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The Dutch Golden Age painter Gerard van Honthorst built his early reputation in Rome as a master of night scenes and artificial lighting. In Samson and Delilah (1621) as the temptress cuts the hero's hair herself, a maid shushes unseen soldiers. Remarkable is the chiaroscuro, which enhances the scene's serenity and dramatizes the balance of power between the biblical figures. Samson lies sleeping in darkness, symbolizing his fall from God's grace and his moral and imminent physical weakness. Delilah's face and bosom are fully illuminated by the candle flame, her expression not haughty but precise, even maternally gentle. This contrasts her duplicity with the virtue of true mother figures such as Mary but also implies that Samson's humbling occurs within the framework of God's intent and compassion. In this issue of Immunohematology St-Louis et al. discuss alloimmunization of D− patients by red cells with the DEL variant phenotype and exceedingly weak D expression.

David Moolten, MD
Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary-care facility

H.M. Smith, R.S. Shirey, S.K. Thoman, and J.B. Jackson

More than 50 red blood cell (RBC) alloantibodies are known to cause hemolytic disease of the fetus and newborn (HDFN). Although Rh immune globulin (RhIG) prophylaxis has significantly reduced the incidence of pregnancies complicated by anti-D, the need to detect and monitor maternal alloantibodies capable of causing HDFN is still a concern. The prevalence and specificity of these alloantibodies were determined. In this retrospective study, the prevalence and specificities of unexpected RBC alloantibodies known to cause HDFN in pregnant women at a tertiary-care facility during a 5-year period were compiled and analyzed. Patient selection was carried out by computerized search of patient data based on an obstetric location and the presence or history of RBC antibody between January 1, 2007, and December 31, 2011. The information was organized by ABO and D status of the patient, antibody specificity, and transfusion needs. Of the 8894 obstetric patients identified during the 5-year period, 264 (3.0%) had one or more unexpected RBC antibodies. Of these 264 women, 107 (40.5%), or 1.2 percent overall, had an alloantibody known to cause HDFN, with a total of 15 different alloantibodies identified. The most common alloantibody found was anti-E (n = 33), followed by anti-M (n = 26) and anti-D (n = 20). In pregnancies of D− women, the most common clinically significant antibodies found were anti-D (n = 20), anti-C (n = 11), and anti-E (n = 2). In pregnancies of D+ women, the most common antibodies were anti-E (n = 31), anti-M (n = 25), and anti-K (n = 16). A total of eight pregnancies with alloantibodies required intrauterine transfusions with the specificities of anti-D; anti-D−C (n = 2); anti-D−C−E; anti-D−C−K; anti-D−C−Jkα; anti-D−S; and anti-E−c. At a large academic tertiary-care center, approximately 1 in 83 obstetric patients had one or more RBC alloantibodies capable of causing HDFN. Anti-E, -M, and -D were the most frequent specificities, respectively. **Immunohematology 2013;29:127–130.**

**Key Words:** prevalence, red blood cell alloantibodies, pregnant women

Hemolytic disease of the fetus and newborn (HDFN) results from the destruction of fetal and newborn red blood cells (RBCs) targeted by maternal RBC alloantibodies that have the capability of crossing the placenta and entering the fetal circulation. Directed to inherited paternal RBC antigen(s), these antibodies are able to bind to the corresponding antigens, marking them for destruction by the fetal spleen, resulting in fetal distress. There are more than 50 RBC alloantibodies that have the capability of crossing the placenta and causing HDFN, with anti-D followed by anti-c and anti-K having the highest probability of causing severe HDFN.1,2

Although advances have been made in the past 50 years, including the implementation of Rh immune globulin (RhIG) to prevent anti-D HDFN in the 1960s, HDFN caused by anti-D as well as by non-D antibodies still remains a serious concern. Because women can be alloimmunized to RBC antigens through transfusion and pregnancy, it is still necessary to detect and monitor maternal alloantibodies that may put the fetus at risk for HDFN.3

The prevalence of alloantibodies in pregnancy has been investigated in multiple studies during the past few years in various countries, such as Sweden, Nigeria, Croatia, and the Netherlands.1,4–6 A compilation of similar data from the United States is limited. Although the frequency of alloantibodies at our institution would not necessarily reflect the prevalence in the United States, an evaluation of such data at a large hospital with high-risk obstetric patients would help to reiterate the importance of screening for and monitoring those pregnancies that present with antibodies that may put the fetus at risk for HDFN. Therefore, we determined the prevalence and specificity of unexpected maternal RBC alloantibodies associated with HDFN in a large obstetric population at our hospital. The associations between ABO group and D type and the presence of clinically significant RBC alloantibodies associated with HDFN were also determined.

**Materials and Methods**

Data collection and analysis were performed at the Johns Hopkins Hospital in Baltimore, Maryland, and included all women seen in the hospital’s obstetrics department between January 1, 2007, and December 31, 2011, who had an ABO and D type and antibody screen performed. Patient selection was carried out by computerized search of patient data based on the parameters of obstetric location and the presence of RBC alloantibody. Only female patients who had a record of,
or presented with, one or more RBC alloantibodies during pregnancy were included in the search. Each patient’s ABO and D type and any RBC transfusions given were then compiled from the blood bank database and analyzed. The identification of passive maternal alloantibody in the baby was detected by a positive direct or indirect antiglobulin test. For those babies with passive antibody of maternal origin, the occurrence of transfusion of antigen-negative units was determined.

The methods used to detect the antibodies included routine screening and antibody identification in accordance with AABB guidelines. A history of RhIG administration was verified by review of medical records at the time of presentation. All mothers with only anti-D as a result of RhIG were excluded from the study. Patients with demonstrable alloantibodies or a history of demonstrable alloantibodies were included. The antibodies were then evaluated and sorted according to their ability to cause HDFN as outlined in the AABB guidelines for prenatal and perinatal immunohematology.

The total number of antenatal cases was determined using the institution’s laboratory information system. All duplicate samples on the same patient and all cancelled and rejected specimens were removed from the total, and only specimens with completed ABO and D types and antibody screens were included. Approximately 9 percent of the women identified ended up delivering elsewhere, but they were included in the analysis.

The association between ABO type and D types and the presence of clinically significant RBC alloantibodies associated with HDFN was determined using $\chi^2$ statistics for proportion and frequency comparisons.

**Results**

Between January 1, 2007, and December 31, 2011, 8894 different obstetric patients were typed for ABO and D and screened for RBC alloantibodies. Approximately 91 percent of these women delivered at Johns Hopkins Hospital, but all 8,894 women were included in the analysis. During this same period, there were 9,734 deliveries at Johns Hopkins Hospital with approximately 20 percent being one or more repeat deliveries from the same woman.

The percentage of the 8894 women who had clinically significant alloantibodies by group O, A, B, and AB blood types were 1.1 percent, 1.4 percent, 1.2 percent, and 1.5 percent, respectively (Fig. 1). No significant association ($p = 0.64$) was found between the ABO group and the presence of clinically significant RBC alloantibodies associated with HDFN.

Of the 8894 obstetric patients screened, 8147 (91.6%) were D+ (Fig. 2) and 747 (8.4%) were D−. A total of 264 (3.0%) women demonstrated or had a history of one or more RBC antibodies at the time of pregnancy. Of the 264 patients with antibodies, 157 (59.5%) had antibodies typically considered clinically benign in regard to HDFN, and 107 (40.5%), or 1.2 percent overall, had at least one antibody known to cause HDFN. The most frequent antibody identified was anti-E ($n = 33$), followed by anti-M ($n = 26$) and anti-D ($n = 20$). A total of 145 antibodies, with 15 different specificities, that are known to cause HDFN were identified. Of the 107 patients with antibodies known to cause HDFN, 28 (26.2%) had multiple antibody specificities; a breakdown of those antibodies is shown in Table 1.
Of the 8147 D+ women, 82 (1.0%) had one or more alloantibodies capable of causing HDFN compared with 25 (3.3%) of the 747 D– women (p < 0.0001). Of the 82 D+ pregnant women with antibodies, anti-E (n = 31), anti-M (n = 25), and anti-K (n = 16) were the most frequent. Anti-D was seen in 20 of the 25 pregnancies in D– women with antibodies, followed by anti-C (n = 11) and anti-E (n = 2).

Of those obstetric cases identified in the study with antibodies known to cause HDFN, 14 women required RBC transfusions during their pregnancy or at delivery because of sickle cell disease (n = 5) or postpartum hemorrhage (n = 9). Eight women required one or more intrauterine transfusions (IUTs). The antibody specificities requiring an IUT were anti-D; anti-D,-C (n = 6); anti-D,-C,-E; anti-D,-C,-K; anti-D,-C,-Jk\textsuperscript{b}; anti-D,-S; and anti-E,-c. The most frequent antibody was anti-D, in seven of the eight pregnancies requiring an IUT.

Approximately 91 percent, or 7457, of the pregnant women in this study delivered at our facility. Of those infants born at our institution, 24, or 0.3 percent, were identified as having passive alloantibody of maternal origin. Anti-D (n = 17), with or without other antibody specificities, was the most common. Of those infants with passive alloantibody of maternal origin, five infants received transfusion of antigen-negative RBCs within 24 hours of birth. All five infants had passively acquired anti-D, with four of five infants demonstrating one to two additional alloantibodies (anti-S, anti-C, anti-K, or anti-Jk\textsuperscript{b}).

**Table 1.** Antibody specificities in pregnant women with clinically significant antibodies between January 1, 2007, and December 31, 2011.

<table>
<thead>
<tr>
<th>Antibody Specificity</th>
<th># of pregnancies in D+ women with antibody specificity</th>
<th># of pregnancies in D– women with antibody specificity</th>
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<tr>
<td>D</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>D+C</td>
<td>2</td>
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<td>D+C+E</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>D+C+K</td>
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<td>1</td>
</tr>
<tr>
<td>D+C+Jk\textsuperscript{b}</td>
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<td></td>
</tr>
<tr>
<td>D+S</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D+M</td>
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<td>1</td>
</tr>
<tr>
<td>C</td>
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</tr>
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<tr>
<td>C+e</td>
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<td></td>
</tr>
<tr>
<td>C+K</td>
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<td></td>
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<td>C+K+S</td>
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<td></td>
</tr>
<tr>
<td>C+S</td>
<td>1</td>
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</tr>
<tr>
<td>C+K+Fa+M+S</td>
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<td></td>
</tr>
<tr>
<td>E</td>
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<tr>
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</tr>
<tr>
<td>E+K+Kp\textsuperscript{a}</td>
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</tr>
<tr>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

This study revealed that of the 8894 pregnant women screened, the percentage of women who demonstrated or had a history of an RBC alloantibody at the time of pregnancy was 3.0 percent, with 1.2 percent of women having at least one alloantibody known to cause HDFN. The most frequent alloantibody found in patients with at least one antibody known to cause HDFN was anti-E (30.8%), followed by anti-M (24.3%) and anti-D (18.7%). In one study evaluating the prevalence of antibodies in Dutch women, anti-E was also found to be the most common antibody detected.\textsuperscript{7}

Nearly half (46.8%) of these women typed as blood group O, followed by 30.2 percent, 18.5 percent, and 4.5 percent...
typing as blood groups A, B, and AB, respectively, with 91.6 percent typing as D+. These percentages are similar to those in a black non-Hispanic population in the United States as reported by Garratty et al., in which 50.2 percent, 25.8 percent, 19.7 percent, and 4.3 percent typed as blood groups O, A, B, and AB respectively, with 92.9 percent typing as D+.

These findings at our institution are most likely attributable to the fact that approximately 62.5 percent of the patients who deliver at our hospital are African American and 4.9 percent are Asian American.

In our study, 1.0 percent of D+ women had one or more alloantibodies known to cause HDFN compared with 3.3 percent of D− women. The most common alloantibodies in D+ women with at least one antibody known to cause HDFN were anti-E (37.8%), anti-M (30.5%), and anti-K (19.5%); in D− women they were anti-D (80%), anti-C (44.0%), and anti-E (8%).

Five infants required transfusion of antigen-negative units within 24 hours of birth, and eight women required at least one IUT. Anti-D was implicated in seven of eight (87.5%) of the IUT cases, which is comparable to the 85 percent of IUT cases in which it was implicated in a previous study, although the numbers are small. Nearly all the cases requiring transfusion in which HDFN was implicated involved Rh antibodies and multiple antibody specificities, which complicated both the identification and monitoring of those antibodies and the providing of antigen-negative units.

Although rare, there have been reports of anti-M causing HDFN. For this reason, in this study all pregnancies with anti-M were considered to have the potential to cause HDFN. There were no cases in this study in which an IUT was required because of anti-M.

The most common alloantibody known to cause HDFN found in pregnancy in this study was anti-E, followed by anti-M and anti-D. Despite efforts to eliminate HDFN caused by anti-D, that alloantibody remains the third most common one found in pregnant women and the most common antibody found in D− pregnant women at our institution. The manner in which D alloimmunization occurred in these patients was beyond the scope of our study.

It is possible that the 1.2 percent prevalence of RBC alloantibody clinically significant for HDFN in our obstetrics population is higher than that found in the general obstetrics population in the United States. Given that Johns Hopkins Hospital is a tertiary-care center, it is likely that a proportion of obstetric patients were referred from other hospitals because they were known to be alloimmunized and thus at high risk, resulting in a higher prevalence. Also, the number of women and babies requiring transfusion in this report is likely an underestimate, as approximately 9 percent of the 8894 pregnant women screened delivered elsewhere. Nevertheless, these findings help to reinforce the importance of educating pregnant women and physicians concerning the risks of HDFN and the need to detect and monitor alloantibodies, not only anti-D but also other alloantibodies known to cause HDFN in pregnant women.

References


8. Garratty G, Glynn SA, McEntire R; Retrovirus Epidemiology Donor Study. ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United States. Transfusion 2004;44:703–6.


LAN (Langereis) was officially recognized by the International Society of Blood Transfusion in 2012 as being the 33rd human blood group system. It consists of one single high-prevalence antigen, Lan (LAN1). The ABCB6 protein is the carrier of the Lan blood group antigen. The ABCB6 gene (chromosome 2q36, 19 exons) encodes the ABCB6 polypeptide (ATP-binding cassette protein, subfamily B, member 6), known as a porphyrin transporter. The exceptional Lan– people do not express ABCB6 (Lan null phenotype), owing to several different molecular mechanisms affecting ABCB6: frameshift leading to a premature stop codon (deletion, insertion, or nonsense mutation of nucleotides); missense mutation; or intronic mutation responsible for RNA splicing defect. Despite the Lan antigen’s being reported to play a key role in erythropoiesis and detoxification of cells, Lan– people do not appear to demonstrate susceptibility to any disease or seemingly physiologic disorder. Anti-Lan has been described as having variable clinical significance, either for hemolytic transfusion reactions (none to severe) or hemolytic disease of the fetus and newborn (none to mild). Despite challenging conditions caused by the scarcity of Lan– donors worldwide, Lan– blood should ideally be given to patients with anti-Lan, especially those with a high-titer antibody. Immunohematology 2013;29:131–135.

Key Words: immunohematology, transfusion, red blood cells, rare blood type, Lan blood group, ABCB6, ABC transporter, ATP binding cassette

History

In 1962, van der Hart and collaborators described an antibody to a high-prevalence red blood cell (RBC) antigen responsible for a severe and acute hemolytic transfusion reaction.1 The corresponding antigen, Lan, was named for the index case, Mr. Langereis (Dutch origin), whose brother was also found to be Lan–. The antibody of the proband was described to be nonreactive with only one random donor in 4000 tested in the population of the Netherlands.

Many examples of anti-Lan in people with the rare Lan– phenotype were later reported.2–10 Two high-prevalence antigens, Gnα and So, described in 1969 and 1970, respectively, were subsequently shown to be the same as Lan.4,11–13

Terminology and Nomenclature

In 1990, the Working Party on Terminology for Red Cell Surface Antigens of the International Society of Blood Transfusion (ISBT) decided to place Lan in the 901 series of RBC antigens, with reference number 901.002.14 The 901 series corresponds to a family of RBC antigens with a prevalence greater than 90 percent in the general population, for which data about their genetic independence from other known blood group antigens and about their molecular basis are nonexistent.15

As a result of the discovery of its molecular basis in January 2012 by Helias and collaborators,16 LAN was officially moved in July 2012 from the 901 series to the novel 33rd blood group system, LAN, by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology (ISBT World Meeting, Cancun, Mexico).17 The LAN blood group system contains one single antigen to date, LAN1.

Genetics and Inheritance

Lan is a high-prevalence antigen in most populations (> 99.9%), and Lan– is considered a rare blood type worldwide.18,19 Prevalence of the Lan– type was estimated to be approximately 1 in 20,000 in Caucasians,18,20 1 in 50,000 in Japanese,6 and 1 in 1500 in black people from South Africa.8 Anti-Lan has also been described in two African Americans.3,10 The Lan– phenotype is inherited as a recessive character.

In France, 10 of the 29 Lan– people (35%) registered in the national rare blood database originate from the Maghreb area in North Africa (unpublished observations).

Molecular Basis of Lan

Helias and collaborators16 used a monoclonal anti-Lan available from a Japanese team, clone OSK43,21 to elucidate the molecular basis of Lan (of note, no commercial anti-Lan typing reagent is available on the market). A biochemical approach, combining an immunoprecipitation test and mass spectrometry analysis, showed that the ABCB6 transporter carries the Lan antigen.

The adenosine triphosphate (ATP)-binding cassette (ABC) superfamily represents the largest and most broadly expressed class of proteins in all kingdoms of life.22 ABC proteins are able to transport a variety of biologic compounds through ATP hydrolysis. ABCB6 (initially named MTABC3
for mammalian-mitochondrial ABC protein 3) is an ATP-binding cassette molecule, subfamily B, member 6, known as a porphyrin transporter, which was initially described to be located in the outer membrane of mitochondria.\textsuperscript{23,24} It was later reported that ABCB6 expression could be restricted to the Golgi apparatus,\textsuperscript{25} lysosomes, and plasma membranes.\textsuperscript{26} However, this appears controversial because ABCB6 was recently purified from mitochondrial membrane extracts.\textsuperscript{27} Until the discovery of the molecular basis of LAN, ABCB6 was quite surprisingly not reported to be present in the erythrocyte membrane.

The ABCB6 gene is located on chromosome 2q36. It spans approximately 9.2 kb of genomic DNA, consists of 19 exons (3021-bp cDNA; GenBank access number NM_005689; Entrez Gene ID 10058), and encodes an 842 multipass amino acid protein (80 kDa), ABCB6, with eight membrane-spanning α-helices.\textsuperscript{28} ABCB6 belongs to the so-called half-transporter family within ABC transporters; it contains one transmembrane domain (TMD) and one nucleotide-binding domain (NBD; Fig 1). The NBD domain binds and hydrolyzes ATP; it is located at the C-terminal end of the ABCB6 polypeptide and includes three characteristic motifs: an LSGGQ conserved signature sequence (specific to ABC transporters)\textsuperscript{29} flanked by Walker A and Walker B consensus motifs (common to many nucleic acid–dependent ATPases).\textsuperscript{30} The minimal functional molecular unit of ABCB6 has been suggested to be a homodimer.\textsuperscript{23,31} Because ABCB6 has been proposed to regulate heme synthesis by shuttling coproporphyrinogen III from the cytoplasm into the mitochondria, it is believed to play a crucial role in erythropoiesis.\textsuperscript{23,27,22} ABCB6 has also been reported to represent a protective mechanism against oxidative cellular stress.\textsuperscript{32} Finally, ABCB6 has been suggested to be involved in cell growth and proliferation by targeting the cell cycle.\textsuperscript{34}

The precise role of ABCB6 in the RBC membrane remains unclear. Because the plasma level of porphyrins was found to be very low in four Lan– individuals, ABCB6 is thought to export porphyrins out of RBCs, which may prevent their intracellular accumulation.\textsuperscript{16}

People with the rare Lan– phenotype are actually Lan null or ABCB6–/– (two nonfunctional ABCB6 alleles) and may therefore be considered as “human knockouts” for ABCB6. Lan– individuals demonstrate different inactivating molecular mechanisms of ABCB6 at homozygous or compound heterozygous states: frameshift leading to a premature stop codon (deletion, insertion, or nonsense mutation of nucleotides), missense mutation, or intronic mutation responsible for RNA splicing defect.

Multiple null alleles of ABCB6 have been reported to date, as shown in Table 1. In addition, a few altered alleles of ABCB6 were recently shown to encode a weak expression of Lan (Table 2).\textsuperscript{33} This may explain why a significant proportion of people who are only weakly positive for Lan may be mistyped as Lan– if a potent anti-Lan is not used.\textsuperscript{38}

The ISBT has proposed a numbering system for ABCB6 alleles encoding for null and altered phenotypes (see Web site Names for LAN Blood Group Alleles v2.0 130208 http://www.isbtweb.org/fileadmin/user_upload/WP_on_Red_Cell_Immunogenetics_and/LAN_Aleles_v2_0___130211.pdf). The reference allele encoding Lan is named ABCB6*01. As of today, ABCB6 null alleles are numbered from ABCB6*01N.01 to ABCB6*01N.15 (N for null) and altered alleles ABCB6*01W.01 to ABCB6*01W.04 (W for weak); for an update, see Web site http://www.isbtweb.org/workng-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/ from the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology.

ABCB6 mutations have been found in some rare genetic diseases, such as ocular coloboma,\textsuperscript{39} autosomal dominant familial pseudohyperkalemia,\textsuperscript{40} and dyschromatosis universalis hereditaria (so called “gain-of-function” mutations).\textsuperscript{41} However, all Lan– people reported to date were seemingly healthy, experiencing no clinical symptoms of porphyria or abnormal complete blood count.\textsuperscript{16} As a result, ABCB6 is known not to be a protein essential for life in humans, and it does not appear to be fully required for erythropoiesis.\textsuperscript{16} An alternative pathway for porphyrin transport very likely exists, probably through ABCG2, another ABC protein that recently has been shown to carry the JR blood group sytem antigens.\textsuperscript{42}

**Biochemistry and Physiology**

LAN shows a wide tissue distribution and has been reported to be highly expressed in fetal liver, heart, eye, and
skeletal muscle. Cord RBCs show a higher Lan reactivity than adult cells.

Lan is resistant to the treatment of RBCs by papain, ficin, trypsin, α-chymotrypsin, dithiothreitol (DTT) 200 mM, pronase, neuraminidase, and EDTA/glycine/acid.

Antibodies in the System and Clinical Significance

Anti-Lan may be stimulated by RBC transfusion or pregnancy. No naturally occurring anti-Lan has been reported to date. Lan alloantibodies are mostly a mix of immunoglobulin (Ig) G1 and IgG3, but IgG1 or IgG3 alone has also been described, as well as IgG2 and IgG4 components. Anti-Lan may fix complement.

One case of autoanti-Lan has been reported in a female patient with a mild autoimmune hemolytic anemia. Anti-Lan was reactive 3+ by indirect antiglobulin test. The direct antiglobulin test was weakly positive (1+), and anti-Lan could be eluted from autologous RBCs. The patient’s RBCs showed a significant weakening of Lan expression.

Anti-Lan has been described as having variable clinical significance. It may cause hemolytic transfusion reactions, ranging from none to severe, and hemolytic disease of the fetus and newborn, ranging from none to mild. The ability of anti-Lan to cause hemolytic transfusion reaction may be studied by in vitro functional assays, and many examples of anti-Lan were found to have the potential to destroy Lan+ RBCs in vivo. Anti-Lan has been described as having variable clinical significance.

Table 1. List of reported ABCB6 null alleles that encode the Lan– phenotype

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Location</th>
<th>Predicted protein change</th>
<th>Ethnic background</th>
<th>References</th>
<th>Allele number proposed by ISBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.197_198insG</td>
<td>Exon 1</td>
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<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.01</td>
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<td>p.Phe29del</td>
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<td>Saison et al.</td>
<td>ABCB6*01N.14</td>
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<td>p.Gln239 stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.02</td>
</tr>
<tr>
<td>c.953_956delGTGG</td>
<td>Exon 4</td>
<td>p.Gly318Ala fs stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.03</td>
</tr>
<tr>
<td>c.1236G&gt;A</td>
<td>Exon 6</td>
<td>p.Trp412 del</td>
<td>African</td>
<td>Reid et al.,</td>
<td>ABCB6*01N.11</td>
</tr>
<tr>
<td>c.1533_1543dupCGGTCCCTGCG</td>
<td>Exon 9</td>
<td>p.Leu515Pro fs stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.04</td>
</tr>
<tr>
<td>c.1558_1559insT</td>
<td>Exon 9</td>
<td>p.Val520Cys fs stop</td>
<td>Unknown</td>
<td>Reid et al.,</td>
<td>ABCB6*01N.12</td>
</tr>
<tr>
<td>c.1799_1710delAG</td>
<td>Exon 11</td>
<td>p.Glu570Gly fs stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.05</td>
</tr>
<tr>
<td>c.1690_1691delAT</td>
<td>Exon 11</td>
<td>p.Met564Val fs stop</td>
<td>Japanese</td>
<td>Helias et al.</td>
<td>ABCB6*01N.06</td>
</tr>
<tr>
<td>c.1867delinsAACAGGTGA</td>
<td>Exon 14</td>
<td>p.Gly623Asn fs stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.07</td>
</tr>
<tr>
<td>c.1942C&gt;T</td>
<td>Exon 14</td>
<td>p.Arg648 stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.08</td>
</tr>
<tr>
<td>c.1985_1986delTC</td>
<td>Exon 15</td>
<td>p.Leu662Pro fs stop</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.09</td>
</tr>
<tr>
<td>c.2256 + 2t&gt;g</td>
<td>Intron 16</td>
<td>RNA splicing defect</td>
<td>Caucasian</td>
<td>Helias et al.</td>
<td>ABCB6*01N.10</td>
</tr>
</tbody>
</table>

Table 2. List of reported ABCB6 alleles that encode the Lan weak phenotype

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Location</th>
<th>Predicted protein change</th>
<th>Ethnic background</th>
<th>References</th>
<th>Allele number proposed by ISBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.826C&gt;T</td>
<td>Exon 3</td>
<td>p.Arg276Trp</td>
<td>Caucasian</td>
<td>Reid et al.,</td>
<td>ABCB6*01W.01</td>
</tr>
<tr>
<td>c.1028G&gt;A</td>
<td>Exon 5</td>
<td>p.Arg343Gln</td>
<td>African</td>
<td>Reid et al.,</td>
<td>ABCB6*01W.02</td>
</tr>
<tr>
<td>c.1762G&gt;A</td>
<td>Exon 12</td>
<td>p.Gly588Ser</td>
<td>Caucasian</td>
<td>Reid et al.,</td>
<td>ABCB6*01W.03</td>
</tr>
<tr>
<td>c.2216G&gt;A</td>
<td>Exon 16</td>
<td>p.Arg739His</td>
<td>Hispanic</td>
<td>Reid et al.,</td>
<td>ABCB6*01W.04</td>
</tr>
</tbody>
</table>

Lan blood group system review
Summary and Perspectives

The ABCB6 protein recently has been shown to carry the Lan antigen. The new system LAN (Langereis), number 33 according to ISBT, currently contains one single antigen, Lan (LAN1), with a prevalence greater than 99.9 percent in most populations. Lan− (LAN:−1) people do not express ABCB6 (Lan null phenotype). LAN remains a difficult blood group system to investigate because no commercial anti-Lan is available and no routine molecular testing exists for Lan genotyping. Implementation of Lan typing in the currently available high-throughput genotyping devices will be quite challenging because of the high number of null alleles of ABCB6 to be simultaneously tested (15 reported to date). In addition, full sequencing of ABCB6 is costly and labor intensive (19 exons). The so-called next-generation sequencing platforms will likely be helpful to overcome this problem in the future. Finally, the LAN blood group system, as well as JR, turns out to be a typical and striking example of the quite unexpected close relationships between the immunohematology, pharmacology, and oncology fields.

References


Alloimmunization of patients by blood units harboring distinct DEL variants

M. St-Louis, A. Lebrun, M. Goldman, and M. Lavoie

The alloimmunization potential of many RHD variants is unknown, and it can be explored by lookback and traceback studies. Héma-Québec (HQ) investigated the RHD status of 3980 D− repeat blood donors. Thirteen were found to be RHD positive: 4 RHD*ψ, and 1 RHD*487delACAG, which show a D− phenotype; and 1 RHD*885T and 7 RHD*(93–94insT) causing a DEL phenotype when C antigen is present. Lookback studies were done to verify the alloimmunization potential of these eight DEL donors. Coincidentally, Canadian Blood Services (CBS) performed a traceback study by investigating the RHD status of donors after a D− recipient developed anti-D after transfusion of two D− red blood cell (RBC) units. Donor genotyping was done either manually (HQ) or using the Progenika Bloodchip platform (CBS). Donations were traced through computer records. Letters were sent to hospital blood bank physicians to verify the presence of anti-D in recipients and to donors to request repeat samples. A total of 118 RBC units were transfused, 82 to D− recipients. Anti-D was found in three patients transfused with RHD*(93–94insT) DEL red blood cells. One donor presenting the same DEL variant was involved in the traceback study. Even without strong evidence clearly demonstrating the alloimmunization potential of DEL variants, whenever HQ or CBS identifies a donor harboring a DEL phenotype, his or her D status will be changed from D− to D+ to protect against the potential alloimmunization risk. *IMMUNOHEMATOLOGY 2013;29:136–140.

Key Words: DEL, RHD variant, alloimmunization, transfusion reaction

RH blood group D antigen is the second most immunogenic, after ABO antigens. Antibodies to D are considered clinically significant because they have been associated with hemolytic transfusion reactions and hemolytic disease of the newborn.1–3 The D antigen expression is polymorphic and varies among populations. In whites, the D− phenotype is observed in 15 to 17 percent compared with 5 percent in black Africans and less than 3 percent in Asians. Many variants accounting for the D− phenotype have been observed and they vary among populations.1,4

The D antigen's polymorphic nature is reflected by a wide range of partial or weak antigen expression, which led to the classification of variant D antigens: partial and weak. Partial D−expressing individuals could be alloimmunized when transfused with normal D+ red blood cells (RBCs), because some D epitopes are absent from their RBC membrane. On the other hand, weak D antigens are thought to cause less alloimmunization because the polymorphisms are located within transmembrane domains or intracellular regions, although these changes might alter the overall protein structure.5,6

A weak Ds subcategory consists of extremely weak RHD variants, termed DEL. A small amount of anti-D can be eluted from DEL RBCs after incubation with anti-D, although there is no agglutination by indirect antiglobulin test (IAT).7 Normal D individuals might have as many as 20,000 sites per erythrocyte,8 whereas DEL individuals have between 20 and 40.8 DEL individuals are mainly found in the Far East (10–30% in China and Japan), although DEL phenotypes resulting from different RHD variants have been observed in whites. These variants are caused by RHD missense mutations, splice site mutations, or deletion of RHD exon(s).8–19

It is well established that some weak Ds and most DEL units are mistyped as D− by routine automated serologic methods used to type blood donors, posing a potential risk of anti-D immunization when transfused to D− recipients.1,4,7,14,17,20 To address this potential risk, blood centers may implement testing of every apparently D− donor. This can be accomplished by adapting the adsorption-elution technique used to detect the DEL phenotype to a higher throughput setting or by using molecular testing.14,20,21

A Vox Sanguinis International Forum published in 2006 summarized the practices of several blood centers regarding the use of molecular testing to detect RHD variants missed by IAT.22 The majority of centers did not routinely use molecular typing, but would consider it for C+ or E+ donors, implying that all D− donors would have to be typed for C and E before molecular analysis. Molecular typing was also considered when a discrepancy was observed between monoclonal reagents on serologic testing.

As for demonstrating the risk of alloimmunization, very few studies have been reported to date.4,7,15,22 Yasuda and collaborators published a clear case of secondary immunization after transfusion of DEL RBCs.7 Shao’s and Kim’s groups published alloimmunization cases involving East Asians.4,16

In one of the cases, the recipient showed an anti-D after only 9
days. Other studies have focused on D immunization in weak D types.\textsuperscript{15}

Alloimmunization potential can be identified by two different means: lookback and traceback studies. Lookbacks start by molecular typing of donors and subsequently investigate whether D– recipients are alloimmunized after a transfusion involving D variant donors. On the other hand, traceback studies are initiated when a D– recipient develops an anti-D after an apparent D– transfusion. Identification of the donors and molecular typing are performed.

In 2007, Héma-Québec (HQ) launched a large genotyping project for repeat whole blood donors.\textsuperscript{23–25} Four $RHD$ variants were found among the D– donors: $RHD^{*(93–94insT)}, RHD^{*\psi}, RHD^{*(487delACAG)}$, and $RHD^{*885T}$. $RHD^{*\psi}$ and $RHD^{*(487delACAG)}$ alleles have always been associated with a D– phenotype. The other two alleles, $RHD^{*(93–94insT)}$ and $RHD^{*885T}$, are considered DEL in the presence of the C antigen. Nothing is known about their alloimmunogenicity.

Interestingly, the most frequent DEL phenotype found to date in Canadian blood donors is the result of variant allele $RHD^{*(93–94insT)}$ (7 of 3980 D– donors, 0.18%). All donors with this allele are likely of French Canadian ancestry. This allele was first reported in 2006 in one German donor,\textsuperscript{26} and examples reported since then include three in Spain,\textsuperscript{16} two in Germany,\textsuperscript{17} and one in Denmark.\textsuperscript{19} However, there is no information on the clinical importance of the allele in terms of alloimmunization potential.

The additional T in $RHD^{*(93–94insT)}$ causes a frameshift mutation that leads to a stop codon at amino acid position 35. Such mutation early in a protein would be predicted to completely suppress antigen expression, but replication slippage may explain traces of functional protein.\textsuperscript{17}

Preventing alloimmunization is a daily challenge for blood banks. However, little is known about the antigen density sufficient to cause an anti-D alloimmunization. Many D– recipients transfused with weak D type 1 and 2 were alloimmunized.\textsuperscript{27–29} The density of these weak Ds has been estimated to be between 400 and 1200.\textsuperscript{28,30} Gassner and colleagues published an alloimmunization case involving a weak D type 26 with an antigen density of 29 to 70.\textsuperscript{15}

To evaluate the risk of alloimmunization from the eight DEL blood donors described earlier, HQ undertook a lookback study going back to 2000. Coincidently, a traceback study was performed at Canadian Blood Services (CBS) to investigate anti-D alloimmunization in an elderly female recipient, reported by a hospital blood bank, involving two D– donors.

Materials and Methods

Donor Testing

Genotyping was originally done using the SNPstream Genotyping System (Beckman-Coulter, Fullerton, CA) and is described in detail elsewhere.\textsuperscript{23} Donor serologic typing was initially done using the Olympus PK7200 (Beckman-Coulter, anti-D P3X61+P3X2123B10+P3X290+P3X35 and PK2, Diagast, Loos, France). The D– status was confirmed using standard serologic methods (e.g., IAT). The DEL status of the eight HQ suspected DEL donors was confirmed by adsorption-elution, following manufacturer’s instructions (Elukit Plus, Dominion Biologicals Limited, Dartmouth, Nova Scotia, Canada; anti-D D175–2+D415 1E4, Novaclone, Dominion Biologicals Limited).

Lookback Study

For D– recipients, hospital blood banks were asked to verify the presence of anti-D, and if it was identified before the transfusion of interest. Finally, they were asked whether any other blood products were transfused during the same period. Standard serology methods were used to identify anti-D.

Traceback Case

An 88-year-old woman with no previous transfusions and one pregnancy had a negative antibody screen on her admission at the hospital with an abdominal aortic aneurysm. Four weeks after she received two O, D– RBC units, her antibody screen tested positive and anti-D was identified. Both transfused units typed D– C+E-c+. An investigation was initiated including extended serologic D antigen testing by IAT using reagents from three different manufacturers (Novaclone; Gamma-clone, Immucor, Norcross, GA; and Bioclone, Ortho Diagnostic Systems, Markham, Ontario, Canada; using ALBAclone Advanced Partial RhD Typing kit, Penicuik, UK), and adsorption-elution (Elukit Plus, Dominion Biologicals Limited). $RHD$ genotyping was performed by Progenika using the BLOODCHIP system (Novartis, Cambridge, MA).

Results

Lookback Study

Blood donations made by HQ’s eight DEL donors were traced through Progesa (Mak-System, Paris, France) as far as year 2000. Hospital blood banks that received the RBC units were notified in writing of the unusual D status of these donors. They were asked whether or not the RBC units had been transfused, and if so, to D+ or D– recipients.
Letters were sent to 27 hospitals that received the 171 traced RBC units. Information was available for 118 units that were transfused to 36 D+ recipients and 82 D– recipients. No further follow-up was pursued for the D+ recipients.

Antibody screening done several weeks after transfusion showed anti-D in three patients transfused with three units from different donors bearing the same RHD*(93–94insT) variant (Table 1). Recipients with anti-D also received other blood products during the same transfusion episode (Table 2). These patients were hospitalized for surgery (abdominal aortic aneurysm [n = 2] and cardiac surgery in a case of Marfan syndrome). Some of these products were platelets from D+ donors. None of the blood donors had anti-D, and we are not aware of their transfusion or pregnancy history.

Little information is available on the alloimmunization potential of RHD*11 RBC units in a CDe haplotype. This donor gave 11 units. We have information for seven of these units. Two were transfused to D– patients. The first patient had no anti-D, and the second was lost to follow-up.

Traceback Case

Of the two donors involved in this possible alloimmunization event, one was confirmed D– by serologic methods and by genotyping. The other typed D– by IAT and the ALBA kit, but was determined serologically to be DEL by adsorption-elution. His genotype was found to be RHD*(93–94insT). This donor lives in Nova Scotia (Canada) and is of Acadian descent, 17th-century French colonists in Canada. The lookback showed that three other RBC units from earlier donations were transfused. Two recipients remained antibody negative, and one was lost to follow-up.

Table 1. Red blood cell units from the three RHD*(93–94insT) donors possibly involved in antigen D alloimmunization

<table>
<thead>
<tr>
<th>Donor</th>
<th>Total RBC units delivered to hospitals</th>
<th>Available information for transfused units</th>
<th>Units transfused to D– recipients</th>
<th>Number of recipients with anti-D</th>
<th>Number of recipients without anti-D</th>
<th>Recipients lost to follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>23</td>
<td>19</td>
<td>1</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>21</td>
<td>14</td>
<td>1</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>21</td>
<td>14</td>
<td>1</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>65</td>
<td>47</td>
<td>3</td>
<td>28</td>
<td>16</td>
</tr>
</tbody>
</table>

RBC = red blood cell.

Table 2. Alloimmunized recipients and blood products received

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Sex/Age</th>
<th>Pregnancy</th>
<th>RHD*(93–94insT)</th>
<th>Whole blood platelets</th>
<th>Apheresis platelets (year)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F/87</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>1 D+ (2006)</td>
</tr>
<tr>
<td>B</td>
<td>F/88</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>1 D+ (2004)</td>
</tr>
<tr>
<td>C</td>
<td>F/44</td>
<td>Unknown</td>
<td>1</td>
<td>6</td>
<td>1 D+ (2006)</td>
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<tr>
<td>CBS case</td>
<td>F/88</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The apheresis process was modified at Héma-Québec in 2006. Before 2006, the Spectra system was used. After 2006, the Trima Accel apparatus (Terumo BCT) was implemented.

Recipients, Sex/Age, Pregnancy, RHD*(93–94insT): Whole blood platelets, Apheresis platelets (year)*: RBCs = red blood cells, CBS = Canadian Blood Services.

Discussion

This study demonstrates the difficulties inherent in determining immunization potential in clinical settings. Investigators in the four cases reported could not completely determine the definitive cause of the observed alloimmunization because DEL RBCs were involved as well as apheresis platelets from D+ donors and a possible D+ pregnancy. At the very least, this DEL variant appears able to trigger a secondary immune response. Additionally, the frequency of alloimmunization appears higher in a traceback study, which starts with a potentially alloimmunized patient, compared with a lookback study, which starts with genotyped donors. As a precaution, HQ and CBS have adopted the policy that all known DEL donors will be considered as D+.

From this study, we might speculate about the potential risk of DEL to immunize D– individuals. Even with a density of 20, a DEL RBC unit might contain on average $4 \times 10^{13}$ D antigens, equivalent to 200 to 400 µL of normal D+ blood. Ogasawara et al. observed that anti-D– sensitized DEL erythrocytes were not phagocytosed in a monocyte phagocytosis assay, suggesting a low alloimmunization risk.

Studies have shown that 200 mL of D+ blood could alloimmunize D– healthy individuals when booster doses were injected (500–1000 µL) after a 6-month rest period. The first three alloimmunization cases described in this work were also transfused with apheresis platelets. A study done at HQ showed an equivalent of 3 µL or $1.8 \times 10^7$ residual RBCs in apheresis platelets prepared on the Trima apparatus (Dr. Louis Thibault, personal communication). Recipient B received platelets before 2006; these platelets were prepared with the Spectra system. They might have contained more residual RBCs, but data are not available for our center.
As for the CBS’ case, the antibody screen was positive 4 weeks after transfusion. The earliest antibody response reported by Frohn and collaborators was 14 days$^{33}$ and for Wagner et al., it might be as fast as 11 days.$^{8}$ This patient gave birth to one child more than 40 years previously. The child’s D status is unknown to us; therefore, we are unsure whether this case represents primary alloimmunization or an anamnestic response.

Even without strong evidence clearly demonstrating the alloimmunization potential of DEL variants, whenever a donor harboring a DEL phenotype is identified, HQ and CBS practices will be to change the donor’s D status from D– to D+ to prevent the potential alloimmunization risk.

**Acknowledgments**

The authors would like to acknowledge the hospital’s transfusion services’ personnel for providing the information concerning the transfusions and for performing the antibody screen. We are indebted to the blood donors and the recipients for their participation in this study.

**References**

12. Shao C-P, Xiong W, Zhou Y-Y. Multiple isoforms excluding normal RhD mRNA detected in Rh blood group D\textsubscript{a} phenotype with RHD 1227A allele. Transfus Apher Sci 2006;34:145–52.


The GIL blood group system was added to the list of systems already recognized by the International Society for Blood Transfusion in 2002. It was designated as system 29 after the antigen was located on the aquaglyceroporin 3 (AQP3) protein and the gene encoding the protein was identified in 2002. There is only one antigen in the system, GIL, and the antigen, as well as the system, was named after the antigen-negative proband identified in the United States who had made anti-GIL. It was later shown to be the same as an unidentified high-incidence antigen lacking from the red blood cells of a French woman. Coincidentally all the antibodies found have been produced as a result of pregnancy. While there has not been a direct link to a disease, the absence of the AQP3 protein may result in a worse than expected rate of survival of patients with bladder cancer as compared with patients with the same disease who express the protein. Future work may center on using GIL as a marker for AQP3 and involving it in targeted cancer therapies. *Immunohematology* 2013;29:141–144

**Key Words:** aquaglyceroporin 3 (AQP3), water channel, high-incidence antigen, GIL antigen, GIL blood group system

### History

The GIL blood group was designated as system 29 by the International Society for Blood Transfusion (ISBT) Committee on Terminology for Red Cell Surface Antigens (current name: Working Party for Red Cell Immunogenetics and Blood Group Terminology) after its meeting in Vancouver, Canada, in August 2002 (Table 1). GIL takes its name from the first proband, the American reported by Frederick et al. in 1981. Thanks to an exchange of cells and sera between Daniels, working in England, and the French group, a sample of cells and sera from a French woman (Boi) collected in 1979 was found to be compatible with the cells and sera from the American proband, Gill. The French woman, born in 1925, had 10 pregnancies before her antibody was detected. Gill was born in 1956, and her antibody was detected after her first child was delivered in 1979. GIL had not met the ISBT requirements to be recognized as a system as the antigen, or the antigen-negative phenotype, had not been shown to be inherited, until the antigen was located on aquaglyceroporin 3 (AQP3) by Roudier et al. in 2002.

### Serology

#### GIL

GIL is the only antigen in the GIL system and has an incidence of almost 100 percent in French, German, and American populations. Daniels et al. used examples of anti-GIL to determine the effect of enzyme and chemical treatment on the antigen. The expression of GIL is not affected by treatment with dithiothreitol. However, GIL was enhanced by treatment with papain, ficin, α-chymotrypsin, and trypsin. Expression of the antigen appears slightly weaker on cord cells when compared with reactivity with red blood cells (RBCs) from adults.

GIL has not been found on platelets, but it is expressed on tissue from the kidney medulla and cortex, basolateral membrane of collecting duct cells, small intestine, stomach, colon, spleen, skin, airways, and eye.

#### Anti-GIL

Anti-GIL did not react by the direct agglutination method but did react by the indirect antiglobulin test. The serum from the French proband (Boi) was obtained after a hemolytic transfusion reaction. Using a two-stage complement method, this antibody was found to bind complement, although the antibody from the American proband (Gill) did not. Anti-GIL was not inhibited by plasma, saliva, hydatid cyst fluid, Lewis substance, milk, urine, or pigeon protein.

Three samples of the first five GIL antibodies were available in sufficient quantity for complement fixation testing. Only the antibody from the French proband fixed complement when tested using a two-stage method. As this sample was then exhausted, immunoglobulin subclassing and monocyte monolayer assays (MMA) were performed on only two of the anti-GIL samples (Gill and Hun). The antibody from the American proband tested as IgG1. The antibody from the third sample (Hun) was found to be IgG1 plus IgG2. All testing was carried out by methods previously described. Both samples

<table>
<thead>
<tr>
<th>ISBT Name</th>
<th>System</th>
<th>Antigen</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>GIL</td>
<td>AQP3 (GIL if inferred by hemagglutination)</td>
<td></td>
</tr>
<tr>
<td>Symbol</td>
<td>GIL</td>
<td>GIL1</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>029</td>
<td>029001 (or 29.1)</td>
<td></td>
</tr>
</tbody>
</table>
of anti-GIL appeared clinically significant with MMA results of 22 percent and 23 percent with one sample and 32 percent and 27 percent with the other. The MMA normal range is 0 to 3 percent.\(^5\)

Before the genetic basis of GIL was elucidated, immunoblotting was carried out using an eluate prepared with the Gill antibody. No bands were detected under reducing or nonreducing conditions.\(^5\) Because neither the genetic basis nor the pattern of inheritance could be provided, initially no ISBT number was assigned.

**Genetics**

The \textit{AQP3} gene (NCBI accession \# NM_004925) is composed of six exons distributed over more than 7 kb of DNA located on human chromosome 9p13 (Fig. 1A).\(^8\) The gene encodes a multipass transmembrane protein of 292 amino acids that spans the cell membrane six times with amino and carboxyl terminals located in the cytoplasm (Fig. 1B).\(^3\) The protein has an N-glycosylation site at Asn141 located in the third extracellular loop and exists as dimers, trimers, and tetramers.\(^9\) Roudier et al.\(^3\) used transcript analysis to investigate \textit{AQP3} expression in the GIL– probands. They demonstrated an abnormal transcript, missing exon 5, that they termed \textit{AQP3} (delta5). DNA sequence analysis showed a single-nucleotide polymorphism (SNP) in a splice site (IVS5+1g>a) that would result in exon skipping. The aberrant mRNA is predicted to encode a truncated protein lacking amino acids 165 to 237 and thus have a unique C-terminus as a result of a frameshift.\(^3\)

The IVS5+1g>a polymorphism destroys the recognition site for the restriction enzyme \textit{PmlI}, such that a polymerase chain reaction restriction fragment length polymorphism assay can be used to screen DNA for the presence of the variant. A sibling and the ten children of the French proband were found to be heterozygous for the IVS5+1g>a SNP.

No \textit{AQP3} was detected in RBC membranes from the probands, supporting the characterization of this allele as a null allele. Sera from the American and French probands reacted specifically with COS-7 cells transiently expressing full-length \textit{AQP3} cDNA, demonstrating that the \textit{AQP3} gene encodes the GIL antigen. The normal \textit{AQP3} gene that encodes the GIL antigen has been assigned \textit{GIL*01} as the allele name by the ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology, the IVS5+1g>a allele is assigned \textit{GIL*01N.01}.\(^4\)

**Biochemistry**

Aquaporins are integral membrane proteins that facilitate the transport of water and other solutes across the cell membrane. \textit{AQP3} is one of two AQP proteins that have been found on human RBCs. Whereas \textit{AQP1} is responsible for the high permeability of the RBC membrane to water, \textit{AQP3} allows permeability to glycerol and, to a lesser extent, urea. \textit{AQP1} has been shown to express Colton blood group antigens.\(^10,11\) Roudier et al.\(^3\) hypothesized that \textit{AQP3} also could be encoded by a blood group gene, and their work elucidated the molecular basis of GIL.

By collecting RBC samples from 24 individuals who lacked a high-incidence antigen and had made the corresponding antibody, and testing them by immunoblotting using anti-\textit{rAQP3}, only the American and French GIL– probands were nonreactive. RBC membranes from both probands lacked both the glycosylated and deglycosylated forms of \textit{AQP3} protein that were visualized on the control cell membranes.\(^3\)

The absence of the \textit{AQP3} protein on the two samples of GIL– RBCs was supported by the lack of glycerol permeability shown by both samples. This was determined by stopped-flow analysis. Interestingly, the controls (GIL+ RBCs) could have their glycerol transport inhibited to the same level shown by the probands lacking \textit{AQP3} by the addition of CuCl\(_2\). This implies that there is no alternative channel for glycerol permeability in the RBC membrane.\(^3\)
Incidence and Relationship to Disease

The incidence of GIL is almost 100 percent in the German, French, and American populations. The screening of 26,391 American donors included 23,499 white donors, 2841 black donors, and 101 Asian donors. All of these were GIL+. Combined with the understanding that AQP3 is a very common protein on RBCs, could there be polymorphisms within a specific race that would be associated with a specific disease?

Bahamontes-Rosa et al. identified five SNPs in the AQP3 gene in a group of African children affected by malaria. The three SNPs located in the coding regions were synonymous, having no impact on the amino acid composition of the protein. The two SNPs located in the promoter region were examined for their impact on AQP3 expression; none was found.

Testing of mRNA content in patients with severe malarial anemia or uncomplicated malaria did show a significantly lower amount of AQP3 mRNA in the anemic patients when compared with either the uncomplicated malaria group or the control group. The difference in AQP3 expression in the severely anemic group could be accounted for by the use of quinine in their treatment and the abnormal erythropoiesis with inadequate reticulocyte response. Therefore, unlike the mouse model in which mice deficient for AQP9 are partially protected from infection with Plasmodium berghei, AQP3 deficiency in the black population is not likely to confer even partial protection from malaria.

More recently, AQP3 has been investigated for its role in cancer. Loss of AQP3 expression was associated with a worse progression-free survival in urothelial bladder cancer. Evidence suggests that AQP3 participates in the cytotoxic response to nucleoside analogs, such as gemcitabine, used as chemotherapeutic agents, as well as arsenite. AQP3 expression levels may be of diagnostic utility in some cancers shown to be linked to colorectal, gastric carcinoma, and breast cancer cell migration. Demonstration of the AQP3-specific inhibitor CuSO₄ in cell culture models suggests that AQP3 may be a target of cancer therapies.

Summary

From the original antibody found compatible with the second proband in 1979 to acceptance by the ISBT as a unique blood group system in 2002, it took 23 years for the GIL system to be given its own place among the other unique blood group systems. The assignment of the GIL antigen to AQP3 was based on a gene-centric approach in which Roudier et al. sequenced the coding regions of the AQP3 gene in probands lacking the GIL antigen, hypothesizing that AQP3 could harbor a blood group antigen as was the case with AQP1 and the Colton blood group system. The GIL system has one antigen. The null phenotype is rare, being found in French and American (one of German ancestry) probands. The exchange of antisera and cells among reference laboratories was critical to the completion of the work to establish GIL as a blood group system. Collaboration among reference laboratories is invaluable and must continue so that we may continue to explore new relationships between serologic and molecular test findings. Molecular testing has opened the door to confirming suspicions long held but never proven, and it is likely that the cloning of genes will elucidate so much more information about the function, structure, and relationship to disease of all blood group systems.

References


A novel JK null allele associated with typing discrepancies among African Americans


The JKnull (Jk-3) phenotype, attributable to null or silenced alleles, has predominantly been found in persons of Polynesian descent. With the increased use of molecular genotyping, many new silencing mutations have been identified in persons of other ethnic backgrounds. To date, only two JK null alleles have been reported in African Americans, JK*01N.04 and JK*01N.05. A comparative study was undertaken to determine whether JK mutations were present in the regional African American population. Results of donor genotyping were compared with previously recorded results of serologic tests, and discrepant results were investigated. Although the two previously identified polymorphisms were not detected in the discrepant samples, a novel allele (191G>A) was identified and was assigned the ISBT number JK*02N.09. This study illustrates a limitation of using single-nucleotide polymorphisms for prediction of blood group antigens. *Immunohematology* 2013;29:145–148.

Key Words: blood group, null allele, Jk antigens, Jk:a–b–

The Kidd blood group system was first described in 1951 when anti-Jka was found to cause hemolytic disease of the fetus and newborn (HDFN). Anti-Jkb was reported in 1953, also as a case of HDFN, and in 1959, anti-Jk3 was described in a posttransfusion patient whose red blood cells (RBCs) typed Jk(a–b–). Since this time, many examples of anti-Jka and anti-Jkb have been reported. Owing 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 caused by anti-Jka and anti-Jkb remain among those most reported to the U.S. Food and Drug Administration.

The Kidd glycoprotein is found on RBCs as well as on endothelial cells of the vasa recta, the vascular supply of the renal medulla. It is an integral protein of the RBC, spanning the membrane ten times. The glycoprotein functions as a urea transporter for the cells and helps maintain osmotic stability. Individuals lacking the glycoprotein are known to have decreased urine concentrating ability, but they have no known disease associations or RBC abnormalities. The Kidd glycoprotein consists of 389 amino acids and is encoded by the SLC14A1 gene located on chromosome 18. Eleven exons are distributed across 30 kb of DNA. As exons 1 through 3 are not translated, the mature protein is encoded by exons 4 through 11. The SLC14A1 gene has two major codominant alleles, JK*A (JK*01) and JK*B (JK*02), which result from a single-nucleotide polymorphism (SNP), 838A>G (rs1058396).

To date, 14 polymorphisms causing JKnull phenotypes have been reported: 6 on the JK*A allele and 8 on the JK*B allele (Table 1). Of the known polymorphisms, only two have been associated with individuals of African American ethnicity (JK*01N.04 and JK*01N.05). Investigation into samples whose genotype results for JK*A and JK*B differed from results previously obtained with serologic testing led to the identification of an additional mutation that resulted in the lack of expression of the JK protein.

Materials and Methods

Sample Selection

After informed consent was obtained, whole-blood samples from African American blood donors and patients from the tristate service area (Louisiana, Texas, and Arkansas) were obtained for testing. Genomic DNA was extracted and prepared using either a manual or an automated method (Gentra PureGene or QIAcube, Qiagen, Valencia, CA).

Serologic Studies

All of the discrepant donor samples were tested either on multiple donations or, if confirmation testing was performed on the same sample, with multiple antisera. Of the three donors with genotype or phenotype discrepancies, donor one was tested on two different donations using a polyclonal reagent (Anti-Jkb, Immucor, Norcross, GA). Donor two was tested twice on the same donation: once with a polyclonal reagent (anti-Jkb, Immucor) and once with an in-house human source of anti-Jkb. Finally, donor three was tested twice, first with a polyclonal serum (anti-Jkb Immucor) and then with a monoclonal reagent (Anti-Jkb, Ortho Clinical Diagnostics, Raritan, NJ). No weakened expression was observed with any of the antisera; in fact, all serologic results were negative. As for the patient, because his discrepancy was discovered on a retrospective genotype and phenotype comparison of
results from 250 patients with sickle cell disease, testing was not confirmed by additional serology. The patient's complete phenotype was performed as part of the immunohematology reference laboratory (IRL) serologic workup that led to the identification of alloanti-Jk^b^.

**Molecular Assays**

RBC blood group genotyping was performed using a microarray kit (HEA BeadChip assay version 1.2, BioArray Solutions, LLC, Warren, NJ) according to the manufacturer’s directions. This microarray detects the 838G>A polymorphism corresponding to JK^A^ or JK^B^, as well as 23 additional polymorphisms associated with 34 RBC antigens. Genotype results were compared with previous serologic typing results for concordance. For samples with discordant results, the genotype was confirmed using another kit (Red Cell EZ Type KDK assay, GTI Diagnostics, Milwaukee, WI), according to the manufacturer’s directions. Samples that remained discordant were sent to Puget Sound Blood Center for DNA sequencing.

The coding regions of the JK genes (exons 4–10) along with the promoter region were enzymatically amplified in a total volume of 50 µL with 100 ng of DNA, 1.25 units of polymerase (Taq, 1× Green GoTaq Flexi Buffer, Promega, Madison, WI), 1.5 mM MgCl\_2, 0.125 mM deoxyribonucleoside diphosphate, and 100 ng each forward and reverse primer. Polymerase chain reaction conditions included denaturation for 2 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, and then a final elongation for 5 minutes at 72°C. Polymerase chain reaction products were purified with a kit (QIAquick PCR purification kit, Qiagen) and sequenced on an automated system (ABI Prism 3100 Applied Biosystems, Foster City, CA). The amplified products were sequenced (ABI Prism 3100 Applied Biosystems), they were aligned to the reference JK allele, and the electropherograms were scanned for SNPs. The sequences were aligned in FASTA format using a software program (BLAST, http://blast.ncbi.nlm.nih.gov/).

**Results**

Data were collected from January 1, 2009, through September 30, 2010. Identified during the 21-month study period were 1079 genotyped donors who had been previously typed for Jk^a^ and 1235 genotyped donors who had been previously typed for Jk^b^ by serologic methods. On comparison of serologic results with molecular typing results, three samples were found to be discordant. All three were heterozygous for the 838G>A SNP and were predicted to be Jk^a+b^; however, the samples typed Jk^b–^ by serologic methods.

DNA sequencing of the three samples indicated that all had wild-type promoter regions and confirmed heterozygosity at 838G>A. However, a 191G>A (R64Q) polymorphism was also present in the heterozygous state (Fig. 1). Given the serologic phenotype in the three donors, it is most likely that the 191G>A polymorphism is carried on the JK^B^ allele. Further, this missense SNP is proposed to result in no or undetectable levels of Jk^b^ on the RBC surface. Further

---

**Table 1. International Society for Blood Transfusion terminology for Kidd blood group alleles**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Allele name</th>
<th>Nucleotide change</th>
<th>Intron/exon</th>
<th>Amino acid change</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*01N.01</td>
<td>Exons 4 and 5 deleted</td>
<td>4 and 5</td>
<td>Initiation Met absent</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*01N.02</td>
<td>202C&gt;T</td>
<td>5</td>
<td>Gln68Stop</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*01N.03</td>
<td>582C&gt;G</td>
<td>7</td>
<td>Tyr194Stop</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*01N.04</td>
<td>956C&gt;T</td>
<td>10</td>
<td>Thr319Met</td>
<td>6</td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*01N.05</td>
<td>561C&gt;A</td>
<td>7</td>
<td>Tyr187Stop</td>
<td>7</td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*01N.06</td>
<td>IVS5–1g&gt;a</td>
<td>Intron 5</td>
<td>Exon 6 skipped; in frame</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.01</td>
<td>IVS5–1g&gt;a</td>
<td>Intron 5</td>
<td>Exon 6 skipped; in frame</td>
<td>8</td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.02</td>
<td>IVS5–1g&gt;c</td>
<td>Intron 5</td>
<td>Exon 6 skipped; in frame</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.03</td>
<td>222C&gt;A</td>
<td>5</td>
<td>Asn74Lys</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.04</td>
<td>IVS7+1g&gt;t</td>
<td>Intron 7</td>
<td>Exon 7 skipped; frameshift→Leu223Stop</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.05</td>
<td>723delA</td>
<td>8</td>
<td>Frameshift→Ile262Stop</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.06</td>
<td>871T&gt;C</td>
<td>9</td>
<td>Ser291Pro</td>
<td>8</td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.07</td>
<td>896G&gt;A</td>
<td>9</td>
<td>Gly299Glu</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.08</td>
<td>956C&gt;T</td>
<td>10</td>
<td>Thr319Met</td>
<td></td>
</tr>
<tr>
<td>JK:–3 or Jk(a–b–)</td>
<td>JK*02N.09</td>
<td>191G&gt;A</td>
<td>4</td>
<td>Arg64Gln</td>
<td>9, this study</td>
</tr>
</tbody>
</table>

investigation, including cDNA analysis and protein modeling, would be necessary to demonstrate these interpretations. The allele has been assigned the ISBT name JK*02N.09.

A retrospective review of patient testing also yielded a discrepancy between serology and molecular typing. Of the 250 patient records reviewed, one patient genotyped JK*A/JK*B, but phenotyped Jk(b−) and had produced alloanti-Jk\(^b\). The DNA sample was sequenced, and the patient was confirmed to carry the 191G>A substitution. This previously unpublished SNP has thus far only been detected in Asians (Gaur LK, unpublished data) using a primer set JK-E3-FP1: 5′ACAGCCCCACTATGGTTAGA3′ and JK-E4-RP1: 5′CTGGCTGAGCAAGAGGGC3′ that encompasses codons 10 to 111 (872 bp); so this is the first report of this polymorphism in African Americans.

**Discussion**

Although no longer in its infancy, the role of molecular technologies in the blood bank environment continues to evolve. With the advent of high-throughput molecular assays that are often more economical and efficient than routine serology, donor screening has been revolutionized.\(^\text{10}\) Although some microarray assays have incorporated JK null allele detection (IDCore+, Progenika, Derio, Spain), these are usually limited to the more common Polynesian and Finnish alleles, JK*02N.01 and JK*02N.06, respectively.\(^\text{9}\) Two JK nulls (JK*01N.04 and JK*01N.05) have previously been found in African Americans, but their occurrence is rare.\(^\text{5,6,7}\) However, this new allele, JK*02N.09, occurred in 1 of 412 donors tested. As shown by this study, antigen screening that uses only SNP analysis may not detect blood group mutations caused by silencing mutations and may lead to erroneous testing results. Of note, this method of donor screening will not increase the risk to alloimmunized 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 mistyping patients, and if a patient’s genotype will be used for prophylactic antigen matching, as in the case of sickle cell disease or warm autoimmune hemolytic anemia, limitations of molecular assays must be considered.

Given the prevalence of the 191G>A SNP in the study population, a comparison of previously typed patients with sickle cell disease was conducted, and indeed the new JK allele was detected in one patient who had produced alloanti-Jk\(^b\). This knowledge has led to a change in LifeShare Blood Centers protocol, such that any African American patient who genotypes as JK*A/JK*B will have the phenotype confirmed by serology whenever possible.

African Americans are known to have silencing mutations in other blood group genes, e.g., FY and GYPB, and detection of these 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 frequency of the 191G>A polymorphism in African Americans in other geographic locations may be of interest. Additionally, given that anti-Jk\(^a\) and anti-Jk\(^b\) can be weakly reactive as well as show a dosage effect, those donors used for reagent RBC panels should be confirmed as truly homozygous for the JK*A or JK*B gene. As with many other blood group systems, what once appeared to be a simple blood group system with two common antigens and one rare null type has increasingly become more complex.

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References


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Program Director
E-mail: lblagg1@jhmi.edu
Phone: (410) 502-9584

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- Transfusion-related acute lung injury (TRALI)

Methodologies employed:
- Granulocyte agglutination (GA)
- Granulocyte immunofluorescence by flow cytometry (GIF)
- Monoclonal antibody immobilization of neutrophil antigens (MAINA)

TRALI investigations also include:
- HLA (PRA) Class I and Class II antibody detection

For further information, contact:

Neutrophil Serology Laboratory (651) 291-6797
Randy Schuller (651) 291-6758
Randy.Schuller@redcross.org

American Red Cross Biomedical Services
Neutrophil Serology Laboratory
100 South Robert Street
St. Paul, MN 55107

National Neutrophil Serology Reference Laboratory

CLIA licensed
What is a certified Specialist in Blood Banking (SBB)?

• Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) Board of Certification (BOC) examination for the Specialist in Blood Banking.

• This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:

• Serve as regulatory, technical, procedural, and research advisors

• Perform and direct administrative functions

• Develop, validate, implement, and perform laboratory procedures

• Analyze quality issues preparing and implementing corrective actions to prevent and document nonconformances

• Design and present educational programs

• Provide technical and scientific training in transfusion medicine

• Conduct research in transfusion medicine

Who are SBBs?

Supervisors of Transfusion Services

Executives and Managers of Blood Centers

LIS Coordinators

Educators

Supervisors of Reference Laboratories

Research Scientists

Consumer Safety Officers

Technical Representatives

Reference Lab Specialists

Why become an SBB?

Professional growth

Job placement

Job satisfaction

Career advancement

How does one become an SBB?

CAAHEP-accredited SBB Technology program or grandfather the exam based on ASCP education and experience criteria.

Fact: In recent years, a greater percentage of individuals who graduate from CAAHEP-accredited programs pass the SBB exam compared to individuals who grandfather the exam. The BEST route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program.

Which approach are you more compatible with?

Contact the following programs for more information:

Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org
I. GENERAL INSTRUCTIONS
Before submitting a manuscript, consult current issues of *Immunohematology* for style.
Number the pages consecutively, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW
A. Each component of the manuscript must start on a new page in the following order:
1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

B. Preparation of manuscript
1. Title page
   a. Full title of manuscript with only first letter of first word capitalized (bold title)
   b. Initials and last name of each author (no degrees; all CAPs), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
   c. Running title of ≤40 characters, including spaces
   d. Three to ten key words
2. Abstract
   a. One paragraph, no longer than 300 words
   b. Purpose, methods, findings, and conclusion of study
3. Key words

4. Text (serial pages): Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections
   a. Introduction — Purpose and rationale for study, including pertinent background references
   b. Case Report (if indicated by study) — Clinical and/or hematologic data and background serology/molecular
   c. Materials and Methods — Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patient’s names or hospital numbers.
   d. Results — Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
   e. Discussion — Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
5. Acknowledgments: Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
6. References
   a. In text, use superscript, Arabic numbers.
   b. Number references consecutively in the order they occur in the text.
7. Tables
   a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of….) use no punctuation at the end of the title.

b. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.

c. Place explanation in footnotes (sequence: *, †, ‡, §, ‡‡, ††).

8. Figures
   a. Figures can be submitted either by e-mail or as photographs (5 ×7” glossy).
   b. Place caption for a figure on a separate page (e.g. Fig. 1 Results of…), ending with a period. If figure is submitted as a glossy, place first author’s name and figure number on back of each glossy submitted.
   c. When plotting points on a figure, use the following symbols if possible: ○ △ □ ▼.

9. Author information
   a. List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable. Provide e-mail addresses of all authors.

III. EDUCATIONAL FORUM
A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:
1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills
2. Annotated conference proceedings

B. Preparation of manuscript
1. Title page
   a. Capitalize first word of title.
   b. Initials and last name of each author (no degrees; all CAPs)
2. Text
   a. Case should be written as progressive disclosure and may include the following headings, as appropriate
      i. Clinical Case Presentation: Clinical information and differential diagnosis
      ii. Immunohematologic Evaluation and Results: Serology and molecular testing
      iii. Interpretation: Include interpretation of laboratory results, correlating with clinical findings
      iv. Recommended Therapy: Include both transfusion and nontransfusion-based therapies
      v. Discussion: Brief review of literature with unique features of this case
      vi. Reference: Limited to those directly pertinent
      vii. Author information (see II.B.9.)
      viii. Tables (see II.B.7.)

IV. LETTER TO THE EDITOR
A. Preparation
1. Heading (To the Editor)
2. Title (first word capitalized)
3. Text (written in letter [paragraph] format)
4. Author(s) (type flush right; for first author: name, degree, institution, address [including city, state, Zip code and country]; for other authors: name, degree, institution, city and state)
5. References (limited to ten)
6. Table or figure (limited to one)

Send all manuscripts by e-mail to immuno@redcross.org
### A. For describing an allele which has not been described in a peer-reviewed publication and for which an allele name or provisional allele name has been assigned by the ISBT Working Party on Blood Group Allele Terminology (http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/)

### B. Preparation

1. **Title**: Allele Name (Allele Detail)
   
   ex. RHCE*01.01 (RHCE*ce48C)

2. **Author Names** (initials and last name of each (no degrees, ALL CAPS)

### C. Text

1. **Case Report**
   i. Clinical and immunohematologic data
   ii. Race/ethnicity and country of origin of proband, if known

2. **Materials and Methods**
   - Description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer’s name, city, and state. Do not use patient names or hospital numbers.

3. **Results**
   
   Complete the Table Below:

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Allele Name</th>
<th>Nucleotide(s)</th>
<th>Exon(s)</th>
<th>Amino Acid(s)</th>
<th>Allele Detail</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>e weak</td>
<td>RHCE*01.01</td>
<td>48G&gt;C</td>
<td>1</td>
<td>Trp16Cys</td>
<td>RHCE*ce48C</td>
<td>1</td>
</tr>
</tbody>
</table>

   - Column 1: Describe the immunohematologic phenotype (ex. weak or negative for an antigen).
   - Column 2: List the allele name or provisional allele name.
   - Column 3: List the nucleotide number and the change, using the reference sequence (see ISBT Blood Group Allele Terminology Pages for reference sequence ID).
   - Column 4: List the exons where changes in nucleotide sequence were detected.
   - Column 5: List the amino acids that are predicted to be changed, using the three-letter amino acid code.
   - Column 6: List the non-consensus nucleotides after the gene name and asterisk.
   - Column 7: If this allele was described in a meeting abstract, please assign a reference number and list in the Reference section.

4. **Additional Information**
   i. Indicate whether the variant is listed in the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/); if so, provide rs number and any population frequency information, if available.
   ii. Indicate whether the authors performed any population screening and if so, what the allele and genotype frequencies were.
   iii. Indicate whether the authors developed a genotyping assay to screen for this variant and if so, describe in detail here.
   iv. Indicate whether this variant was found associated with other variants already reported (ex. RHCE*ce48C,1025T is often linked to RHD*DIVa-2)

### D. Acknowledgments

### E. References

### F. Author Information

List first name, middle initial, last name, highest degree, position held, institution and department, and complete address (including ZIP code) for all authors. List country when applicable.
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