anti-KEL1. Strong expression of KEL2 and KEL4 antigens indicated that the donor did not have a McLeod phenotype and strong expression of Ge3 indicated that he did not have depression of KEL1 due to the Ge:-2,-3 phenotype. DNA sequencing revealed that this person had a normal *KEL*2* gene and a *KEL*2* gene with an A to T substitution at position 577 nucleotide which

is adjacent to the *KEL**1/2 polymorphism. Although this change predicts a Thr to Ser substitution at amino acid 193 which is within the N-glycosylation consensus sequence, the Asn-Arg-Ser193 sequence would be expected to support N-glycosylation of Asn191 since both Asn-X-Thr and Asn-X-Ser signal the N-glycosylation of Asn. Poole and colleagues found *KEL**1-Ser193 in two related Swiss German blood donors and in a third unrelated Swiss German donor.¹³ Interestingly our donor's ancestors are German and Scottish Irish, but they have lived in the US for many generations.

Although the product of the gene *KEL**1-Ser193 is reactive with some anti-KEL1, it is more similar to *KEL**2 than *KEL**1. At position 578 it is the same as *KEL**2, and it is likely N-glycosylated site at position 191. RBCs from donors with *KEL**1-Ser193 reacts weakly with some anti-KEL1, however, since *KEL**1-Ser193 homozygous or *KEL**1/1-Ser193 donors have not been identified, it is not certain if this allele reacts with anti-KEL2. The analysis of RBCs from one donor heterozygous for *KEL**1-Ser193 and *KEL**2 by flow cytometry with anti-KEL2 indicate that this atypical allele reacted with anti-KEL2.¹³ These results suggest that RBCs from donors with *KEL**1-Ser193 should not be transfused to patients requiring KEL1 or KEL2 negative RBCs.

Prior to the realization that our donor's RBCs were reactive with some anti-KEL1 three RBC components from this donor were transfused to a *KEL**2 homozygous patient. Although the patient was not alloimmunized, this does not mean that this KEL1 variant is not immunogenic.

Neither the sequence specific primer genotyping method we, nor Poole and colleagues used identified the *KEL*1-Ser193 allele. These methods typed the allele as *KEL**2. Sequence specific probes that identify *KEL**1-Ser193 have been developed.¹³ Since *KEL**1-Ser193 RBCs may be immunogenic it maybe worthwhile to include probes that recognize this allele in *KEL**1 and *KEL**2 genotyping assays. It may not be necessary to include probes or primers that recognize *KEL**1-Ser193 for all applications, but if genotyping is used to select donors for patients who will require regular transfusions throughout their life including probes or primers that recognize *KEL**1-Ser193 in addition to *KEL**1 and *KEL**2 may be important. It may also be worthwhile for reagent manufacturers to characterize the reactivity of their KEL1 antibodies to *KEL**1-Ser193. If a donor with variable reactivity to KEL1 antibodies is identified and is confirmed by sequence based typing or other genotyping methods to have *KEL**1-Ser193, their RBC components could be excluded from transfusion to patients requiring antigen matched units.

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Figure 1. KEL*1/2 genotyping of the donor with the KEL1 variant RBC phenotype

A blood donor with KEL*1/2 phenotype (Panel A), KEL2 homozygous (Panel B) and KEL1 variant phenotype (Panel C) were genotyped using SSP. Results of testing with the *KEL*1* specific primers are shown in lane 1 and the *KEL*2* specific primer in lane 2. The *KEL*1* and *KEL*2* specific amplicons are 141 bp and the control amplicon 434 bp.

Table 1

Results of testing the donor's first 5 donations with anti-KEL1

Donation	Lot #/Manufacturer of anti-KEL1	Type of reagent	Result
1	Immucor 5A75171	IgM human monoclonal	Negative
2	Immucor 5A75171	IgM human monoclonal	Negative
3	Immucor 5H7518	IgM human monoclonal	Negative
4	Immucor 5H7518-1	IgM human monoclonal	Negative
5	Immucor 6A7519	IgM human monoclonal	Weak positive

Table 2 Primers used to amplify and sequence exon 6 of the *KEL* gene

Primer	Use	Sequence
K1K2LPA	PCR+ Sequencing	5' TTTAGTCCTCACTCCCATGCTTCC 3'
K1K2RPA	PCR+ Sequencing	5' TATCACACAGGTGTCCTCTCTTCC 3'
K1K2LIntA	Sequencing	5' CCCCTCTCTCTCCTTTAAAG 3'
K1K2IntB	Sequencing	5' CCCCTCTCTCTCCTTTAAAGCTT 3'
K1K2LNoint	Sequencing	5' CTTGGAGGCTGGCGCATCTCTGG 3'

PCR = polymerase chain reaction

Table 3

Results of typing for the donor's red blood cells for KEL1, KEL2, kpa, kpb, and Ge3

Antibody			RBC Donations	
Specificity	Source	Type	5	6
Anti-KEL1	Immucor 6A7519	IgM human monoclonal	1+	NT
Anti-KEL1	Immucor 6H7521	IgM human monoclonal	1+	1+w
Anti-KEL1	Immucor 5A7517-1	IgM human monoclonal	Negative	NT
Anti-KEL1	Ortho KC141D	IgG human polyclonal	1+w	weak micro.
Anti-KEL1	MIMA-22	IgG2 murine monoclonal	3+	3+
Anti-KEL1	Immucor 9N7511	IgM human monoclonal	NT	Negative
Anti-KEL1	Gamma KM25-1	IgM human monoclonal	NT	1+w
Anti-KEL1	In house 017T	IgG human polyclonal	NT	Negative
Anti-KEL1	In house 047T	IgG human polyclonal	NT	1+
Anti-KEL2	Immucor KC52A-1	IgG human polyclonal	3+	3+
Anti-Kpa	Immucor KPA49HJ-1	IgG human polyclonal	Negative	NT
Anti-Kpa	Gamma KPA5OH-1	IgG human polyclonal	NT	Negative
Anti-Kpb	Gamma KPB49D-1	IgG human polyclonal	NT	4+
Anti-Ge3	in house 622R	IgG human polyclonal	NT	4+

NT = not tested