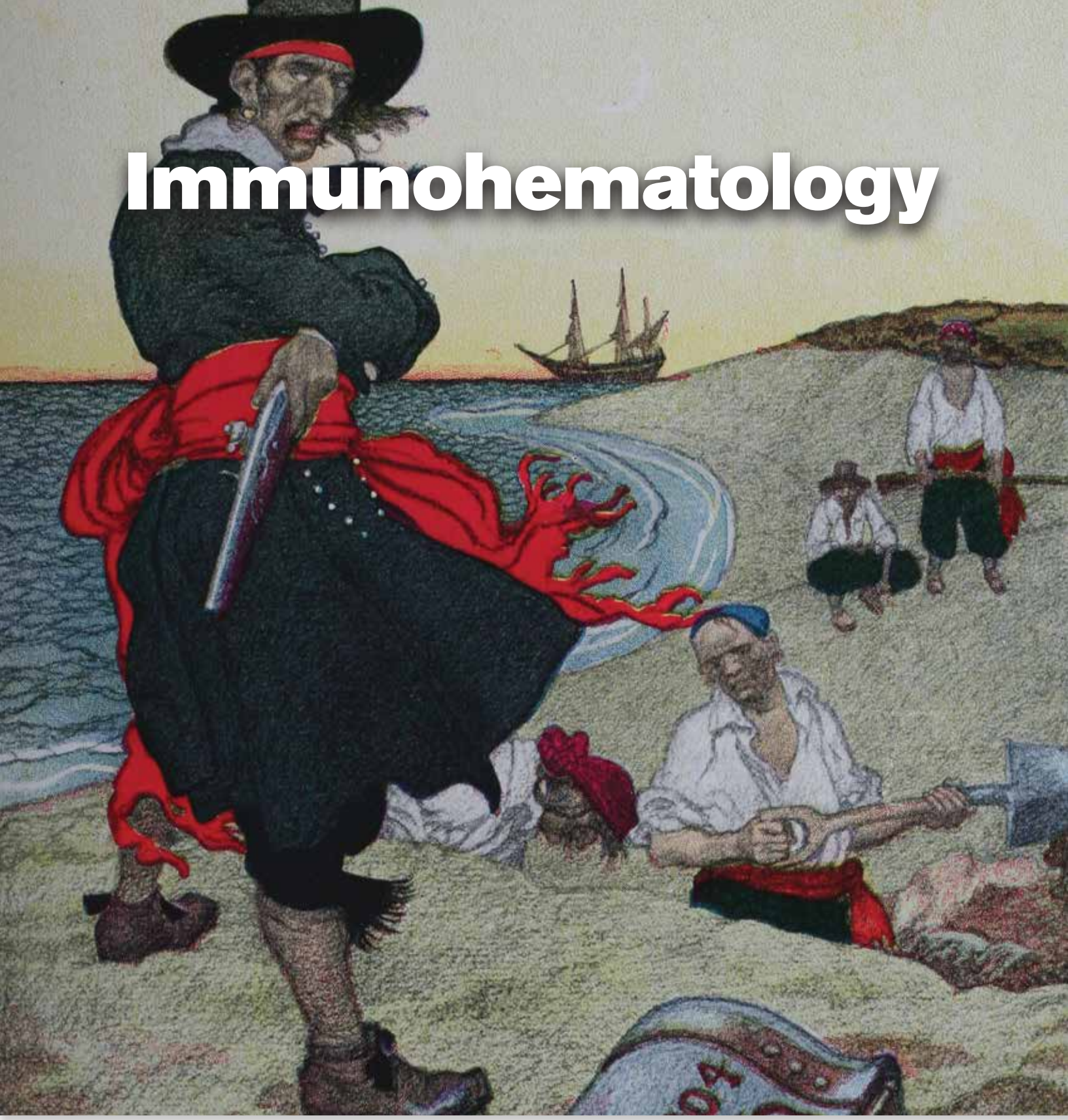

Immunohematology

Journal of Blood Group Serology and Molecular Genetics

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On Our Cover

Howard Pyle was an American illustrator and author of children's books. He had an affinity for stories of adventure, and his many subjects included medieval knights, Robin Hood, as well as pirates such as Captain William Kidd, pictured here burying treasure during the "Golden Age of Piracy" in the mid-17th to early-18th centuries. Since few details regarding the sartorial preferences of these outlaws survived, Pyle created his own flamboyant fashion largely out of whole cloth. This somewhat impractical style with its Romani-like flair soon became archetypal as exemplified by films such as *Captain Blood* and *Pirates of the Caribbean*. An article by Ramsey et al. concerning the Kidd blood group appears in this issue.

David Moolten, MD

Review

A Caucasian *JK*A/JK*B* woman with Jk(a+b–) red blood cells, anti-Jk^b, and a novel *JK*B* allele c.1038delG

G. Ramsey, R.D. Sumugod, P.F. Lindholm, J.G. Zinni, J.A. Keller, T. Horn, and M.A. Keller

The Kidd blood group on the red blood cell (RBC) glycoprotein urea transporter-B has a growing number of weak and null alleles in its gene *SLC14A1* that are emerging from more widespread genotyping of blood donors and patients. We investigated a 64-year-old Caucasian woman of Polish-Czech descent who developed anti-Jk^b detected in solid-phase RBC adherence testing within 12 days after 7 units of RBCs were transfused. Her RBCs subsequently typed Jk(a+b–) by licensed reagents and human antisera. Nevertheless, in RBC genotyping (BioArray HEA BeadChip, Immucor, Warren, NJ) performed in our transfusion service on all patients with alloantibodies, her Kidd typing was *JK*A/JK*B* based on the Jk^a/Jk^b single nucleotide polymorphism in exon 9 (c.838G>A, p.Asp280Asn). Genomic analysis and cDNA sequencing of her *JK*B* allele revealed a novel single-nucleotide deletion of c.1038G in exon 11, predicting a frameshift and premature stop (p.Thr346Thrfs*5) after translation of nearly 90 percent of the expressed exons 4–11. This allele has been provisionally named *JK*02N.14,* subject to approval by the International Society of Blood Transfusion Working Party. The site of this variant is closer to the C-terminus than that of any allele associated with the Jk(a–b–) phenotype reported to date. Routine genotyping of patients with RBC alloantibodies can reveal variants posing potential risk of alloimmunization. Continuing investigation of Kidd variants may shed light on the structure of Kidd antigens and the function of urea transporter-B. ***Immunohematology* 2016;32:91–95.**

Key Words: Kidd blood group system, human urea transporter, genotyping, antigen phenotype

The Kidd blood group resides on human urea transporter B (UT-B).[1,2] Red blood cells (RBCs) genetically lacking Kidd antigens are characteristically resistant to lysis with 2*M* urea. UT-B is also expressed in the renal medulla and bladder, and individuals with the Jk(a–b–) phenotype have a mild deficit in urine concentration. The Kidd/UT-B gene, *SLC14A1,* is at chromosome 18q12.3, and exons 4–11 encode the protein. The three-dimensional structure of a mammalian homolog of UT-B has been resolved, permitting the modeling of UT-B as a 10-pass membrane glycoprotein with a transport pore.[3,4] Recently, UT-B also was identified as a water channel, like aquaporin 1, which carries the Colton blood group.[5]

There are three Kidd antigens, Jk^a, Jk^b, and Jk3, with Jk^a and Jk^b differing at amino acid Asp280Asn attributable to the presence of the single nucleotide polymorphism (SNP) 838G>A in exon 9. Several genetic variants with weak or null phenotypes have been identified.[6–8] We report a Caucasian patient with anti-Jk^b and Jk(a+b–) RBCs, in whom we identify a novel *JK*B* nonsense mutation predicting truncation of the protein in the transmembrane region closest to the protein C-terminus.

Case Report

A 64-year-old Caucasian woman of Polish-Czech descent was brought to the emergency department after syncope. Past medical history was remarkable only for hypertension and ovarian cyst resection. To her knowledge, she had never been pregnant or transfused. Upon arrival, she had a cardiac arrest. After successful resuscitation, extensive coronary artery disease was found, and she subsequently had two coronary stents and a pacemaker placed. Her postoperative course was complicated by *Klebsiella pneumoniae* pneumonia and deep venous thromboemboli. During anticoagulation, a large retroperitoneal hematoma (13 × 13 × 25 cm in largest dimensions) developed, and her hemoglobin (Hgb) fell from 8.0 g/dL to 4.2 g/dL (normal 11.6–15.4 g/dL). Three units of RBCs were transfused before arterial embolization of the bleeding site, and 4 more units were given afterward. Her Hgb rose to 8.8 g/dL and stabilized (Fig. 1). Her cardiac condition improved, her pneumonia and thromboses resolved, and she was discharged on day 17 after transfusion.

Materials and Methods

Antibody screening and identification was done by solid-phase red blood cell adherence (SPRCA) (Immucor, Norcross, GA), supplemented by polyethylene glycol (PEG) screening. RBC Kidd serologic typing was performed with multiple

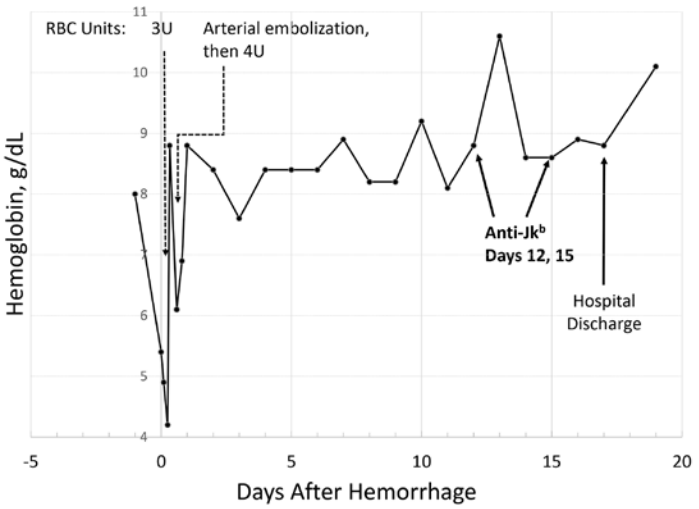

Fig. 1 The time course of the patient's RBC transfusions, hemoglobin levels, and anti-Jk^b detection. RBC = red blood cell.

antisera (see Results). A direct antiglobulin test (DAT) was done manually (IgG + C3) and by SPRCA (IgG). Genomic DNA was isolated using standard methods. Genotyping was carried out using the HEA BeadChip kit (Immucor BioArray, Warren, NJ) (Northwestern Memorial Hospital, Chicago, IL).[9] Additional molecular analysis was performed at the American Red Cross National Molecular Laboratory (Philadelphia, PA). Briefly, amplification and sequencing of *JK* exon 11 was performed (BigDye Terminator Kit, Life Technologies, Carlsbad, CA), and the results were compared with the consensus sequence using Sequencher 5.0 (GeneCodes, Ann Arbor, MI). cDNA was prepared using the Superscript III kit (Life Technologies) and amplified using *JK*-specific primers; the polymerase chain reaction product was isolated using the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA) and ligated into plasmids using the TOPO TA Cloning kit (Life Technologies). Plasmid DNA was prepared using the QIAprep Miniprep kit (QIAGEN) and sequenced using the BigDye Terminator Kit and vector primers.

Results

The patient's sample tested as group A, D+, with negative antibody screens from pre-transfusion through day 6 after the 7 units of RBCs were transfused on day 0–1. On days 12 and 15, her plasma reacted to homozygous Jk(a–b+) reagent RBCs in SPRCA tested on Echo and NEO instruments (Immucor, Norcross, GA). The PEG screen was nonreactive, including testing with Jk(a–b+) reagent RBCs. The pre-transfusion specimen was no longer available. On day 12, her circulating RBCs typed Jk(a+b–) with polyclonal antisera (Immucor). There was no laboratory evidence of delayed hemolysis. RBC unit segments were not typed for Jk^b. In retrospect, the Kidd typing of only one donor was available: one of the 4 units after embolization was from a Jk(a–) donor and thus presumed Jk(b+). Manual and SPRCA DATs were negative, her Hgb had been stable, and her total bilirubin, which was slightly elevated (2.2 mg/dL, normal ≤1.0 mg/dL) after the hematoma, had declined to normal. On day 86, her RBCs typed Jk(a+b–) with licensed monoclonal antisera (Ortho Clinical Diagnostics, Rochester, NY; and Immucor) and with human anti-Jk^a and anti-Jk^b (American Red Cross National Reference Laboratory). Adsorption and elution of anti-Jk^b from her RBCs was not performed at that time because of her transfusions less than 90 days before. An antibody screen 16 months after transfusion was negative.

The HEA BeadChip interrogates for the Jk phenotype at SNP c.838G>A, and this patient's RBCs were predicted to type Jk(a+b+). The discrepancy between her serologic phenotype and antibody versus the genotype-predicted phenotype was investigated by cDNA analysis. The patient's *JK*A* and *JK*B* transcripts matched the consensus sequences except for a novel single-nucleotide deletion of c.1038G in *JK*B,* confirmed by gDNA sequencing of exon 11 and cDNA analysis. The deletion of c.1038G is predicted to result in a frameshift and premature stop five amino acids downstream from the codon affected by the deletion (Thr346Thrfs*5) (Table 1). This SNP is in the transmembrane region closest to the C-terminal of the Kidd/UT-B protein (Fig. 2). Our patient's allele is under consideration for the designation *JK*02N.14* by the International Society of Blood Transfusion Working Party.[7]

Table 1. Segment of Kidd gene, *SLC14A1,* showing location of c.1038delG mutation

Codon	…346	347	348	349	350	351	352	353…
Consensus cDNA	AC**G**	CTA	TTG	TTC	CTC	ATC	ATG	ACC
Amino acid	Thr	Leu	Leu	Phe	Leu	Ile	Met	Thr
c.1038delG cDNA	AC-C	TAT	TGT	TCC	TCA	TCA	TGA	
Amino acid	Thr	Tyr	Cys	Ser	Ser	Ser	**STOP**	

Nucleotides c.1036–1038 code for amino acid 346. The frameshift at Thr346 resulting from c.1038delG creates a stop codon after Ser351. Consensus cDNA and amino acids are from the dbRBC Sequence Alignment Viewer.[8]

Discussion

In the absence of prior pregnancy or transfusion (as reported by the patient), our patient's anti-Jk^b may have

represented a rapid primary immune response detected by SPRCA within 12 days after transfusion. In a similar scenario, Crews et al. reported on a patient with a *JK*A* null variant (*JK*01N.07*) revealed after anti-Jk^a was detected by SPRCA 20 days after transfusion.[12]

Our patient received 7 units of RBCs before anti-Jk^b was detected, of which 74 percent would have been predicted to be Jk(b+) (Caucasian prevalence).[2] However, most of the first 3 units likely were hemorrhaged into the large hematoma before arterial embolization. Only 4 of the 7 units were given after the bleeding was controlled. These events may partly explain why there was no clinically evident hemolysis, and why no Jk(b+) RBCs were detected in the circulation when anti-Jk^b developed. The anti-Jk^b was not detected 16 months later, in keeping with the evanescent tendency of Kidd antibodies.[13]

Variants in the *JK* gene were traditionally sought in Jk(a–b–) individuals who were identified either by the presence of anti-Jk3 or by resistance to urea lysis. The growing use of RBC genotyping in donors and patients will reveal discrepancies between the Kidd phenotype and genotype and in some cases, such as the one described here, will lead to the discovery of novel alleles in heterozygous individuals.[14,15] Our patient's anti-Jk^b with a Jk(a+b–) serologic RBC phenotype would have been unremarkable without the discrepancy between the serologic phenotype and the genotype-predicted phenotype. Our transfusion service routinely performs HEA BeadChip analysis in patients with alloantibodies.[16] This case illustrates that genetic investigations of individuals with anti-Jk^a or anti-Jk^b can reveal heterozygous alleles that carry risk for alloimmunization.

More than 40 *JK*A* and *JK*B* variants cause silenced or null phenotypes (Table 2). Our patient's predicted *JK*B* truncation in the transmembrane region of exon 11 after p.351 results in a Jk(a+b–) phenotype. By comparison, a recently reported *JK*A* allele[11] has a predicted stop codon after p.366, in *cis* with a marker associated with weak Jk expression, p.Glu44Lys[10] (Fig. 2). This predicted truncation after p.366 is 15 codons downstream from our patient's putative stop codon, but is in the cytoplasmic region of the protein and yields a Jk(a+^{weak}) phenotype.

Continuing investigation of null and weak *JK* variants may shed light on how diverse mutations across the *SLC14A1* gene affect the immunological expression of Kidd antigens and the physiological transporter functions of UT-B.

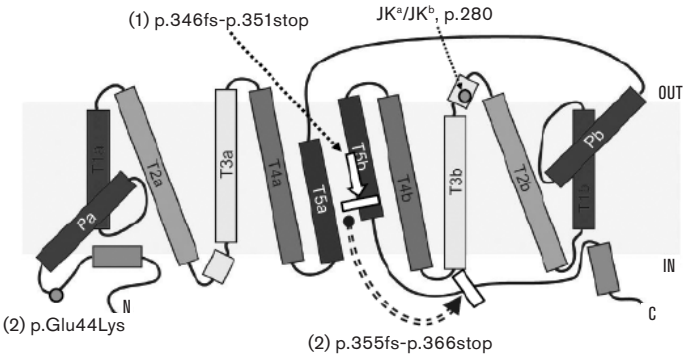

Fig. 2 Two *JK* exon-11 stop-codon variants with different phenotypes, mapped to the urea transporter protein model of Levin et al.[4] (1) *JK*B* variant in our patient, with predicted truncation in the T5b transmembrane region, expressing Jk(a+b–) red blood cell (RBC) phenotype. (2) *JK*A* variant with p.Glu44Lys (associated with weak Jk expression when alone[10]), in *cis* with p.355fs-p.366stop, predicting truncation in the C-terminal cytoplasmic region; this variant expresses a Jk(a+^{weak}) RBC phenotype.[11] The Jk^a/Jk^b polymorphism at p.280 is located near the external end of the T3b transmembrane helix in exon 9; exon 11 begins at the external end of T4b.[3] fs = frameshift; T1a–5a, T1b–5b = transmembrane helices; Pa–Pb = pore helices.

Acknowledgments

We thank the technical staff of the American Red Cross National Molecular Laboratory.

References

1. Daniels G. Human blood groups. 3rd ed. Oxford: Wiley-Blackwell, 2013:325–35.
2. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. London: Elsevier, 2013:373–81.
3. Levin EJ, Quick M, Zhou M. Crystal structure of a bacterial homolog of the kidney urea transporter. Nature 2009;462: 757–61.
4. Levin EJ, Cao Y, Enkavi G, et al. Structure and permeation mechanism of a mammalian urea transporter. Proc Natl Acad Sci U S A 2012;109:11194–9.
5. Azouzi S, Gueroult M, Ripoche P, et al. Energetic and molecular water permeation mechanisms of the human red blood cell urea transporter B. PLoS One 2013;8:e82338.
6. Hamilton JR. Kidd blood group system: a review. Immunohematology 2015;31:29–35.
7. Storry JR, chair, Working Party, Red Cell Immunogenetics and Blood Group Terminology. Names for JK (ISBT 009) blood group alleles, v3.0 131028. Amsterdam: International Society of Blood Transfusion, 2013 [accessed December 18, 2015, at http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology].
8. Patnaik SK, Helmberg W, Blumenfeld OO. BGMUT: NCBI dbRBC database of allelic variations of genes encoding antigens of blood group systems. Nucleic Acids Res 2012;40:D1023–9 [accessed December 18, 2015, at http://www.ncbi.nlm.nih.gov/projects/gv/rbc].
9. Hashmi G, Shariff T, Seul M, et al. A flexible array format for large-scale, rapid blood group DNA typing. Transfusion 2005;45:680–8.

Table 2. Mutations in human urea transporter-B (*SLC14A1*) that result in the Jk(a–b–) phenotype, listed sequentially from N-terminus to C-terminus

Amino acid change*	*JK*A* or *JK*B*	ISBT alleles,[7] dbRBC,[8] or other	Exon	Ethnicities
No protein (deletion exons 4–5)	A	*01N.01*	4–5	Tunisian, English, Bosnian
p.Val10_Arg17del	A	*01N.09*	4	African
p.Pro53fs, 60*	B	Burgos et al.[11]	5	African
p.Arg64Gln	B	*02N.09*	5	African, Japanese
p.Gly65Asp, p.Pro196Pro	B	*02N.10*	5	French-Canadian
p.Gln68*	A	*01N.02*	5	U.S. Caucasian
p.Asn74Asp (+p.Glu44Lys, weak Jk[9])	A	dbRBC	5	Chinese
p.Asn74Lys, p.Met167Val	B	02N.03	5	Taiwanese
p.Leu109Phefs, 117*	A	dbRBC	5	Japanese
p.Arg114_Thr156del (exon 6) (intron 5 mutation)	A	*01N.06*	6	Indian Asian
p.Arg114_Thr156del (exon 6) (intron 5 mutation)	B	*02N.01*	6	Polynesian, Chinese
p.Arg114_Thr156del (exon 6) (intron 5 mutation)	B	*02N.02*	6	Chinese, Vietnamese
p.Trp144*	A	dbRBC	6	Japanese
p.Leu146Pro, p.Met167Val	B	*02N.12*	6	Chinese
p.157-221del (exon 7), fs, 223* (intron 7 mutation)	B	*02N.04*	7	French Caucasian
p.Met167Val, Trp171*	B	*02N.11*	7	Chinese
p.Met167Val, p.Pro179Arg	B	*02N.13*	7	Chinese
p.173-177del	A	Henny et al.[17]	7	Swiss
p.Tyr187*	A	*01N.05*	7	African
p.Tyr187*	B	dbRBC	7	Japanese
p.Tyr194*	A	*01N.03*	7	Swiss, English
p.Asp216Alafs, 237*	B	dbRBC	7	Japanese
p.222-270del (exon 8), fs* (intron 8 mutation)	A	dbRBC	8	Chinese
p.Trp240*	B	dbRBC	8	Japanese
p.241fs, 262*	A	*01N.07*	8	—
p.241fs, 262*	B	*02N.05*	8	U.S. Hispanic
p.Leu246Arg	A	Ma et al.[18]	8	Chinese
p.Leu246Arg	B	dbRBC	8	Chinese
p.Ala248Thr[†]	A	dbRBC	8	Austrian
p.Ser253del[‡]	A	Onodera et al.[19]	8	Japanese
p.Asn269Ser	A	*01N.08*	8	—
p.Ala270fs (intron 8 mutation)	A	*01N.10*	8	Chinese
p.Ala270Ala[§]	B	Henny et al.[17]	8	Swiss
p.Leu272Phe	A	Keller et al.[15]	9	—
p.286fs, 352* (+ p.Glu44Lys, weak Jk)	B	DePalma et al.[20]	4, 9	U.S. Caucasian
p.Ser291Pro	B	*02N.06*	9	Finnish
p.Gly298Glu	A	dbRBC	9	Japanese
p.Gly299Glu	B	*02N.07*	9	Taiwanese, Japanese
p.Thr319Met	A	*01N.04*	10	African, Indian
p.Thr319Met	B	*02N.08*	10	Indian, Pakistani
p.Thr319Met (+ p.Glu44Lys, weak Jk)	A	dbRBC	4, 10	Thai
IVS10+5C (intron 10)	A	Bub et al.[21]	Pre-11	Brazilian
p.Thr346fs, 351*	B	This report (tentative *02N.14*)[¶]	11	U.S. Polish-Czech

Exons 4–11 are normally expressed (389 amino acids). Alleles are listed in order of first variant amino acid from N-terminus to C-terminus. *JK*A/JK*B* = p.Asp280Asn, exon 9; ISBT = International Society of Blood Transfusion; del = deletion; fs = frameshift.

*Asterisk in amino acid changes represents stop codon predicted in amino acid sequence.

[†]p.Ala248Thr: listed as Jk(a–) in dbRBC and weak Jk(a+) (*JK01.W05*) in ISBT.

[‡]p.Ser253del: erroneously listed as *JK*B* allele in dbRBC.

[§]p.Ala270Ala: same amino acid, but nucleotide change near end of exon may affect splicing.

[¶]*JK*02N.14* was originally used as an erroneous duplicate of *JK*02N.07*.

10. Wester ES, Storry JR, Olsson ML. Characterization of Jk(a+^{weak}): a new blood group phenotype associated with an altered *JK*01* allele. Transfusion 2011:51:380–92.
11. Burgos A, Vege S, Velliquette RW, Lomas-Francis C, Westhoff CM. Serologic and molecular investigation of novel Kidd system alleles in African-Americans (abstract). Transfusion 2013;53(2S):39A–40A.
12. Crews WS, Gould JM, Crowley J, Keller MA, Herman JH. A novel *JK*A* variant detected only by solid-phase testing (abstract). Transfusion 2013;53(2S):164A.
13. Tormey CA, Stack G. The persistence and evanescence of blood group alloantibodies in men. Transfusion 2009;49:505–12.
14. Guo Z, Wang C, Yan K, et al. The mutation spectrum of the JK-null phenotype in the Chinese population. Transfusion 2013;53:545–53.
15. Keller MA, Crowley JA, Horn T. Kidd antigen discrepancies: genotype-predicted phenotype vs serologic phenotype (abstract). Vox Sang 2014;107(S1):37.
16. Ramsey G, Zinni J, Sumugod RD, Lindholm PF. Utility of routine RBC genotyping for RBC alloantibody problems (abstract). Transfusion 2014;54(2S):47A–48A.
17. Henny C, Lejon Crottet S, Gowland PL, Niederhauser C, Hustinx H. Three novel JK alleles detected in Swiss blood donors (abstract). Vox Sang 2014;107(S1):188.
18. Ma L, Liu YC, Zhu SW, et al. A novel missense mutation nt737T>G of JK gene with Jk(a–b–) phenotype in Chinese blood donors. Transfus Med 2015;25:38–41.
19. Onodera T, Sasaki K, Tsuneyama H, et al. *JK* null alleles identified from Japanese individuals with Jk(a–b–) phenotype. Vox Sang 2014;106:382–4.
20. DePalma H, Vege S, Hu Z, et al. Identification of novel *JK*B* variants (abstract). Transfusion 2014;54(2S):45A–46A.
21. Bub CBB, Torres KBT, Aravechia MGA, et al. A novel *JK*A* allele associated with a typing discrepancy in a Brazilian patient with sickle cell disease (SCD) (abstract). Vox Sang 2015;109(S1):296–7.

Glenn Ramsey, MD (corresponding author), Professor of Pathology, Feinberg School of Medicine, Northwestern University, and Medical Director, Blood Bank, Northwestern Memorial Hospital, Feinberg 7-301, 251 E. Huron St., Chicago, IL 60611, g-ramsey@northwestern.edu; Ricardo D. Sumugod, MS, MT(ASCP)SBB, Manager, Blood Bank, Northwestern Memorial Hospital; Paul F. Lindholm, MD, Associate Professor of Pathology, Feinberg School of Medicine, Northwestern University, and Medical Co-Director, Blood Bank, Northwestern Memorial Hospital; and Jules G. Zinni, MLS(ASCP)^{CM}MBCM, Senior Medical Technologist, Blood Bank, Northwestern Memorial Hospital; Jessica A. Keller, MS, Supervisor; Trina Horn, MLT(ASCP)SBB^{CM}, Manager; and Margaret A. Keller, PhD, Director, National Molecular Laboratory, American Red Cross, Philadelphia, PA.

Review

How to recognize and resolve reagent-dependent reactivity: a review

G.C. Patch, C.F. Hutchinson, N.A. Lang, and G. Khalife

Reagent-dependent reactivity can be described as agglutination of red blood cells (RBCs) in serologic testing that is not related to the interaction of RBC antigens and antibodies that the test system is intended to detect. In other words, reagent-dependent reactivity results in false-positive agglutination reactions in serologic testing. These false-positive reactions can cause confusion in antigen typing and RBC antibody detection and identification procedures, and may result in delays in patient transfusion. It is imperative that reagent-dependent reactivity is recognized and resolved during the investigation of ABO discrepancies, positive RBC antibody screens and antibody identification panels, and crossmatch reactivity. ***Immunohematology* 2016;32:96–99.**

Key Words: reagent-dependent reactivity, solid-phase red blood cell adherence, gel, LISS, antibody identification, false-positive reactions, panreactivity

Chapter 43 of the fourth edition of *Applied Blood Group Serology*[1] is titled "Miscellaneous Conditions That May Affect Results in the Blood Transfusion Laboratory." In the introduction to this chapter, authors P.D. Issitt and D.J. Anstee write, "For those who watch *Seinfeld,* this is a chapter about nothing." In the same vein, this article is about nothing: seemingly positive serologic results that generally can be proven to be nothing upon further investigation.

The AABB Standards for Immunohematology Reference Laboratories mandate that "the laboratory shall recognize and have a process to investigate reagent-dependent reactivity."[2] The need for this standard is a result of several testing platforms, both manual and automated, that are used throughout the United States for red blood cell (RBC) antibody detection and identification, and for the performance of ABO and other antigen testing of RBCs. Commercially prepared antisera, RBCs, and enhancement media have all been implicated in cases of reagent-dependent reactivity. Our immunohematology reference laboratory (IRL) regularly reports various causes of reagent-dependent reactivity to our client hospital transfusion services. The purpose of this article is to review multiple causes of reagent-dependent reactivity and how to recognize and resolve them.

Dyes Used in ABO Typing Reagents

Anti-A reagents contain a blue dye (such as FD&C Blue No. 1 and Patent Blue), and anti-B reagents contain a yellow dye (such as acriflavin, FD&C Yellow No. 5, and Naphthol Yellow);[3,4] antibodies to these dyes have been reported in the literature.[1,5] Antibodies in the patient's plasma can combine with the dye in the reagent to form antigen–antibody complexes; in the presence of these complexes, RBCs may agglutinate. Alternately, the dye may bind to the RBCs, which are then agglutinated by an antibody directed towards the dye. It has also been postulated that the dye might somehow modify the RBC membrane so that spontaneous agglutination of the RBCs occurs.[1] If unexpected reactions are observed in the ABO front (forward) type and an antibody to a dye is suspected, the patient's RBCs should be washed one to two times to remove all traces of plasma, and the testing should be repeated with the washed cells.[1]

Additives to ABO and Other Antisera

Most commercial antisera also contain bacteriostatic or other preservative agents. Patient plasma may contain antibodies to these agents, or immune complexes may be formed in the presence of these reagents.[1,5,6] These agents include chloramphenicol, gentamicin, neomycin, vancomycin, paraben, thimerosal, sodium azide, tetracycline, hydrocortisone, and other corticosteroids.[1,5–7] Reactions caused by these additives are an in vitro phenomenon and have no clinical significance in transfusion therapy, other than causing laboratory problems that delay transfusions.[7] If unexpected reactions are observed with any antisera, the patient's RBCs should be washed one to two times to remove all traces of plasma, and testing should be repeated with the washed cells.

B(A) Phenomenon

A leading manufacturer of one source of anti-A includes the following message in the package insert for this product: "Anti-A may detect previously unrecognized A antigen in a

small number (0.1%) of Group B people. The agglutination is weak, mixed field, and easily dispersed."[3] This phenomenon is known as B(A). The package insert states that this problem can be resolved by testing with polyclonal anti-A or another monoclonal anti-A derived from a cell line other than MH04.[3]

RBC Preservative Problems

The package insert from one leading manufacturer of screening cells used in antibody detection procedures contains the following warning: "Infrequently, falsely positive results may occur in the presence of antibodies directed to components of the red blood cell diluent."[8] The chemicals in most of these commercial RBC diluents are adenine, chloramphenicol, glucose, inosine, neomycin sulfate, sodium chloride, and sodium citrate.[5] All manufacturers add antibiotics to their RBC suspension media to reduce or prevent bacterial contamination;[5] antibodies to any of the antibiotics could be present in a patient's plasma. Some manufacturers also add hydrocortisone to their RBC diluents, and numerous examples of IgM antibodies directed towards hydrocortisone have been described.[5] Finally, RBC diluents contain some source of sugar, and antibodies to one or more sugars in the diluent have been reported.[5]

An antibody to a commercial RBC diluent should be suspected if a laboratory reports panreactivity with screening or panel RBCs, but not with donor RBCs (which would lack the components of the commercial RBC diluent), at the same phase of testing. An IRL might suspect a RBC diluent problem if the referring laboratory uses a different source of commercial RBCs, and the IRL cannot duplicate the panreactivity seen in testing by the referring lab. Alternatively, the IRL might see panreactivity with one commercial source of RBCs and no reactivity with another commercial source of RBCs.

RBC diluent formulas are "proprietary" and vary from one manufacturer to the next. If an antibody to a component of the commercial RBC diluent is suspected, the commercial cells should be washed one to two times to remove the diluents, and the testing should be repeated. The components of the diluent may be washed away easily. If washing the cells is unsuccessful in resolving the problem, an alternate commercial source or fully phenotyped donor cells should be used in testing.

Antibodies to Chemicals in Commercial Antibody Potentiators

Albumin is rarely used as a potentiator in RBC antibody screening or identification procedures, but autoagglutinins reacting only in the presence of bovine albumin have been reported. In 1969, a group of researchers showed that the "albumin autoagglutinin phenomenon" was caused by antibodies in the patient's sera reacting with sodium caprylate, which was added as a stabilizer during the heating phase of the manufacturing of bovine albumin.[5] Other researchers point to patients with antibodies directed to the bovine albumin or contaminants in the bovine albumin.[5]

Low-ionic-strength saline (LISS) is a common potentiator used in tube testing as well as in gel testing and solid-phase RBC adherence (SPRCA) assays. LISS-dependent autoagglutinins are detected with some regularity in the IRL. A LISS panagglutinin is typically suspected when all reagent RBCs, including the autocontrol, react at 37°C and/or the antihuman globulin phase, but the direct antiglobulin test (DAT) is negative. Thimerosal and paraben are LISS additives that have been implicated in this phenomenon.[1,9–13] Antibody identification tests and the autocontrol should be repeated using a different potentiator such as polyethylene glycol (PEG). LISS-dependent reactivity should be reported when all cells including the autocontrol are reactive in the presence of one potentiator, but are nonreactive in the presence of others. The recommendation to the hospital is to transfuse RBCs that are crossmatch-compatible using a method that does not use LISS or another implicated potentiator.

Solid-Phase-Only Reactivity

SPRCA assays are generally automated and are well suited to antibody screening and antibody identification for transfusion services and blood bank laboratories with a high volume of testing and/or limited staff. Nonetheless, a small percentage of false-positive reactivity has been acknowledged by the U.S. manufacturer of this testing platform. The package insert reads under Specific Performance Characteristics: "Some patient and donor specimens were evaluated that reacted by Capture-R Ready-Screen, but were nonreactive by reference hemagglutination techniques. Most of these specimens were shown to contain solid-phase-only autoantibodies."[14]

Our laboratory began using a manual SPRCA assay (Capture-R; Immucor, Norcross, GA) as an adjunct to manual tube RBC antibody detection and identification methods approximately 10 years ago. We soon realized that the test seemed to be "too sensitive," as we could not detect antibody activity by any other method in many donor and patient samples. A call was made to the manufacturer's technical support department, and we were told that SPRCA assays can detect an antibody directed at the cryptantigens of the

RBC membrane; these are antigens that can be exposed when RBC stroma is present, but not usually seen with intact RBCs. Therefore, we launched a joint project with the American Red Cross IRL in Columbus, Ohio, to investigate this so-called "SPRCA-only reactivity" in normal, healthy blood donors. A total of 283,971 donor antibody screens were performed in an 8-month period, and 694 donors were identified as having a SPRCA-only antibody; this translated to a rate of 0.24 percent.[15] The criteria used to categorize an antibody as SPRCA-only included the following: panreactivity in SPRCA, negative antibody screen in gel, negative antibody screen in tube using PEG, and a negative DAT.

Our laboratory regularly receives samples referred from client hospitals that use automated solid-phase platforms for antibody screens and antibody identification. Anecdotally, it seems that automated platforms detect more of these so-called SPRCA-only antibodies than does the manual SPRCA assay used by our laboratory; it also leads to the speculation that these antibodies might also be detected more frequently in a patient population than in a healthy, donor population. In one study characterizing the performance of the 2003 walk-away analyzer (Galileo, Immucor), the authors cited a false-positive rate of 1.4 percent.[16] A false positive in their study was defined as a positive screen with a negative panel.[16]

A SPRCA-only antibody should be suspected if panreactivity is seen in an antibody identification procedure using a solid-phase platform and the DAT is negative. Although an antibody to a high-prevalence antigen or the presence of multiple alloantibodies would act in the same manner, it may be prudent to initially repeat an antibody screen on the sample in question with a tube method using either LISS or PEG, or test in a gel platform if available. If the antibody screen is negative with a method other than solid-phase, it is most likely that the solid-phase results are false positives. Obviously, if the antibody screen is positive by a second test method, the presence of antibodies must be further explored.

Practical Applications: Why Recognizing Reagent-Dependent Reactivity Is Important

The overview of reagent-dependent reactivity in this article is not meant to be all-inclusive, and the information given is largely based on the kinds of problems referred to our IRL. As such, the main reason for this article is to educate readers to simple solutions for the resolution of these problems.

Recognizing and subsequently resolving reagent problems is important because it prevents the time and expense of referring samples to an outside laboratory. Unfortunately, many transfusion services have only one way of performing a test or approaching a problem and are reluctant to try anything else, even if suggested to them. The reflex decision is to send the sample to an IRL, which delays patient treatment and adds expense.

Many transfusion services have come to rely on automation—and rightfully so—for high-throughput testing and the ability to cross-train employees. Unfortunately, the cost of automation often means that the transfusion service has limited back-up resources for problem-solving. For example, when a client hospital reports panreactivity in a sample using either gel or solid-phase technology, an IRL will ask (after confirming that the DAT is negative), "Did you repeat the testing in tube?" Common responses include, "We don't do that" or "We don't do tube testing." When tested by tube method by the IRL, no atypical antibodies are detected. In contrast, some hospitals request that the IRL attempt to duplicate their results using a solid-phase method even when tube testing is nonreactive. These unnecessary delays in patient treatment and charges could be prevented if the referring transfusion service would simply take the time to perform a tube antibody screen. It is a simple, low-cost test that may solve the problem in less than 30 minutes—yet many transfusion services are unwilling or unable to do this follow-up testing.

For those readers who are blood bank educators as well as technical specialists, perhaps it should be your role to teach students and employees about reagent-dependent reactivity. Blood bank curricula contain lectures about potentiators and how they work, but may not cover LISS-dependent autoagglutinins. The principle of SPRCA is covered and students learn how to operate the instrument, but the phenomenon of SPRCA-only antibodies may not be addressed. Discussion of common reagent-dependent problems and their resolution should perhaps be as important as discussing how to identify a true RBC antibody. After all, a "false positive" can only be called that after you prove it is actually a "Seinfeld" nothing.

References

1. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:1127.
2. AABB. Standards for immunohematology reference laboratories. 9th ed. Bethesda, MD: American Association of Blood Banks, 2015:25.
3. Ortho Bioclone package insert for murine monoclonal anti-A, anti-B and anti-A,B; revision July 2010. Hampton, NH: Fisher Scientific.

4. Gamma-Clone package insert for murine monoclonal anti-A, anti-B and anti-A,B; revision February 2013. Norcross, GA: Immucor.
5. Garratty G. In vitro reactions with red blood cells that are not due to blood group antibodies: a review. Immunohematology 1998;14:3–15.
6. Judd JW, Johnson ST, Storry JR. Judd's methods in immunohematology. 3rd ed. Bethesda, MD: American Association of Blood Banks Press, 2008:519.
7. AABB. Technical manual. 18th ed. Bethesda, MD: American Association of Blood Banks Press, 2014:416–7.
8. Immucor package insert for Panoscreen Cells; revision February 2013. Norcross, GA: Immucor.
9. Shulman IA, Simpson RB, Farmer CF, Lam HT. Thimerosal-dependent agglutination complicating the serologic evaluation for unexpected antibodies. Transfusion 1984;4:365–7.
10. Judd WJ, Steiner EA, Cochran RK. Paraben-associated auto-, anti-Jk^{a} antibodies: three examples detected using commercially prepared low-ionic-strength saline containing parabens. Transfusion 1982;22:31–5.
11. Shulman IA, Hasz LA, Simpson RB. Thimerosal dependent agglutination, a newly described blood bank problem. Transfusion 1983;22:241–3.
12. Mason JM, Osborne PT, Hall AJ, et al. Example of a thimerosal-dependent antibody without apparent blood group specificity. Vox Sang 1985;48:313–6.
13. Spencer GT, McDowell MA. Thimerosal-dependent antibody enhanced in the presence of LISS. Vox Sang 1985;49:422–3.
14. Capture-R Ready-ID package insert; revision February 2013. Norcross, GA: Immucor.
15. Lang N, Sulfridge DM, Hulina J, et al. Solid phase reactive only antibodies. Transfusion 2011;51(Suppl):172A.
16. Quillen K, Caron J, Murphy K. Performance characteristics of two automated solid-phase red cell adherence systems for pretransfusion antibody screening: a cautionary tale. Immunohematology 2012;28:137.

Gavin C. Patch, BS, MT(ASCP)SBB^{CM}, IRL Supervisor; Charles F. Hutchinson, BS, MT(ASCP)SBB, IRL Lead Technologist; Nancy A. Lang, MS, MT(ASCP)SBB (corresponding author), IRL Lead Technologist, nlang@cbccts.org; and Ghada Khalife, MD, Associate Medical Director, Community Blood Center, 349 South Main St., Dayton, OH 45402.

The Augustine blood group system, 48 years in the making

G. Daniels

The high-prevalence antigen, At^a, was first identified in 1967, but it was not until 2015 that At^a became AUG1 of a new blood group system, Augustine (AUG). The new system was established after the identification of the gene encoding At^a and the recognition of a null phenotype (AUG:–1,–2) in an At(a–) patient with an antibody (anti-AUG2) reactive with At(a–) red blood cells. The At(a–) phenotype is very rare and, with the exception of the one family with the null phenotype, has only been found in individuals of African origin. Anti-At^a has been implicated in immediate and delayed hemolytic transfusion reactions, but not in severe hemolytic disease of the fetus and newborn. The Augustine gene is *SLC29A1,* which encodes the equilibrative nucleoside transporter ENT1. At(a–) (AUG:–1,2) results from homozygosity for c.1171G>A, encoding Glu391Lys, whereas the AUG_{null} (AUG:–1,–2) phenotype results from homozygosity for a splice site mutation, c.589+1G>C, in the only family where it has been found. Absence of ENT1 in that family may be associated with pseudogout and abnormal bone calcification. ***Immunohematology* 2016;32:100–103.**

Key Words: blood groups; At^a; Augustine; *SLC29A1;* ENT1; equilibrative nucleoside transporter

In 1967, Applewhaite et al.[1] described the first example of anti-At^a, an antibody to a common antigen. Six more examples were reported in 1973,[2] and an antigen, originally reported[3] as El, was subsequently shown to be of the same specificity.[4] At^a was numbered 901003, as part of the 901 series of high-prevalence antigens, until 2015, when Daniels et al.[5] identified the gene encoding At^a as *SLC29A1,* and thus, a new blood group system containing At^a (AUG1) and a new antigen, AUG2, was established.[6] Augustine (AUG) became the 36th blood group system (Table 1).

Table 1. Antigens of the Augustine system and their molecular backgrounds

Antigens			Molecular basis *(SLC29A1)**		
Name	ISBT number		Nucleotides	Exon/intron	Amino acids
At^a	AUG1	036001	c.1171G (A)	Exon 12	Glu391 (Lys)
–	AUG2	036002	c.589+1G (C)	Intron 6	Splice site mutation

*Molecular basis of antigen-negative phenotype provided in parentheses.

At^a (AUG1) and Anti-At^a

At^a is a common antigen. All reported At(a–) individuals have been of African origin, and in several surveys, only 1 of about 16,450 African Americans was At(a–).[1,3,7,8] Family analyses provided strong evidence that the At(a–) phenotype results from homozygosity for a recessive gene.[1–3,9–11] At^a is not destroyed by treatment of At(a+) red blood cells (RBCs) with trypsin, α-chymotrypsin, papain, pronase, sialidase, 200 mmol/L dithiothreitol, or 2-aminoethylthiouronium bromide and is expressed strongly on cord RBCs.[12,13]

Anti-At^a may be stimulated by transfusion or pregnancy; no example of "naturally occurring" anti-At^a is known. At^a antibodies are mostly immunoglobulin G (IgG), reacting by an antiglobulin test, although one serum also contained some directly agglutinating IgM.[2] IgG anti-At^a was subtyped in two sera: one contained IgG1; the other contained IgG1, IgG3, and IgG4.[14]

AUG2 and Anti-AUG2

In 1994, an antibody in an At(a–) pregnant woman with consanguineous parents (Fig. 1) reacted with all RBCs tested, with the exception of those of her two At(a–) siblings. The positively reacting RBCs included RBCs of many phenotypes lacking high-prevalence antigens that were available in several international reference laboratories. Furthermore, several examples of At(a–) RBCs reacted with the serum, demonstrating that the antibody was not anti-At^a. At that time, it was speculated that the patient may have a null phenotype (Daniels G, unpublished 1994). This speculation was confirmed 21 years later by molecular genetics[5] (see "Molecular Genetics of the Augustine Blood Group System" later in this review), and the antibody was named anti-AUG2.[6]

Anti-AUG2 reacted with RBCs treated with papain, trypsin, chymotrypsin, pronase, sialidase, or 2-aminoethyl-thiouronium bromide (Daniels G, unpublished 1994).

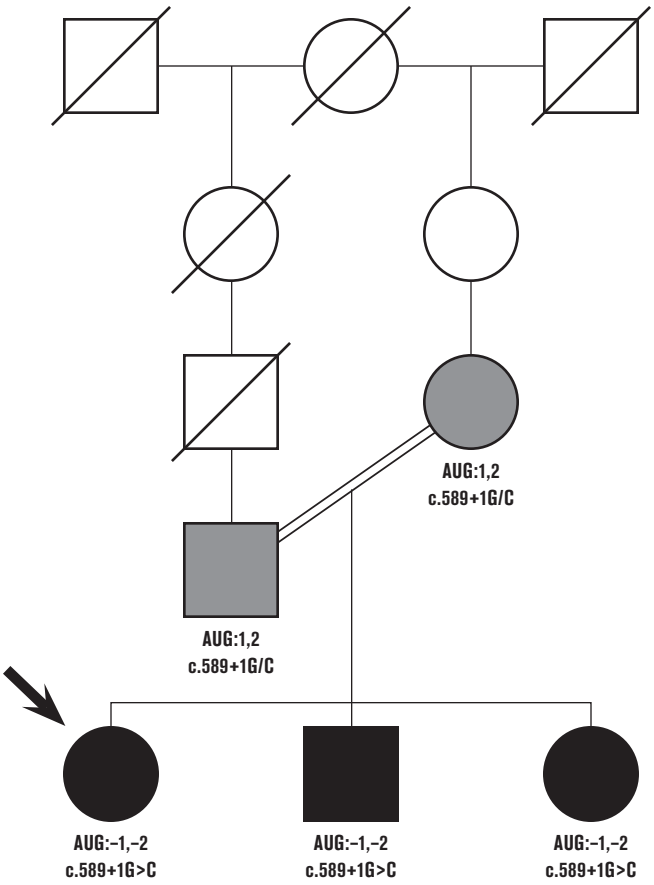

Fig. 1 Pedigree of AUG_{null} propositus (arrow), showing Augustine phenotype and *SLC29A1* intron 6 +1 genotype. Shaded squares and circles represent heterozygosity for the null allele; black squares and circles represent homozygosity for the null allele.

Clinical Significance of Augustine Antibodies

There are two reports of hemolytic transfusion reactions (HTRs) caused by anti-At^a: one was an immediate HTR with chills and nausea during a RBC survival study;[10] the other was a severe delayed HTR after transfusion of multiple units of At(a+) RBCs.[11] At^a antibodies facilitate rapid destruction of ^{51}Cr-labelled At(a+) RBCs in vivo and give positive results in in vitro functional assays.[9–10,14] Ideally, At(a–) RBC units should be selected for transfusion to patients with anti-At^a, although obtaining these units may not always be possible.

Despite numerous pregnancies involving anti-At^a, there has been no report of severe hemolytic disease of the fetus and newborn (HDFN). One case is described in which mild HDFN was treated with phototherapy.[15] In a case of twins whose RBCs gave a 3+ direct antiglobulin reaction, molecular testing was applied as an aid to antibody identification.[16]

The only known example of anti-AUG2 was probably responsible for a positive direct antiglobulin test on neonatal RBCs, but there was no evidence of HDFN.

Molecular Genetics of the Augustine Blood Group System

In 2015, the antibody, now called anti-AUG2, was used to purify a RBC membrane protein by immunoprecipitation and polyacrylamide gel electrophoresesis.[5] Three tryptic peptides were analyzed by mass spectrometry, and their sequences were identified as being encoded by *SLC29A1,* the gene for the equilibrative nucleoside transporter 1 (ENT1, or solute carrier 29A1).

Sequencing of *SLC29A1* of five At(a–) individuals showed that they were all homozygous for c.1171G>A in exon 12 (Table 1), encoding Glu391Lys in the fifth extracellular loop of ENT1 (Fig. 2).[5] Homozygosity for this nucleotide change was also found in DNA isolated from eight samples of serum containing anti-At^a, including that of the original At(a–) propositus, Mrs. Augustine. The c.1171G>A transition in *SLC29A1* corresponds to the minor (A) allele of rs4548701, which has only been detected in people of African ancestry. The U.S. National Institutes of Health Heart, Lung, and Blood Institute–sponsored Exome Sequencing Project found 49 heterozygotes in 2203 African Americans, suggesting

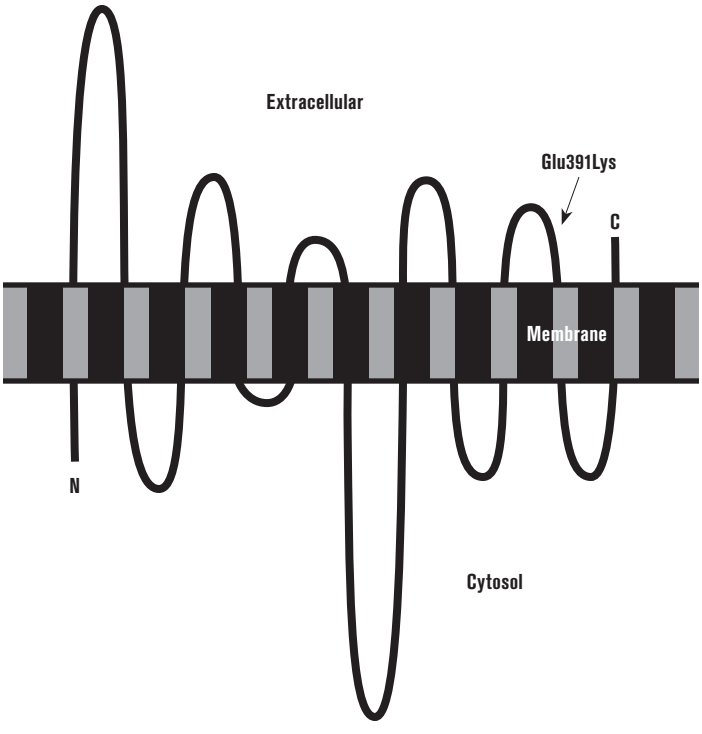

Fig. 2 Diagrammatic representation of the conformation of equilibrative nucleoside transporter (ENT1) in the red blood cell membrane, with 11 membrane-spanning domains, internal amino terminal domain (N), and external carboxy-terminal domain (C), and showing the approximate position of the At^a polymorphism.

a frequency of about 1 percent for the rare allele, but none were found in 4300 European Americans,[5] From the allele frequency, we can predict that the At(a–) phenotype would be present in about 1 in 10,000 African Americans. The A allele of rs4548701 creates the absence of a Taq$^{\alpha}$1 restriction site. Screening for rs4548701 A allele homozygosity could be used to search for At(a–) blood donors. No antibody specific for ENT1 with Lys391, which would represent anti-At^{b}, has been found.

Sequencing of *SLC29A1* of the patient who had produced anti-AUG2 and who was suspected of having the AUG_{null} phenotype revealed that she was homozygous for a G-to-C transversion at the +1 position of intron 6 (c.589+1G>C) (Table 1), a splice-site mutation that was absent from the DNA of controls and in public databases. Her two AUG:–1,–2 siblings were also homozygous for the mutation, and her consanguineous parents (Fig. 1), who were first cousins once removed, were both heterozygous for the mutation. Furthermore, immunoblotting revealed no ENT1 protein in the RBC membranes of the propositus.[5] This testing confirmed that the patient does indeed have a null phenotype and that her antibody, now called anti-AUG2, is an antibody equivalent to anti-Ku, -JK3, -FY3, and -IFC of other blood group systems.

SLC29A1 and Its Product, ENT1

SLC29A1, the gene encoding the nucleoside transporter ENT1, was initially isolated from a human placental DNA library in 1997.[15] The gene is located on chromosome 6p21.1 and is organized into 13 exons.

ENT1, a member of the equilibrative or bidirectional family of nucleoside transporters, is a 456–amino acid glycoprotein that spans the membrane 11 times and has a cytosolic N-terminal domain and an external C-terminal domain (Fig. 2).[17] ENT1, which is almost ubiquitously distributed in human tissues, facilitates the transfer of both purine and pyrimidine nucleosides, and is responsible for the majority of adenosine transport across the plasma membrane.[18] Although mice lacking ENT1 have been bred,[19] the AUG:–1,–2 propositus and her siblings were the first ENT1-deficient humans found.[5]

Because the AUG:–2 phenotype arises from homozygosity for an inactivating mutation in *SLC29A1,* it can be surmised that the AUG:–2 propositus and her siblings are deficient in ENT1 from all tissues, and some resultant morbidity might be expected. When these individuals were studied, at the ages of 45–50 years, they had no obvious developmental abnormalities, and they all had normal lifestyles.[5] Nevertheless, all had suffered frequent attacks of pseudogout, a form of arthritis, since the age of 18–20 years. In addition, X-ray analysis revealed multiple calcifications around their hand joints and, in the propositus, ectopic calcification in the hips, pubic symphysis, and lumbar discs.[5] We cannot confirm that ENT1 deficiency is responsible for these symptoms from the study of only one family, but adenine transport does appear to be an important factor in the regulation of bone metabolism,[20] and recent information obtained from *Slc29a1*$^{-/-}$ ("knockout") mice supports this premise.[21,22]

ENT1 plays an important role in cellular uptake of nucleoside analog drugs, such as ribavirin, which is often used as front-line treatment of hepatitis C virus infection.[23,24] The efficacy of such drugs could be compromised in patients with the extremely rare AUG_{null} phenotype.

SLC29A1 is highly expressed in early erythroid progenitor cells,[5] and ENT1 is abundant in the membranes of mature RBCs.[17] However, the biological importance of ENT1 in RBC function or in erythropoiesis remains to be resolved in light of the absence of any obvious erythroid abnormality associated with the AUG_{null} phenotype.

References

1. Applewhaite F, Ginsberg V, Gerena J, et al. A very frequent red cell antigen, At^{a}. Vox Sang 1967;13:444–5.
2. Gellerman MM, McCreary J, Yedinak E, Stroup M. Six additional examples of anti-At^{a}. Transfusion 1973;13:225–30.
3. Frank S, Schmidt RP, Baugh M. Three new antibodies to high-incidence antigenic determinants (anti-El, anti-Dp, and anti-So). Transfusion 1970;10:254–7.
4. Brown A, Harris P, Daniels GL, Frank S, Moore BPL, Berger R. At^{a} (August) and El (Eldr) are synonymous. Transfusion 1983;44:123–5.
5. Daniels G, Bailif BA, Helias V, et al. Lack of nucleoside transporter ENT1 results in the Augustine-null blood type and ectopic mineralization. Blood 2015;125:3651–4.
6. Storry JR, Castilho L, Chen Q, et al. International Society of Blood Transfusion Working Party on Red Cell Immunogenetics and Terminology: Report of the Seoul and London meetings. ISBT Sci Ser 2016;11:118–22.
7. Winkler MM, Hamilton JR. Previously tested donors eliminated to determine rare phenotype frequencies [abstract]. In: Proc 21st Joint Congr Int Soc Blood Transfus Am Ass Blood Banks 1990:158.
8. Vengelen-Tyler V. Efficient use of scarce sera in screening thousands of donors [abstract]. Transfusion 1985;25:476.
9. Sweeney JD, Holme S, McCall L, et al. At(a–) phenotype: description of a family and reduced survival of At(a+) red cells in a proposita with anti-At^{a}. Transfusion 1995:35:63–7.
10. Ramsey G, Sherman LA, Zimmer AM, et al. Clinical significance of anti-At^{a}. Vox Sang 1995;69:135–7.

11. Cash K, Brown T, Sausais L, et al. Severe delayed hemolytic transfusion reaction secondary to anti-At[a]. Transfusion 1999; 39:834–7.
12. Daniels G. Human blood groups. 3rd ed. Oxford, UK: Wiley-Blackwell, 2013.
13. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. London, UK: Elsevier, 2012.
14. Arndt PA, Garratty G. A retrospective analysis of the value of monocyte monolayer assay results for predicting clinical significance of blood group alloantibodies. Transfusion 2004;44:1273–81.
15. Culver PL, Brubaker DB, Sheldon RE, et al. Anti-At[a] causing mild hemolytic disease of the newborn. Transfusion 1987;27:468–70.
16. McBean R, Liew Y-W, Wilson B, et al. Genotyping confirms inheritance of the rare At(a–) type in a case of haemolytic disease of the newborn. J Pathol Clin Res 2016;2:53–5.
17. Griffiths M, Beaumont N, Yao SY, et al. Cloning of a human nucleoside transporter implicated in the cellular uptake of adenosine and chemotherapeutic drugs. Nat Med 1997;3: 89–93.
18. Young JD, Yao SY, Sun L, et al. Human equilibrative nucleoside transporter (ENT) family of nucleoside and nucleobase transporter proteins. Xenobiotica 2008;38:995–1021.
19. Grenz A, Bauerle JD, Dalton JH, et al. Equilibrative nucleoside transporter 1 (ENT1) regulates postischemic blood flow during acute kidney injury in mice. J Clin Invest 2012;122:693–710.
20. Mediero A, Cronstein BN. Adenosine and bone metabolism. Trends Endocrinol Metab 2013;24:290–300.
21. Warraich S, Bone DB, Quinonez D, et al. Loss of equilibrative nucleoside transporter 1 in mice leads to progressive ectopic mineralization of spinal tissues resembling diffuse idiopathic skeletal hyperostosis in humans. J Bone Miner Res 2013;28:1135–49.
22. Hinton DJ, McGee-Lawrence ME, Lee MR, et al. Aberrant bone density in aging mice lacking the adenosine transporter ENT1. PLoS One 2014;9:e88818.
23. Endres CJ, Moss AM, Ke B, et al. The role of the equilibrative nucleoside transporter 1 (ENT1) in transport and metabolism of ribavirin by human and wild-type or Ent1–/– mouse erythrocytes. J Pharmacol Exp Ther 2009;329:387–98.
24. Ibarra KD, Pfeiffer JK. Reduced ribavirin antiviral efficacy via nucleoside transporter-mediated drug resistance. J Virol 2009;83:4538–47.

Geoffrey Daniels, PhD, Bristol, UK, Geoff.daniels.bris@outlook.com.

Case Report

Autoanti-C in a patient with primary sclerosing cholangitis and autoimmune hemolytic anemia: a rare presentation

M. Bajpai, A. Maheshwari, S. Gupta, and C. Bihari

Primary sclerosing cholangitis (PSC) is rarely associated with autoimmune hemolytic anemia (AIHA), and the presence of specific autoantibodies has not been reported previously. We present a unique case report of PSC associated with AIHA implicating autoanti-C. A 17-year-old girl was admitted to our hospital with PSC along with AIHA. Her blood sample demonstrated a positive direct antiglobulin test and a positive autocontrol in the antihuman globulin phase, confirming the patient had warm-reactive AIHA. Further testing showed the possibility of anti-C. The patient's Rh phenotype was C+D+E−c−e+. Further testing with select cells, serial alloadsorption, and an elution confirmed anti-C specificity. The patient was transfused with two C−, crossmatch-compatible packed red blood cell units. The patient's hemoglobin level and general condition showed improvement. This unique case report shows PSC associated with AIHA caused by autoanti-C. Usually, warm AIHA presents with a panreactive pattern, and it is difficult to find compatible blood. In this rare case, we could determine the specific antibody; efforts should always be made in cases of AIHA to identify the specificity of autoantibody. ***Immunohematology* 2016;32:104–107.**

Key Words: autoimmune hemolytic anemia, primary sclerosing cholangitis, autoantibody

Autoimmune hemolytic anemia (AIHA) is an uncommon condition, with prevalence of approximately 1.7 per 100,000 individuals.[1] Usually, these antibodies are panreactive, but they may rarely exhibit specific reactivity against red blood cell (RBC) antigens. Diagnosis of AIHA is based mainly on the direct antiglobulin test (DAT). AIHA can be classified as warm type (optimal reactivity at 37°C), cold type (optimal reactivity at 4°C), or both, classified as mixed-type AIHA.[2] On the basis of the presence or absence of underlying etiology, AIHA is further divided into primary (idiopathic) or secondary (secondary to lymphoproliferative disorder, autoimmune diseases, drugs, and non-hematological malignancies).[1] Primary sclerosing cholangitis (PSC) is rarely associated with AIHA, and the presence of a specific autoantibody has not been reported previously.[3,4] We present a unique case report of PSC associated with AIHA caused by autoanti-C.

Case Report

A 17-year-old girl was admitted to our hospital with chief complaints of abdominal pain in the upper-right quadrant, breathlessness, jaundice, fatigue, weakness, and falling hemoglobin for the past 2 weeks. She had similar episodes in the past; the first at the age of 5 years (which resolved within 10–12 days), and again, 16 months later, for which she was treated with steroids and supportive therapy. There was no history of RBC transfusions, a fact that was reconfirmed in view of the long history of the disease. Her family stated that previous episodes had been mild. Family history was not relevant to the current disorder. She had no history of pregnancy or transplantation. On physical examination, she had mild hepatosplenomegaly. Lab tests revealed a hemoglobin (Hb) of 5.8 g/dL (normal range 12–16 g/dL) and a hematocrit of 18.6% (normal range 36–48%). Liver function tests revealed a total bilirubin of 2.3 g/dL (normal range 0.3–1.9 mg/dL) and increased serum alkaline phosphatase, up to 152 IU/L (normal range 44–147 IU/L).

On immunofluorescence, her anti-nuclear antibody test was positive; tests for anti-neutrophilc cytoplasmic antibodies, anti-smooth muscle antibodies, and anti-mitochondrial antibodies were all negative. Serum IgG4 levels were within the normal range. Computed tomography of the abdomen showed diffuse fusiform dilatation and narrowing of the common bile duct with multiple areas of focal fusiform dilatation in intrahepatic biliary radicles, with attenuation in both lobes of the liver. Imaging findings were indicative of PSC. Colonic biopsies were done from multiple sites to rule out ulcerative colitis; on pathological examination, these were largely unremarkable, with no evidence of inflammatory bowel disease.

Materials and Methods

The patient's blood type was group O, D+, her DAT was positive with 4+ reactivity using column agglutination

technique (CAT) with an antihuman globulin (AHG) gel card that is IgG-specific (DiaMed, Cressiers/Morat, Switzerland). Her autocontrol was negative at 4°C, room temperature, and 37°C, but showed 2+ reactivity in the AHG phase on CAT gel card, thus confirming this patient as having warm-reactive AIHA. Further testing was performed to identify the autoantibody.

First, the patient's serum was tested against a three-cell antibody screening panel, which showed reactivity in one cell in the AHG phase, and the autocontrol was positive. An 11-cell identification panel was used that showed the possibility of the antibody being of anti-C specificity (Table 1). The patient's Rh phenotype was performed showing C+D+E–/c–e+. For confirmation, three samples of C+ RBCs and three samples of C– RBCs were tested against the patient's serum (rule of 3). The C– cells were compatible and the C+ cells were incompatible with the patient's serum, confirming the specificity as anti-C. For further confirmation, we performed serial alloadsorptions with C+ RBCs; the adsorbed patient serum showed no agglutination with the panel cells, which included C+ RBCs. Furthermore, before alloadsorption, the C+ allogeneic RBCs were DAT– (control), and after alloadsorption, they became DAT+, thus confirming adsorption of anti-C (Table 2). We performed an acid elution (Gamma ELU-KIT, Immucor Medizinische Diagnostik, Rödermark, Germany) of these alloadsorbed RBCs, and the eluate showed agglutination with C+ cells and no agglutination with C– cells (control). This testing confirmed the presence of autoanti-C of warm type reacting at the AHG phase only. An eluate of the patient's RBCs was made and tested against the identification panel. The results showed a nonspecific pattern: stronger reactivity with all C+ RBCs, but weak reactivity with some C– RBCs.

As the patient's hemoglobin was continuously falling, she was treated with immunosuppressants (azathioprine and steroids); the next day, she was transfused with two C–, crossmatch-compatible units of packed RBCs with no untoward reactions. Rh-phenotype matching was not done, since we have a small inventory, and it would not have been possible. The patient's Hb showed an immediate rise from 5.8 to 9.6 g/dL and then dropped to 8.5 g/dL the next day; her general condition improved. Her Hb was maintained at this

Table 2. Reactivity of patient's serum with RBCs before and after alloadsorption

RBC phenotype	Before alloadsorption	After alloadsorption with R_1R_1 RBCs	After alloadsorption with rr RBCs
R_1R_1 (D+C+E–c–e+)	2+	Negative	2+
R_2R_2 (D+C–E+c+e–)	Negative	Negative	Negative
rr (D–C–E–c+e+)	Negative	Negative	Negative
DAT of alloadsorbed RBCs	NT	2+	Negative

RBC = red blood cell; DAT = direct antiglobulin test; NT = not tested.

Table 1. Reactivity of patient's serum with screening cells and identification panel cells

		Screening cells																		
		Rh-hr					Lewis		MNS				P	Kell		Duffy		Kidd		Results
Cell No.	RhType	D	C	c	E	e	Le^a	Le^b	M	N	S	s	P1	K	k	Fy^a	Fy^b	Jk^a	Jk^b	LISS/AHG
1	R_1wR_1	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	2+
2	R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	+	0	0	+	Neg
3	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	Neg
		Identification panel cells																		
1	R_1wR_1	+	+	0	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	2+
2	R_1R_1	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	2+
3	R_2R_2	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	+	Neg
4	r′r	0	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	2+
5	r″r	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	Neg
6	rr	0	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	+	0	Neg
7	rr	0	0	+	0	+	0	0	0	+	0	+	+	0	+	+	+	+	0	Neg
8	R_0r	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	0	+	0	Neg
9	rr	0	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	Neg
10	rr	0	0	+	0	+	+	0	0	+	0	+	+	0	+	+	0	+	0	Neg
11	rr	0	0	+	0	+	0	0	+	0	+	+	0	0	+	+	0	0	+	Neg
Autocontrol																				2+

LISS = low-ionic-strength saline; AHG = antihuman globulin; Neg = negative (no agglutination).

level with minor fluctuations for 1 month. The patient was lost to follow-up beyond that. No more transfusions were required during the rest of her hospital stay.

Discussion

PSC is an uncommon condition characterized by chronic progressive inflammatory fibrosis of the biliary tract, which can lead to biliary cirrhosis, portal hypertension, and liver failure.[5,6] The recommendation for patients younger than 25 years of age with PSC is to perform additional testing for autoimmune hepatitis. The prevalence of autoimmune hepatitis in PSC patients ranges from 1.4 percent to 17 percent.[7] Patients with PSC should also be tested for serum levels of IgG4, since a subset of these patients have markedly high levels of IgG4 (>140 mg/dL).[8] Corticosteroids and other immunosuppressant agents are more effective in patients with IgG4-related PSC than in those with non–IgG4-related PSC. In our reported case, the patient's IgG4 levels were in the normal range, but because of the autoimmune association, she was started on immunosuppressive therapy.

In the literature, a few cases have been reported showing an association between PSC and AIHA, not associated with inflammatory bowel disease, as in our case.[9,10] A study done by Vaglio et al. showed that out of 100 AIHA cases studied, 54 percent were associated with an underlying disorder; of these secondary AIHA cases, autoimmune disorders were most frequent, and the majority (64%) of antibodies detected were of the warm-reactive type.[11] In this case, similar findings were observed, since the autoantibody showed reactivity only at 37°C in the AHG phase.

Detection of the specificity of autoantibody is usually not possible, since most cases show a panreactive pattern with antibody identification cell panels. A few cases of autoantibodies against the Rh blood group system (anti-E, anti-c, anti-D, anti-e) have been reported in the past with a specific autoantibody pattern from cell identification panels.[12] To the best of our knowledge, a confirmed case of autoanti-C has not been reported until now, although it was suspected in two cases in which autoanti-C-mimicking alloantibodies had been reported previously, but phenotyping identified the patients' RBCs as C–.[12,13] Our case is unique because PSC was associated with AIHA caused by autoanti-C.

Blood transfusion support for these patients is a challenging task. Extensive immunohematological workups are required, and even then, the results are often inconclusive. When the antigen panel shows panreactivity and compatible blood is not available, transfusion should not be withheld if the patient is in critical condition and requires an urgent transfusion. Autoadsorption should be done to rule out the presence of alloantibodies in the adsorbed serum; if antibodies are detected, then antigen-negative RBCs may be transfused. In patients who are negative for alloantibodies, phenotype-matched blood may be transfused to prevent the development of alloantibodies.[14] In rare cases, when it is possible to identify the autoantibody, antigen-negative RBCs are the therapy of choice, as illustrated in this case.

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References

1. Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. J Autoimmun 2007;29:1–9.
2. Leger RM. The positive direct antiglobulin test and immune mediated hemolysis. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, eds. AABB technical manual. 18th ed. Bethseda, MD: AABB, 2014;439.
3. Moeller DD. Sclerosing cholangitis associated with autoimmune hemolytic anemia and hyperthyroidism. Am J Gastroenterol 1985;80:122–5.
4. Baker SS, Compton CC. A 17-year-old boy with autoimmune hemolytic anemia and abnormal liver function. N Engl J Med 1991;324:180–8.
5. Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. Gastroenterology 1980;79:200–6.
6. Chapman RW, Arborgh BA, Rhodes JM, et al. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. Gut 1980;21:870–7.
7. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. Lancet 2013;382:1587–99.
8. Mendes FD, Jorgensen R, Keach J, et al. Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol 2006;101:2070–5.

9. Moeller DD. Sclerosing cholangitis associated with autoimmune hemolytic anemia and hyperthyroidism. Am J Gastroenterol 1985;80:122–5.
10. Salagre KD, Sahay RN, Patil A, Joshi A, Shukla A. Leukemoid reaction, a rare manifestation of autoimmune hemolytic anemia in a case of small duct primary sclerosing cholangitis. J Assoc Physicians India 2013;61:494–5.
11. Vaglio S, Arista MC, Perrone MP, et al. Autoimmune hemolytic anemia in childhood: serologic features in 100 cases. Transfusion 2007;47:50–4.
12. Issitt PD, Pavone BG. Critical re-examination of the specificity of auto-anti-Rh antibodies in patients with a positive direct antiglobulin test. Br J Haematol 1978;38:63–74.
13. Weber J, Caceres VW, Pavone BG, Issitt PD. Allo-anti-C in a patient who had previously made an autoantibody mimicking anti-C. Transfusion 1979;19:216–8.
14. Petz LD. A physician's guide to transfusion in autoimmune haemolytic anaemia. Br J Haematol 2004;124:712–6.

Meenu Bajpai, MD, Additional Professor (corresponding author), Department of Transfusion Medicine, Institute of Liver & Biliary Sciences, Sector D-1, Vasant Kunj, New Delhi 110070, India, meenubajpai@hotmail.com; Ashish Maheshwari, MD, Senior Resident, Department of Transfusion Medicine; Shruti Gupta, MD, Senior Resident, Department of Transfusion Medicine; Chhagan Bihari, MD, Associate Professor, Department of Pathology, Institute of Liver & Biliary Sciences, New Delhi, India.

ORIGINAL REPORT

Laboratory management of perinatal patients with apparently "new" anti-D

J.L. Hannon and G. Clarke

Despite the existence of long-standing, well-organized programs for Rh immune globulin (RhIG) prophylaxis, immune anti-D continues to be detected in the D– perinatal population. Between 2006 and 2008, 91 prenatal patients, found to have a previously unidentified anti-D, were followed up with a survey to their treating physician and with additional serologic testing where possible. The physician survey requested pregnancy and RhIG history information, including recent or distant potential alloimmunizing events, and the physicians were asked their opinion on the likely cause for the anti-D. Based on survey responses, updated RhIG information, and results of follow-up serology, anti-D was determined to be attributable to previously unreported RhIG in 44 of 91 (48.3%) cases and to active immunization (immune anti-D) in 36 of 91 cases (39.6%). A probable cause for alloimmunization was reported in 14 of 52 (26.9%) returned surveys. Anti-D alloimmunization continues to occur in our prenatal population despite a comprehensive approach to RhIG therapy. Observations from this prospective patient management strategy include the need for improved application of guidelines for RhIG administration and improved quality of information provided to laboratories assessing RhIG eligibility. A laboratory process for prospective follow-up when unexpected anti-D is detected in pregnancy is recommended. ***Immunohematology* 2016;32:108–111.**

Key Words: pregnancy, prenatal, laboratory testing, alloimmunization, anti-D, Rh immune globulin, anti-D prophylaxis, practice guidelines

Despite the existence of longstanding and comprehensive programs for Rh immune globulin (RhIG) prophylaxis, immune anti-D continues to be detected in the D– perinatal population. Specific reasons for the formation of anti-D in these women are often unclear. Between 2006 and 2008, approximately 70,000 maternal samples were tested per year in our prenatal laboratory. Although exact patient numbers were not obtainable, analysis based on the average number of samples routinely received for alloimmunized and non-alloimmunized pregnancies allowed us to estimate that approximately 65,000 prenatal patients were screened annually during that time period. The majority of patients were D+ (86%) and had one sample tested during their pregnancy. However, approximately 13 percent of the maternal patient population were D–; D– women who returned for testing had an average of two samples tested per pregnancy. Patients with clinically significant antibodies including anti-D had multiple blood samples collected, depending on clinical need. Therefore, the approximately 70,000 maternal samples per year tested represented an estimated 65,000 prenatal patients who, being D– or having clinically significant antibodies, were tested on more than one occasion depending on test protocols and patient compliance.

Materials and Methods

Over a 32-month period between 2006 and 2008, our laboratory identified 91 women with anti-D in their serum in the absence of a report of RhIG prophylaxis or previously identified anti-D. A survey was prospectively distributed to the treating physician for each woman (Fig. 1). Because this quality assurance survey was performed prospectively as part of patient management and was used to assist in the determination of the need for RhIG prophylaxis, informed consent was not sought. This survey was part of the quality management approach taken to ensure that RhIG was administered appropriately. Physicians were asked to provide a history of RhIG administration during this and previous pregnancies and to report the occurrence of sensitizing events, including prior transfusions that could have contributed to alloimmunization. Physicians were also asked to comment on what they considered to be the most likely cause for the patient's anti-D. Updated RhIG information was obtained from physicians' offices for some patients. Serologic follow-up was performed where active immunization was suspected.

Results

Of the 91 surveys sent, 52 were completed and returned, for a response rate of 57.1 percent. Updated RhIG information was obtained by telephone or by survey in 18 cases, and additional serologic findings were available for 9 patients (Fig. 2). Information contained in the survey responses, together with the updated RhIG information obtained from physicians' offices and serologic findings (including titer strength and persistence of anti-D over time), were considered in assessing

Anti-D Detected in Pregnancy Quality Survey

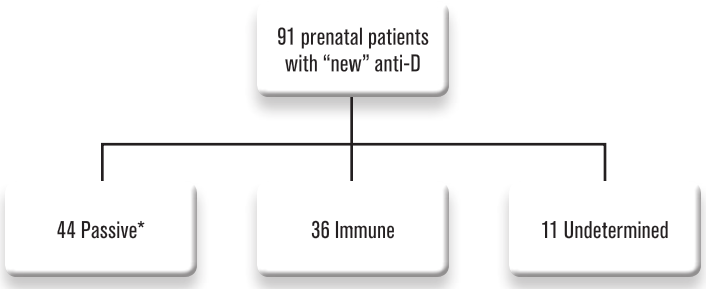
1. During pregnancy, has the patient received a routine antenatal dose of RhIG?
   ☐ Yes Dose: ____ Date: _____ Location: _____
   ☐ No
2. Was this woman known to have anti-D (unrelated to RhIG therapy) prior to this pregnancy?
   ☐ Yes — Reported by: _____ (facility/laboratory)
   ☐ No
   ☐ Unknown
3. Has the patient experienced any potential sensitizing event? If yes, please specify the event:
   ☐ Yes Event: _____ Date: _____
   ☐ No

☐ Chorionic villus sampling (CVS)	☐ Amniocentesis	☐ Vaginal bleeding
☐ Spontaneous abortion (miscarriage)	☐ Therapeutic abortion	☐ Stillbirth
☐ Ectopic pregnancy	☐ Caesarean section delivery	☐ Abdominal pain or trauma
☐ Other:		

4. If the patient experienced a sensitizing event, did she receive RhIG?
   ☐ Yes Dose: ____ Date: _____ Location: _____
   ☐ No
5. Has the patient had any previous pregnancies?
   ☐ Yes Dose: ____ Date: _____ Location: _____
   ☐ No
   ☐ Unknown
6. Has the patient had any transfusions?
   ☐ Yes Dose: ____ Date: _____ Location: _____
   ☐ No
   ☐ Unknown
7. What do you think is the most likely reason for the patient's anti-D?
   ☐ Passively acquired due to RhIG injection on _____
   ☐ RhIG was not administered following a known sensitizing event
   ☐ RhIG was not administered at 28 weeks' gestation
   ☐ RhIG was not administered following delivery of an Rh-positive baby
   ☐ RhIG failure — RhIG was administered appropriately during pregnancy (at 28 weeks and following any potential sensitizing event and following delivery of Rh-positive baby)
   ☐ Patient had an undetected sensitizing event
   ☐ Other: _____
   ☐ Unknown

Please fax completed survey to Canadian Blood Services at 780-431-8770. Thank you.

Fig. 1 Quality survey sent by the Canadian Blood Services to physicians who treated prenatal patients with apparent "new" anti-D. RhIG = Rh immune globulin.



**Based on survey responses (n = 17), updated Rh immune globulin information (n = 18), and follow-up serology (n = 9).*

Fig. 2 Summary of results of Canadian Blood Services survey and follow-up testing.

the likely cause for the newly identified anti-D. In 44 of 91 (48.3%) cases, anti-D was attributed to passive immunization as a result of RhIG administration. This information had not been provided to the laboratory when the samples were originally submitted for testing. Thirty-six of 91 (39.6%) cases were deemed to have active (immune) anti-D, as supported by the high titer of the antibody, persistence of the antibody beyond the timeframe expected for passively acquired anti-D, or survey information. In 11 of 91 (12.1%) cases, despite the fact that anti-D was present, it was not possible, based on the available data, to ascertain whether the anti-D was passive or active in nature.

In most cases in which anti-D was presumed to be immune related, physicians were unable to attribute the presence of anti-D to a specific cause and responded that the reason for the anti-D was unknown (Table 1). In four cases, physicians indicated that the anti-D was a result of RhIG having not been given after a known sensitizing event. In one of these cases, informed consent was withheld because of the religious beliefs of the patient. In the other three cases, miscarriage or termination of pregnancy occurred, and RhIG prophylaxis was not provided. Antenatal RhIG prophylaxis was not given at 28 weeks' gestation in two cases. In two other cases, RhIG was not given at the time of delivery of a D+ neonate. In five cases, anti-D was attributed to RhIG failure despite the fact that RhIG was administered appropriately for the clinical situation. In these cases, it would be interesting to know whether fetal maternal hemorrhage (FMH) screening was performed to ensure that dosing was adequate for the event being covered, but this information was not available. One patient had a previous transfusion in a foreign country, and it was not known whether this could have contributed to the development of anti-D. In 21 cases, the reason for the anti-D was stated to be unknown by the responding physician,

Table 1. Physicians report of most likely cause for anti-D in prenatal patients based on 52 completed surveys at Canadian Blood Services.

Potential cause	Number of patients	%
Unknown/no cause	21	40.4
Passive attributable to RhIG	17	32.8
RhIG failure	5	9.6
RhIG was not administered following known sensitizing event	4	7.7
RhIG was not administered at 28 weeks' gestation	2	3.8
RhIG was not administered following delivery of D+ baby	2	3.8
Other (possible transfusion-related)	1	1.9

RhIG = Rh immune globulin.

and in 17 cases, the survey identified that RhIG had been administered during the current pregnancy. These cases were subsequently classified as passive anti-D.

Based on survey results, 37 patients had a history of pregnancy-related events that could have contributed to anti-D formation. These included vaginal bleeding (n = 13), spontaneous abortion or miscarriage (n = 14), therapeutic abortion (n = 4), stillbirth (n = 1), ectopic pregnancy (n = 2), previous caesarian section (n = 4), amniocentesis (n = 3), abdominal pain of undetermined etiology or trauma (n = 2), and twin-to-twin hemorrhage (n = 2). In some cases, more than one event occurred in the same patient. Of the 36 patients with active immunization, sensitizing events were reported in 12 (33.3%). This history was largely elicited following completion of the questionnaire by the physician and was not provided when the samples were submitted to the laboratory for antenatal testing. This scenario illustrates that sensitizing events are common in pregnancy and that this information is rarely provided to the laboratory performing the prenatal serology.

Discussion

RhIG prophylaxis has been of major clinical benefit in reducing morbidity and mortality attributable to D-related hemolytic disease of the fetus and newborn. Implementation of routine antenatal and postnatal RhIG prophylaxis has led to an alloimmunization rate of less than 0.1 percent in most developed countries, including Canada.[1,2] In the 1970s in Manitoba, Canada, Bowman et al. pioneered the implementation of antenatal RhIG administration.[3,4] This practice has become the standard in North America. In 2003, an international survey questionnaire published in *Vox Sanguinis*[5] concluded that indications for anti-D prophylaxis were relatively standard across jurisdictions, with the exception of the approach to antenatal RhIG prophylaxis where countries varied in the geographic extent to which this treatment was offered. Minor variations were also reported in indications for testing for FMH screening.

Despite these efforts, a significant number of women continue to form anti-D. Between 2010 and 2013 in western Canada, clinically significant antibodies were identified in 4071 of 614,749 (0.66%) prenatal patients tested, and of that number, 489 (12%) of the antibodies reported were anti-D, although many of these were preexisting antibodies.

Persistence of Rh alloimmunization may be partially attributable to failure to adhere to specific guidelines and protocols, including failure to treat with RhIG at the time of delivery or at the time of amniocentesis or abortion, or failure of a single dose of RhIG to protect against massive transplacental hemorrhage.[1,2,6–9]

Based on our estimate of 65,000 prenatal patients tested per year in our laboratory, approximately 175,000 patients would have been tested over the 32-month timeframe of the study. Thirty-six women with previously unreported anti-D in their serum were considered to have been already immunized on the basis of survey and serologic results. This number represents 0.02 percent of the study population. This rate does not include women known to have anti-D at the time a sample was submitted to the prenatal laboratory for testing.

Several significant observations could be made based on our patient follow-up. In 8 of 91 (8.8%) of our surveyed patients, anti-D developed because of failure to administer RhIG for an established indication. In 1985, Huchcroft et al. reported an overall compliance with RhIG administration guidelines of 98.2 percent when considering only postpartum administration, but when other indications for RhIG were analyzed, the compliance rate dropped to 92.6 percent, with administration of RhIG to patients with ectopic pregnancy and antenatal hemorrhage particularly lacking.[1] Audits of adherence to RhIG administration guidelines in the UK in the late 1990s indicated that guidelines were fully adhered to in only 59–79 percent of cases and that RhIG administration was only provided in 25 percent of prenatal patients undergoing amniocentesis.[2] Our data in 2006, 20 years after the Huchcroft et al. report, indicated that a significant number of prenatal women continue to develop anti-D because of omission of RhIG in situations where this treatment is clearly indicated. This result suggests the need for ongoing education of healthcare workers administering this product.

It was notable from our survey that many patients presenting with previously unrecognized anti-D have a

history of sensitizing events that could have contributed to alloimmunization. One-third of patients with suspected active alloimmunization reported a previous sensitizing event. This information is rarely provided to the laboratory. A direct effort must be made by the laboratory to solicit this information from the treating physician.

Forty-four of 91 (48.3%) "new" anti-D cases initially detected in our laboratory were later attributed to passive anti-D from RhIG injection. This information was not provided at the time the sample was submitted, nor was administration of RhIG acknowledged on initial follow-up with the healthcare provider's office. These scenarios demonstrate the difficulties the prenatal laboratory encounters in obtaining accurate and reliable patient information. We recommend that each new prenatal patient with anti-D be followed up at the time the antibody is discovered in an effort to obtain more accurate clinical information. Ideally, records should be kept throughout the patient's pregnancy to document exact dates and details of possible events of sensitization and dosage and timing of RhIG administration. Periodic quality audits or surveys to monitor adherence to policies for RhIG administration would also be useful. The advent of electronic health records may assist in determining whether a dose of RhIG has been given before antibody investigation. Since June 9, 2011, our perinatal laboratory has been transferring results on perinatal patients to the provincial electronic patient health record. Laboratory staff gained access to patient records in autumn 2013. It will be interesting to determine whether access to this database has improved the ability of the laboratory to obtain information on RhIG administration and pregnancy complications that could lead to alloimmunization.

It may not be feasible to eliminate the risk of alloimmunization in pregnancy despite adherence to RhIG guidelines, since minor sensitizing events such as small transplacental hemorrhages may occur undetected.[7] Patients may not appreciate the need to seek medical advice when minor events such as vaginal bleeding or minor trauma occur during pregnancy. Patient education regarding the D– blood type and recommendations for reporting of minor incidents with the intent to receive RhIG prophylaxis may be helpful.

The Serious Hazards of Transfusion (SHOT) organization[10] in the UK is conducting a study of women who have produced immune anti-D that is detectable for the first time during pregnancy. They are requesting detailed information regarding previous pregnancies, sensitizing events, anti-D prophylaxis, and pregnancy outcomes in an effort to understand the cause for anti-D detected in D– prenatal patients despite availability of RhIG prophylaxis. It will be interesting to learn the outcome of this study, directed toward better understanding of a continuing and potentially preventable complication of pregnancy.

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References

1. Huchcroft S, Gunton P, Bowen T. Compliance with postpartum Rh isoimmunization prophylaxis in Alberta. Can Med Assoc J 1985;133:871–5.
2. Lloyd Jones M, Wray J, Wight, J, et al. A review of the clinical effectiveness of routine antenatal anti-D prophylaxis for rhesus-negative women who are pregnant. Br J Obstet Gynaecol 2004;111:892–902.
3. Bowman JM, Chown B, Lewis M, et al. Rh isoimmunization during pregnancy: antenatal prophylaxis. Can Med Assoc J 1978;118:623–7.
4. Bowman JM, Pollock JM. Antenatal prophylaxis of Rh isoimmunization: 28 weeks'-gestation service program. Can Med Assoc J 1978;118,:627–30.
5. De Silva M, Engelfriet CP, Reesink HW. International Forum: current status of immunoprophylaxis with anti-D immunoglobulin. Vox Sang 2003;85:328–37.
6. Bowman JM, Chown B, Lewis M, et al. Rh isoimmunization, Manitoba, 1963–1975. Can Med Assoc J 1977;116:282–4.
7. Tuohy JF, Sangalli M, Kim L. Red blood cell iso-immunization in the Wellington area of New Zealand: the case for antenatal prophylaxis. Aust NZ J Obstet Gynaecol 2004;44:458–9.
8. Hughes RG, Craig JIO, Murphy WG, et al. Causes and clinical consequences of Rhesus (D) haemolytic disease of the newborn: a study of the Scottish population, 1985–1990. Br J Obstet Gynaecol 1994;101:297–300.
9. McSweeney E, Kirkham J, Vinall P, et al. An audit of anti-D sensitization in Yorkshire. Br J Obstet Gynaecol 1998;105: 1091–4.
10. http://www.shotuk.org/reporting/anti-d-immunisation-reporting/. Accessed August 11, 2016.

Judith L. Hannon, MD, FRCPC (corresponding author), Medical Officer, Canadian Blood Services, 8249-114 Street, Edmonton, AB, Canada T6G 2R8, judy.hannon@blood.ca; Gwen Clarke, MD, FRCPC, Associate Medical Director, Canadian Blood Services, Edmonton, AB, Canada.

The H blood group system

E.A. Scharberg, C. Olsen, and P. Bugert

The H blood group system, ISBT symbol H (018), consists of a single antigen (H) defined by a terminal fucose residue found on red blood cells and in secretions formed by the action of α-1,2-fucosyltransferases 1 (α2FucT1) and 2 (α2FucT2), respectively. Mutant alleles of the corresponding *FUT1* and *FUT2* genes result in either a H– phenotype (Bombay phenotype, O_h) or a weak H phenotype (para-Bombay, $H+^w$). In addition, the *FUT2* gene is the molecular basis of the secretor (Se) status, and homozygosity or compound heterozygosity for null alleles is associated with the nonsecretor (se) status. H– individuals have natural anti-H (mostly IgM), which can cause severe hemolytic transfusion reactions with intravascular hemolysis. ***Immunohematology* 2016;32:112–118.**

Key Words: H antigen, Bombay phenotype, O_h, *FUT1, FUT2*

The H blood group system was discovered by Bhende et al.[1] in the early 1950s, when three individuals with a new unusual blood type were encountered in the city of Bombay (Mumbai) in India. The red blood cells (RBCs) of all three individuals typed as blood group O, but their serum samples were found to contain anti-A, anti-A1, anti-B, and anti-H and to agglutinate RBCs of all "ordinary" ABO groups.[1] Their samples were serologically compatible only with the RBCs of one another and contained the first examples of potent human anti-H in individuals with RBCs that, based on tests with anti-A and anti-B, appeared to be homozygous for a "new" allelomorph at the A1 A2 B O locus.[1] The phenotype was called Bombay phenotype. Further test results showed that the RBCs of these individuals did not react with human or animal anti-H sera. After the cloning and characterization of the *FUT1* gene[2] in 1990 and proving that the gene was the molecular basis of the Bombay phenotype[3] in 1994, the H blood group system was established.

This unusual blood group system has only one antigen (H). Complete lack of H is commonly known as the Bombay or O_h phenotype, and weak expression of H is referred to as the para-Bombay phenotype. The lack of H results in production of the corresponding naturally occurring antibodies, anti-A, anti-B, and anti-H.[4,5]

Biochemistry

The H antigen is a terminal fucose residue in an α-1,2-linkage on precursor carbohydrate chains of two different types (Fig. 1). The H antigen on the type 1 precursor is predominantly produced by the α-1,2-fucosyltransferase 2 (α2FucT2) enzyme in secretory cells of the digestive and respiratory tracts. The α-1,2-fucosyltransferase 1 (α2FucT1) enzyme is a single-pass type II transmembrane glycoprotein found in the Golgi apparatus that forms the H antigen on type 2 precursor chains in erythroid tissues and vascular endothelial cells.[6,7] The carbohydrate chain including the H antigen is then the substrate of the glycosyltransferases encoded by the *ABO* gene to produce A and/or B antigens. Subsequently, in blood group O individuals, the H antigen is not converted to A or B and is strongly detectable on RBCs.

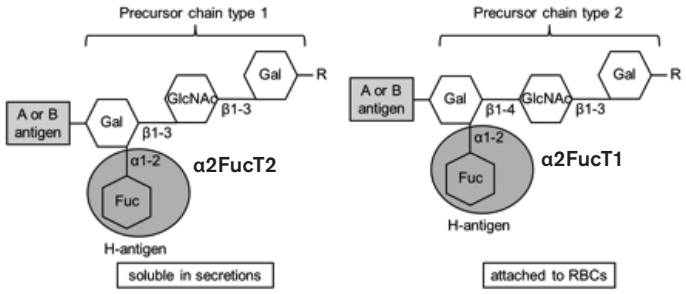

Fig. 1 Schematic illustration of the carbohydrate precursor chains used as substrates by the α2FucT1 and α2FucT2 enzymes encoded by the *FUT1* and *FUT2* genes, respectively. RBCs = red blood cells.

H is found in highest amounts in group O individuals and in least amounts in group A, B, and AB individuals. This is because when the A and/or B enzymes are present, H antigen is converted to A and/or B antigen, resulting in less H antigen present on the cell membrane. H therefore varies in quantity according to the blood group as follows: $O > A_2 > B > A_2B > A_1 > A_1B$.

Antibodies in the H Blood Group System

Anti-H is the only antibody specificity found in the H blood group system. This antibody is naturally occurring in individuals who lack H, as is the case with ABO blood group antibodies. The serum of O_h individuals always contains anti-H, -A, and -B. Occasionally, A_1, A_1B, and (less commonly) B individuals expressing only a low quantity of H can produce anti-H, but the antibody is mostly weakly reactive and usually

considered clinically insignificant, unlike the anti-H in H– individuals, which can cause severe intravascular hemolysis.

Anti-H is most often type IgM, but it can have an IgG component. Like the isoagglutinins, it is best reactive by the saline room temperature method, but can also react in the indirect antiglobulin test (IAT), especially when an IgG component is present. Anti-H can activate complement, and is capable of causing intravascular hemolysis of RBCs. When anti-H is identified in pregnant women, tests using dithiothreitol can be performed to determine the presence or absence of IgG antibodies, and, if present, the titer of the IgG component.

Anti-H produced by Bombay phenotype individuals recognizes H on both type 1 (secretions) and type 2 (RBCs) chains, whereas the anti-H produced by Bombay secretors (hh, Se) reacts preferentially with type 2H. Anti-H lectin (*Ulex europaeus*) can be used to type individuals for the presence of H antigen. It is notable that the lectin reacts best with type 2H individuals, but has some specificity for type 1H. There are other lectins (*Lotus tetragonolobus, Anguilla anguilla, Cysticus sessifolius,* and *Laburnum alpinum*) that recognize H antigen and also monoclonal antibodies with 1H and H specificity.

Autoanti-H and autoanti-IH are primarily autoantibodies that are encountered in a blood transfusion setting often in group A_1 and B patients. These antibodies have been reported in individuals with cold agglutinin syndrome and should be considered in the presence of cold agglutinin testing by including RBCs of different ABO groups and with varying amounts of H and I antigens. The antibody reacts more avidly with samples with enhanced I and H antigens such as group A_2 and O RBCs, and should be most compatible with group A_1 and cord RBCs (having the least quantity of H and I antigen).

Molecular Basis

The *FUT1* and *FUT2* genes are closely linked and located on chromosome 19q13.3.[8] In genomic sequence annotation release 107 from March 2015, the two genes are in opposite orientation with a distance of approximately 42,000 base pairs (bp) (Fig. 2). The *SEC1P* pseudogene upstream of *FUT2* is highly homologous to the *FUT* genes but inactive because of deletions and mutations in the coding region. The molecular cloning and characterization of the *FUT1* gene was first reported in 1990.[2] The genomic structure of the *FUT1* gene includes four exons spanning a region of approximately 7800 bp. The entire coding sequence (CDS) is located within exon 4 and encodes a 365–amino acid polypeptide.[2] The encoded α2FucT1 enzyme adds α-L-fucose to the type 2 precursor chain on RBCs and other cells. The 5′-untranslated region with exons 1–3 contains two distinct promoters with transcription start sites upstream of exon 1 and exon 2.[9] Alternative splicing of the *FUT1* transcripts was reported for different cell types. Recently, it was shown that the transcription factor c-jun binds to an AP-1 site in the promoter region upstream of exon 1 and thereby activates transcription of the *FUT1* gene.[10] The *FUT2* gene has only two exons, and the entire CDS is in exon 2. The encoded α2FucT2 enzyme adds α-L-fucose to the type 1 precursor chain in secretions.[3] Two transcript variants with an upstream and a downstream translation initiation site encode a 343– and a 332–amino acid protein, respectively. The smaller protein is considered to be the active enzyme. This transcriptional regulation by c-jun was not found for the *FUT2* gene.[10]

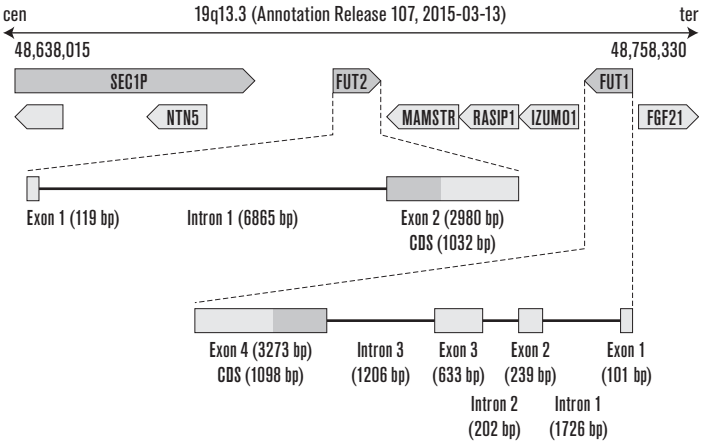

Fig. 2 Gene map of the chromosome 19q13.3 region from centromer (cen; left) to telomer (ter; right) including the *SEC1P, CA11, NTN5, FUT2, MAMSTR, RASIP1, IZUM01, FUT1* and *FGF21* genes according to the National Center for Biotechnology Information (NCBI) genome database annotation release 107 (March 2015). The genomic organization of *FUT1* and *FUT2* indicates that the coding sequence (CDS; dark gray box) of each gene is located within one exon (exon 4 of *FUT1* and exon 2 of *FUT2*).

At least one functioning copy of *FUT1* needs to be present (H/H or H/h) for H to be expressed on RBCs. If both copies of *FUT1* are inactive (h/h), the Bombay phenotype (O_h) results, and there is no expression of A, B, or H antigens on the RBCs. The inactive null alleles of *FUT1* (*FUT1*01N*) are characterized by nonsense mutations causing a stop codon and a truncated protein or by missense mutations causing a single amino acid exchange in the catalytic domain of the enzyme (Table 1).[11,12] Homozygosity or compound heterozygosity for null alleles leads to the h/h phenotype, which is characterized by absence of a functional fucosyltransferase adding fucose to the precursor chain type 1 on RBCs. Many missense

Table 1. Null alleles of the *FUT1* gene leading to a lack of H-antigens on red blood cells

Allele name (ISBT)	Nucleotide change	Amino acid change	Type of mutation	Reference
*FUT1*01N.01*	422G>A	Trp141Ter	Nonsense	27
*FUT1*01N.02*	461A>G	Tyr154Cys	Missense	19, 28
*FUT1*01N.03*	462C>A	Tyr154Ter	Nonsense	29
*FUT1*01N.04*	513G>C	Trp171Cys	Missense	28
*FUT1*01N.05*	538C>T	Gln180Ter	Nonsense	27
*FUT1*01N.06*	547_548delAG	183fs	Frameshift	20
*FUT1*01N.07*	586C>T	Gln196Ter	Nonsense	30
*FUT1*01N.08*	695G>A	Trp232Ter	Nonsense	31
*FUT1*01N.09*	725T>G	Leu242Arg	Missense	19, 32
*FUT1*01N.10*	776T>A	Val259Glu	Missense	28
*FUT1*01N.11*	785G>A; 786C>A	Ser262Lys	Missense	33
*FUT1*01N.12*	826C>T	Gln276Ter	Nonsense	10
*FUT1*01N.13*	880_882delTT	294fs	Frameshift	20
*FUT1*01N.14*	944C>T	Ala315Val	Missense	28
*FUT1*01N.15*	948C>G	Tyr316Ter	Nonsense	10
*FUT1*01N.16*	980A>C	Asn327Thr	Missense	34
*FUT1*01N.17*	1047G>C	Trp349Cys	Missense	28
*FUT1*01N.18*	684G>A	Met228Ile	Missense	12
*FUT1*01N.19*	694T>C	Trp232Pro	Missense	12
*FUT1*01N.20*	764_768delC	256fs	Frameshift	35
*FUT1*02N.01*	423G>A 392T>C 668-670delACT	Trp141Ter Leu131Pro Tyr224del	Nonsense Nonsense In-frame del	35 36 36

ISBT = International Society of Blood Transfusion; del = deletion.

Table 2. Missense mutations of the *FUT1* gene leading to a weak expression of H antigens on red blood cells

Allele name (ISBT)	Nucleotide change	Amino acid change	Reference
*FUT1*01W.01*	293C>T	Thr98Met	37
*FUT1*01W.02*	328G>A	Ala110Thr	30
*FUT1*01W.03*	349C>T	His117Tyr	19
*FUT1*01W.04*	442G>T	Asp148Tyr	31
*FUT1*01W.05.01*	460T>C	Tyr154His	20, 31
*FUT1*01W.05.02*	460T>C; 1042G>A	Tyr154His; Glu348Lys	31
*FUT1*01W.07*	491T>A	Leu164His	10
*FUT1*01W.08*	522C>A	Phe174Leu	38, 39
*FUT1*01W.09*	658C>T	Arg220Cys	20, 39, 40
*FUT1*01W.10*	659G>A	Arg220His	12, 39
*FUT1*01W.11*	661C>T	Arg221Cys	41
*FUT1*01W.12*	682A>G	Met228Val	42, 43
*FUT1*01W.13*	689A>C	Gln230Pro	27
*FUT1*01W.14*	721T>C	Tyr241His	31
*FUT1*01W.15*	801G>C	Trp267Cys	44
*FUT1*01W.16*	801G>T	Trp267Cys	44
*FUT1*01W.17*	832G>A	Asp278Asn	44
*FUT1*01W.19*	917C>T	Thr306Ile	27
*FUT1*01W.21*	235G>C	Gly79Arg	40
*FUT1*01W.22*	991C>A	Pro331Thr	45
*FUT1*01W.23*	424C>T	Arg142Trp	46
*FUT1*01W.24*	649G>T	Val217Phe	35
*FUT1*01W.25*	235G>C	Gly79Arg	40
*FUT1*01W.26*	545G>A	Arg182His	11, 12
*FUT1*01W.27*	958G>A	Gly320Arg	11, 12
*FUT1*01W.28*	896A>C	Gln299Pro	47
*FUT1*01W.29*	655G>C	Val219Leu	48
*FUT1*02W.01*	269G>T	Gly90Val	12
*FUT1*02W.02*	371T>G	Phe124Cys	12
*FUT1*02W.04*	980A>C	Asn327Thr	12, 39
*FUT1*02W.05*	748C>T 366-398del33	Arg250Trp Val123-Pro133del	12 49

ISBT = International Society of Blood Transfusion; del = deletion.

mutations of *FUT1* (*FUT1*01W*) have been described (Table 2), leading to a weak expression of H on RBCs typical for the para-Bombay phenotype ($H+^{W}$).[11,12]

The *FUT2* gene encodes α2FucT2, which synthesizes the H antigen found in body fluids, and is associated with a secretor (Se) status. Homozygosity or compound heterozygosity for null alleles is associated with the nonsecretor (se) status. In Europeans, the 428G>A nonsense mutation (Trp143ter) is the most common cause of the nonsecretor status. All other null alleles and *FUT2* gene deletions are rare variants (Table 3).[11,12] Because of the sequence homology with *SEC1P,* unbalanced recombination leading to *SEC1P-FUT2* hybrid genes or *FUT2* gene deletions have been reported as the molecular basis of the nonsecretor status.[13,14] Additionally, nonsecretors express only the Le^{a} antigen of the Lewis blood group system because the α1,3/4-fucosyltranferase encoded by the *FUT3* gene uses the type 1 precursor. In secretors, the type 1 precursor carries the H antigen, and the carbohydrate structure can be converted to A or B blood group antigens as well as to the Le^{b} antigen. Interestingly, *FUT2* mutations leading to an inefficient α2FucT2 enzyme such as 385A>T (Ile129Phe) are associated with the Le^{ab} phenotype because less H type 1 results in less Le^{b}, and the *FUT3* enzyme more efficiently converts type 1 precursor to Le^{a}.[15]

In summary, for the genotype–phenotype correlation in the H blood group system, it is important to consider the genotype of both genes, *FUT1* and *FUT2*. For the classic

Table 3. Null alleles of the *FUT2* gene conferring the nonsecretor status

Allele name (ISBT)	Nucleotide change	Amino acid change	Type of mutation	Reference
*FUT2*01N.01*	244G>A; 385A>T	Ala82Thr; Ile129Phe	Missense Missense	50
*FUT2*01N.02*	428G>A; 739A>G	Trp143Ter; Gly247Ser	Nonsense Missense	19, 45, 51
*FUT2*01N.03*	569G>A	Arg190His	Missense	50
*FUT2*01N.04*	571C>T	Arg191Ter	Nonsense	52
*FUT2*01N.05*	628C>T	Arg210Ter	Nonsense	14
*FUT2*01N.06*	658C>T	Arg220Ter	Nonsense	53
*FUT2*01N.07*	664C>T	Arg222Cys	Missense	52
*FUT2*01N.08*	685_686delGT	230fs234Ter	Frameshift	
*FUT2*01N.09*	688_690delGTC	del230Val	In-frame del	54, 55
*FUT2*01N.10*	400G>A; 760G>A	Val134Ile; Asp254Asn	Missense Missense	52
*FUT2*01N.11*	778delC	259fs275Ter	Frameshift	56
*FUT2*01N.12*	849G>A	Trp283Ter	Nonsense	57
*FUT2*01N.13*	868G>A	Gly290Arg	Missense	52
*FUT2*01N.14*	950C>T	Pro317Leu	Missense	50
*FUT2*01N.15*	302C>T	Pro101Leu	Missense	58
*FUT2*01N.16*	960A>G	Gly247Ser	Missense	45, 51, 52
*FUT2*01N.17*	412G>A	Gly138Ser	Missense	45
*FUT2*01N.18*	818C>A	Thr273Asn	Missense	59
*FUT2*0N.01*	Gene deletion		Deletion	19, 32, 60
*FUT2*0N.02*	Coding region deleted		Deletion	61
*FUT2*0N.03*	Fusion gene 1 between *FUT2* and *SEC1P*		Recombination	14, 50, 62
*FUT"*0N.04*	Fusion gene 2 between *FUT2* and *SEC1P*		Recombination	50

ISBT = International Society of Blood Transfusion; del = deletion.

Bombay phenotype (O_h), both genes are inactive (genotype: *h/h* and *se/se*) and no H antigens are expressed on RBCs or in secretions. For the para-Bombay phenotype ($H+^W$), two types can be distinguished:

(1) lack of H on RBCs caused by inactive *FUT1* null alleles (*FUT1*01N*) and presence of H in secretions caused by an active *FUT2* gene (Se);
(2) weak expression of H on RBCs caused by *FUT1* mutant alleles (*FUT1*01M*) encoding weakly active fucosyltransferases in combination with active or inactive *FUT2* (Se or se).

Particularly for the type 1 para-Bombay phenotype, it is important to note that the H antigens produced by *FUT2* in secretions can be absorbed by RBCs. The $H+^W$ cells can be converted to $A+^W$ or $B+^W$ when the corresponding glycosyltransferases are encoded by the ABO locus.

Table 4. Distribution of O_h phenotype cases reported from different states of India

State	Number of O_h phenotype cases
Andhra Pradesh	8
Bihar	2
Goa	6
Gujarat	5
Karnataka	14
Kerala	4
Madhya Pradesh	4
Maharashtra	112
North India (unclassified)	2
Orissa	1
Pondichery	1
Rajasthan	2
South India (unclassified)	1
Tamil Nadu	2
Uttar Pradesh	5
Not Known	10
Total	**179**

Compiled from Lowe.[6]

Populations

The Bombay phenotype is almost unique to Indian ethnic groups, with a prevalence of about 0.7 percent, and is rare in most populations. The greater numbers of cases are situated in the southern states of India (Table 4).[16] Further studies published in 2007 of the Bhuyan tribal population of Orissa, India, revealed the average prevalence of the Bombay phenotype to be 1 in 278.[17] The prevalence therefore varies depending on the number of global migrants originating from these regions. A study on people living on Reunion Island (east of Madagascar) revealed a second geographic "hot spot" of the O_h phenotype with a high prevalence (1:1000).[18,19] Interestingly, although the largest series of H– phenotypes in Bombay and Natal were of Indian ethnicity, 85 percent of the Reunion O_h phenotype were of European descent (mostly of French origin). The O_h and para-Bombay phenotype has also been found in other ethnic groups such as Taiwanese (1:8000), Hong Kong Chinese (1:15,620), and those of European origin (1:1,000,000).[20,21]

In summary, *FUT1* mutant alleles are rare, and their geographic distribution varies greatly. The frequency of *FUT2* null alleles may be 20 percent or higher. A linkage disequilibrium of mutations in *FUT1* and *FUT2* was observed, and is attributable to the close proximity of both genes on chromosome 19q13.3.[19] Individuals of particular ethnic groups have a unique set of mutations: in India, *FUT1* 725T>G with *FUT2* deletion; on Reunion Island (Caucasian), *FUT1* 349C>T with *FUT2* 428G>A.

Clinical Significance

Anti-H in O_h individuals can cause severe hemolytic transfusion reactions with intravascular hemolysis. Anti-H and anti-IH, which are sometimes found in the serum of A1 people, although generally not considered clinically significant, have been reported to cause hemolytic transfusion reactions when the antibodies are demonstrable and reactive by the IAT.[22,23] Such patients should receive cross-match compatible A1 (type-specific) RBC concentrates.

Theoretically, anti-H during pregnancy could cause hemolytic disease of the fetus and newborn (HDFN) in a non-O_h fetus. In practice, however, cases of HDFN have not been described. In an evaluation performed in South Africa of 21 O_h pregnancies/births, only one baby required transfusion, and none were seen to have HDFN.

The function of the H antigen, besides being a precursor for the synthesis of ABO blood group antigens, is not well known; although, it may be involved in cell adhesion. Individuals lacking H do not seem to suffer any ill effects.

The leukocyte adhesion deficiency II (LADII) is a rare inherited primary immunodeficiency disorder resulting from a defect in fucose metabolism. Patients with this disorder are unable to fucosylate glycoproteins, including the H blood group polysaccharide and, therefore, present serologically as O_h blood group. This disorder is characterized by recurrent infections in the patients, persistent leucocytosis, and severe mental and growth retardation.[24] Treatment with oral L-fucose reverses most of the symptoms in these patients, but has no effect on true O_h individuals.

Recent reports indicate that *FUT1* significantly contributes to angiogenesis in the context of rheumatoid arthritis and ovarian cancer.[9,25,26] It is speculated that the higher degree of fucosylation of molecules important for cell adhesion, such as the Lewis antigens, promotes inflammatory and angiogenic mechanisms.

Summary

The H blood group system currently consists of the antigen H. Complete lack of H results in the Bombay (O_h) phenotype and weak expression of H is known as the para-Bombay phenotype. There are a number of genetic mutations that have been found to be responsible for the O_h (*FUT1*01N* alleles) and the para-Bombay (*FUT1*01W* alleles) types. The *FUT1* locus and *FUT2* locus are closely linked, which can result in individuals who may have weak expression or no H expressed on their RBCs, although H may be found in secretions. The H antigen is the precursor for the ABO blood group antigens, and therefore plays a significant role in the serologic blood type of an individual. As with ABO, the antibodies produced by individuals who lack H can cause severe hemolytic reactions, although there have not been many reported cases of obstetric significance.

References

1. Bhende YM, Deshpande CK, Bhatia HM, et al. A new blood group character related to the ABO system. Lancet 1952;1: 903–4.
2. Larsen RD, Ernst LK, Nair RP, et al. Molecular cloning, sequence, and expression of a human GDP-L-fucose: beta-D-galactoside 2-alpha-L-fucosyltransferase cDNA that can form the H blood group antigen. Proc Natl Acad Sci U S A 1990;87: 6674–8.
3. Kelly RJ, Ernst LK, Larsen RD, Bryant JG, Robinson JS, Lowe JB. Molecular basis for H blood group deficiency in Bombay (Oh) and para-Bombay individuals. Proc Nat Acad Sci U S A 1994;91:5843–7.
4. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. New York: Elsevier Academic Press, 2012.
5. Daniels G. Human blood groups. 3rd ed. Hoboken, NJ: Wiley-Blackwell, 2013.
6. Lowe JB. Biochemistry and biosynthesis of ABH and Lewis antigens: characterization of blood group-specific glycosyltransferases. In: Cartron J-P, Rouger P, eds. Molecular basis of human blood group antigens. New York: Plenum Press, 1995:5–115.
7. Oriol R. ABO, Hh, Lewis and secretion: serology, genetics and tissue distribution. In: Cartron J-P, Rouger P, eds. Molecular basis of human blood group antigens. New York: Plenum Press, 1995:37–73.
8. Rouquier S, Lowe JB, Kelly RJ, et al. Molecular cloning of a human genomic region containing the H blood group alpha (1,2) fucosyltransferase gene and two H locus–related DNA restriction fragments: isolation of a candidate for the human Secretor blood group locus. J Biol Chem 1995;270:4632–9.
9. Koda Y, Soejima M, Kimura H. Structure and expression of the gene encoding secretor-type galactoside 2-alpha-L-fucosyltransferase (FUT2). Eur J Biochem 1997;246:750–5.

10. Gao N, Liu J, Liu D, et al. c-Jun transcriptionally regulates alpha 1, 2-fucosyltransferase 1 (FUT1) in ovarian cancer. Biochimie 2014;107:286–92.
11. dbRBC. H blood group system. http://www.ncbi.nlm.nih.gov/projects/gv/rbc/xslcgi.fcgi?cmd=bgmut/systems_info&system=hh.
12. ISBT. Red cell immunogenetics and blood group terminology. http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/.
13. Soejima M, Fujihara J, Takeshita H, Koda Y. Sec1-FUT2-Sec1 hybrid allele generated by interlocus gene conversion. Transfusion 2008;48:488–92.
14. Koda Y, Soejima M, Liu Y, Kimura H. Molecular basis for secretor type alpha(1,2)-fucosyltransferase gene deficiency in a Japanese population: a fusion gene generated by unequal crossover responsible for the enzyme deficiency. Am J Hum Genet 1996;59:343–50.
15. Henry S, Mollicone R, Fernandez P, Samuelsson B, Oriol R, Larson G. Molecular basis for erythrocyte Le(a+ b+) and salivary ABH partial-secretor phenotypes: expression of a FUT2 secretor allele with an A-->T mutation at nucleotide 385 correlates with reduced alpha(1,2) fucosyltransferase activity. Glycoconj J 1996;13:985–93.
16. Balgir RS. Detection of a rare blood group "Bombay Oh Phenotype" among the Kutia Kondh primitive tribe of Orissa, India. Indian J Hum Genet 2005;5:193–8.
17. Balgir RS. Identification of rare blood group in Bhuyan tribe of Orissa, India. Indian J Hum Genet 2007;13:109 –13.
18. Gerard G, Vitrac D, Le Pendu J, Muller A, Oriol R. H-deficient blood groups (Bombay) of Reunion Island. Am J Hum Genet 1982;34:937–47.
19. Fernandez-Mateos P, Cailleau A, Henry S, et al. Point mutations and deletion responsible for the Bombay H null and the Reunion H weak blood groups. Vox Sang 1998;75:37–46.
20. Yu LC, Yang YH, Broadberry RE, Chen YH, Lin M. Heterogeneity of the human H blood group alpha(1,2)fucosyltransferase gene among para-Bombay individuals. Vox Sang 1997;72:36–40.
21. Chi Q, Tang W, Wang CQ, Chen Y, Chen GL, Guo YJ. Molecular genetics analysis and frequency survey of H deficient phenotype. Chin J Blood Transfus (Chinese) 2006;19:445–8.
22. Irani MS, Richards C. Haemolytic transfusion reaction due to anti-IH. Transfusion 2011;51:2676–8.
23. Campbell SA, Shirey RS, King KE, Ness PM. An acute haemolytic transfusion reaction due to anti-IH in a patient with sickle cell disease. Transfusion 2000;40:828–31.
24. Becker DJ, Lowe JB. Leukocyte adhesion deficiency type II. Biochim Biophys Acta 1999;1455:193–204.
25. Isozaki T, Ruth JH, Amin MA, et al. Fucosyltransferase 1 mediates angiogenesis, cell adhesion and rheumatoid arthritis synovial tissue fibroblast proliferation. Arthritis Res Ther 2014;16:R28.
26. Wang H, Deng L, Cai M, et al. Annexin A4 fucosylation enhances its interaction with the NF-kB p50 and promotes tumor progression of ovarian clear cell carcinoma. Oncotarget (in press) doi: 10.18632/oncotarget.10226.
27. Storry JR, Johannesson JS, Poole J, et al. Identification of six new alleles at the FUT1 and FUT2 loci in ethnically diverse individuals with Bombay and para-Bombay phenotypes. Transfusion 2006;46:2149–55.
28. Wagner FF, Flegel WA. Polymorphism of the h allele and the population frequency of sporadic nonfunctional alleles. Transfusion 1997;37:284–90.
29. Ogasawara K, Nakata K, Watanabe J, et al. New FUT1(H) alleles identified from H-deficient phenotype individuals. Vox Sang 2000;78(Suppl 1):4.
30. Guo ZH, Xiang D, Zhu ZY, et al. Analysis on FUT1 and FUT2 gene of 10 para-Bombay individuals in China. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2004;21:417–21.
31. Kaneko M, Nishihara S, Shinya N, et al. Wide variety of point mutations in the H gene of Bombay and para-Bombay individuals that inactivate H enzyme. Blood 1997;90:839–49.
32. Koda Y, Soejima M, Johnson PH, Smart E, Kimura H. Missense mutation of FUT1 and deletion of FUT2 are responsible for Indian Bombay phenotype of ABO blood group system. Biochem Biophys Res Commun 1997;238:21–5.
33. Wagner T, Vadon M, Staudacher E, et al. A new h allele detected in Europe has a missense mutation in alpha(1,2)-fucosyltransferase motif II. Transfusion 2001;41:31–8.
34. Luo G, Wei L, Wang Z, et al. The summary of FUT1 and FUT2 genotyping analysis in Chinese para-Bombay individuals including additional nine probands from Guangzhou in China. Transfusion 2013;53:3224–9.
35. Cai XH, Jin S, Liu X, Fan LF, Lu Q, Xiang D. Molecular genetic analysis for the para-Bombay blood group revealing two novel alleles in the FUT1 gene. Blood Transfus 2011;9:466–8.
36. Zanjani DS, Afzal Aghaee M, Badiei Z, et al. Molecular basis of Bombay phenotype in Mashhad, Iran: identification of a novel FUT1 deletion. Vox Sang 2016;111:88–92.
37. Yan L, Zhu F, Xu X, Hong X, Lv Q. Molecular basis for para-Bombay phenotypes in Chinese persons, including a novel nonfunctional FUT1 allele. Transfusion 2005;45:725–30.
38. Yip SP, Chee KY, Chan PY, Chow EY, Wong HF. Molecular genetic analysis of para-Bombay phenotypes in Chinese: a novel non-functional FUT1 allele is identified. Vox Sang 2002;83:258–62.
39. Chen DP, Tseng CP, Wang WT, et al. Two prevalent h alleles in para-Bombay haplotypes among 250,000 Taiwanese. Ann Clin Lab Sci 2004;34:314–8.
40. Xu X, Tao S, Ying Y, et al. A novel FUT1 allele was identified in a Chinese individual with para-Bombay phenotype. Transfus Med 2011;21:385–93.
41. Samuelsson J, Staples N, Condon J, et al. First example of the para-Bombay phenotype in an Australian proband is encoded by a novel FUT1 allele. In: Annual meeting of Australian and New Zealand Society for Blood Transfusion, Sydney, Australia, October 2005: Abstract 47.
42. Yan LX, Xu XG, Hong XZ, Wu JJ, Zhu FM, Fu QH. Identification of a novel FUT1 allele derived from the alpha-(1,2)-fucosyltransferase gene through a nucleotide substitution 682A>G. Transfus Med 2006;16:447–9.
43. Tao SD, He YM, Hong XZ, et al. Study on the expression stability of mutant alpha-1,2 fucosyltransferase gene 293C to T and 658C to T in eukaryotic cells. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2011;28:521–4.
44. Johnson PH, Mak MK, Leong S, et al. Analysis of mutations in the blood-group H gene in donors with H-deficient phenotypes. Vox Sang 1994;67(Suppl 2):25.

45. Matzhold EM, Helmberg W, Wagner T, et al. Identification of 14 new alleles at the fucosyltransferase 1, 2, and 3 loci in Styrian blood donors, Austria. Transfusion 2009;49:2097–108.
46. Chi Q, Zhang A, Ren B, Guo Y. Genetic analysis of 16 para-Bombay phenotype individuals: a novel non-functional FUT1 allele and a new evidence of genetic linkage within FUT1 and FUT2 gene. Vox Sang 2011;101(Suppl 2):108.
47. Liang W, Xu H, Liu YY, et al. Molecular genetic analysis of para-Bombay phenotype in Chinese persons: a novel FUT1 allele is identified. Transfusion 2015;55:1588.
48. He Z, Liu F. Two novel α 1,2-fucosyltransferase alleles in an H-deficient phenotype individual. Transfus Med Hemother 2014;41:375–9.
49. Su Yq, Wei TL, Yu Q, Liang YL, Li DC. Molecular genetic analysis of FUT1 and FUT2 gene in para-Bombay Chinese: a novel FUT1 allele is identified. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2007;24:520–3.
50. Soejima M, Nakajima T, Fujihara J, Takeshita H, Koda Y. Genetic variation of FUT2 in Ovambos, Turks, and Mongolians. Transfusion 2008;48:1423–31.
51. Yip SP, Lai SK, Wong ML. Systematic sequence analysis of the human fucosyltransferase 2 (FUT2) gene identifies novel sequence variations and alleles. Transfusion 2007;47: 1369–80.
52. Koda Y, Ishida T, Tachida H, et al. DNA sequence variation of the human ABO-secretor locus (FUT2) in New Guinean populations: possible early human migration from Africa. Hum Genet 2003;113:534–41.
53. Liu YH, Koda Y, Soejima M, et al. The fusion gene at the ABO-secretor locus (FUT2): absence in Chinese populations. J Hum Genet 1999;44:181–4.
54. Yu LC, Lee HL, Chu CC, Broadberry RE, Lin M. A newly identified nonsecretor allele of the human histo-blood group alpha(1,2)fucosyltransferase gene (FUT2). Vox Sang 1999;76:115–9.
55. Yu LC, Chu CC, Chan YS, et al. Polymorphism and distribution of the Secretor alpha(1,2)-fucosyltransferase gene in various Taiwanese populations. Transfusion 2001;41:1279–84.
56. Liu Y, Koda Y, Soejima M, Pang H, Schlaphoff T, du Toit ED, Kimura H. Extensive polymorphism of the FUT2 gene in an African (Xhosa) population of South Africa. Hum Genet 1998;103:204–10.
57. Yu LC, Broadberry RE, Yang YH, Chen YH, Lin M. Heterogeneity of the human Secretor alpha(1,2)fucosyltransferase gene among Lewis(a+b-) non-secretors. Biochem Biophys Res Commun 1996;222:390–4.
58. Chang JG, Yang TY, Liu TC, et al. Molecular analysis of secretor type alpha(1,2)-fucosyltransferase gene mutations in the Chinese and Thai populations. Transfusion 1999;39:1013–7.
59. Soejima M, Fujimoto R, Agusa T, et al. Genetic variation of FUT2 in a Vietnamese population: identification of two novel Se enzyme-inactivating mutations. Transfusion 2012;52: 1268–75.
60. Koda Y, Soejima M, Johnson PH, Smart E, Kimura H. An Alu-mediated large deletion of the FUT2 gene in individuals with the ABO-Bombay phenotype. Hum Genet 2000;106:80–5.
61. Pang H, Fujitani N, Soejima M, et al. Two distinct Alu-mediated deletions of the human ABO-secretor (FUT2) locus in Samoan and Bangladeshi populations. Hum Mutat 2000;16:274.
62. Mitani T, Tsujita H, Sonoda S, Akane A. Se genotyping following allele-specific polymerase chain reaction amplification. Leg Med (Tokyo) 2002;4:193–6.

Erwin Andreas Scharberg, MD (corresponding author), Director of Immunohematology, Institute of Transfusion Medicine and Immunohematology, German Red Cross Blood Service Baden-Württemberg-Hessen, gGmbH Gunzenbachstr. 35, 76530 Baden-Baden, Germany, A.Scharberg@blutspende.de; Coral Olsen, Senior Biomedical Scientist Immunohaematology Specialised Laboratory Services, South African National Blood Service, South Africa; and Peter Bugert, PhD, Associate Professor, Institute of Transfusion Medicine and Immunology, Heidelberg University, Medical Faculty Mannheim, and German Red Cross Blood Service Baden-Württemberg-Hessen, Mannheim, Germany.

Mary Harrell McGinniss, BB(ASCP)
1925–2016

Mary McGinniss received her A.B. degree in Sociology in 1947 from Trinity College, Washington, DC. In 1956, she began a 30-year career in the Department of Transfusion Medicine (DTM) at the National Institutes of Health (NIH), locating in its Research Division in 1962, where she remained until she retired in 1986. During her tenure at NIH, she continued her theoretical and technical training as a student at NIH's Graduate School, the Foundation for Advanced Education in the Sciences (FAES).

During her career as a research biologist in NIH's DTM, Mary trained and mentored many physicians, fellows, and students working on their Specialist in Blood Banking (SBB) certification. She was an author on more than 60 journal articles, and contributed chapters to several books, coauthoring with many scientists-in-training, who are now established and famous in their fields. Perhaps her most notable publication was that which described the relationship of Duffy blood group determinants as receptors for *Plasmodium knowlesi* (malaria parasite) on the red cell membrane that she coauthored with Dr. Louis H. Miller (1975). As Mary recalled their encounter, she was drawing a map of Africa for a presentation on the distribution of the Fy(a–b–) phenotype when Dr. Miller looked over her shoulder and asked why she was drawing a map of the distribution of malaria in Africa. That coincidental meeting was the beginning of a highly productive partnership that led to the identification and publication on how malaria parasites use Duffy antigen receptors to penetrate and infect human red cells.

In 1986, Mary became the volunteer Managing Editor of the American Red Cross (ARC) journal, *Immunohematology*. She was a talented writer, demanding editor, and a driving force for consistency and excellence. Rather than dismiss submitted

What I will always remember about Mary is her untiring willingness to assist others along their career paths. Whether this was her indefatigable explanations of red cell serology to transfusion medicine fellows and SBB students at NIH or her patient reworking of almost incomprehensible manuscripts until the kernel of important scientific information imbedded within was clearly evident, Mary has always been willing to give of her time, knowledge and experience to help others along their way. We have all been the benefactors of this assistance for which we are very grateful!

I have always been thankful for your work in editing my reviews. You transformed what I had written in broken Japanese-English to help create a better final product. You have done an outstanding job as a Managing Editor and I am sure that your devotion to the journal will be fully rewarded in your retirement.

Thanks for keeping us authors, reviewers and editorial board members on our toes for these past years. Without your past diligence, the Journal would not be where it is today. It has been a distinct pleasure working with you, and I wish you a happy "second" retirement.

Mary McGinniss taught me all I ever needed to know about immunohematology, and quite a bit about life. I was lucky to have her as a teacher, friend and mentor during the many years we spent together at NIH. I will never forget one of the first projects we did together, "The Saline Agglutinating Phenomenon" which actually got a plenary presentation at the AABB. She was an inspiration, not only for the many classes of SBB students who passed through her laboratory, but also for the NIH physician/fellows in transfusion medicine who learned at her side.

Mary was synonymous with everything good about the NIH...teaching, scholarship, research and technical expertise. Her many awards and honors are testimony to her major contributions to the field of blood banking and immunohematology. It is fortunate that she has spent the latter part of her stellar career overseeing *Immunohematology*. I am sure that many others who are not here today would join us in a warm, "thank you" to Mary for a job well done.

You combine the gentle reminder of deadlines with the encouraging word better than anyone I have ever worked with. The success of the journal owes you so much and you must be very proud of your accomplishments. With much love and respect.

You have been one to champion quality and adherence to rules, especially, when there have been challenges. I would like to say how much I have appreciated your energy for the journal. The journal is in great shape because of your efforts.

subpar manuscripts from new authors, she volunteered her time to mentor the authors to ensure that their manuscripts would be of the caliber required by *Immunohematology* and that their data and message would be read. She wanted everyone to succeed. She inspired technologists to write, kept the journal on its production schedule, and set high journalistic standards for all. For the next 18 years, Mary kept *Immunohematology* on course.

Mary received many recognitions and awards for her professional service. NIH recognized her several times for her excellence during her 30-year tenure, including the NIH Directors Award twice. NIH's DTM and the ARC presented her with the Richard J. Davey Lectureship Award, which recognizes an individual whose contributions have significantly advanced the field of transfusion medicine. The AABB conferred the Ivor Dunsford Memorial Award in 1977, and in 1983, she received the Charles E. Walter Memorial Award; both awards recognized her contributions to the field of Immunohematology. In 1983, she was cited in the 13th edition of *Who's Who of American Women*.

Mary loved life. She spoiled her children, enjoyed having a good Scotch before dinner, and rescued and adored her many cats. After her retirement from NIH, she looked forward to visits by friends and long lunches at a favorite nearby French restaurant. She particularly enjoyed keeping up with the many friends she made over the years.

Mary McGinniss was a role model for a generation of medical technologists, researchers in immunohematology, and practitioners of transfusion medicine. She will be missed in the corridors of NIH, in the pages of *Immunohematology,* and whenever and wherever blood bankers congregate and muse about the history of our discipline.

As both an author and as a reviewer, I always got helpful feedback from Mary. She always made me feel as I had her complete attention and that my issues were important! Thanks Mary for being there and making the journal such a professional document. We will miss you!

It takes great organizational skills to manage the many hand-offs associated with moving a manuscript through the publishing process. It also takes a keen eye for detail to edit and fine-tune someone's thoughts, ideas and research into an article suitable for publishing. In addition, it takes initiative and resourcefulness to move a journal through 18 years of changing times in the immunohematology world. You exemplified all of these skills and many more in order to establish and maintain the quality of the journal as it is today.

Thank you so much for all the hard work you put into Immunohematology and for all the help you gave me. I became interested in the production side of scientific publications after seeing what you went through to help a writer submit a well-constructed paper. I always wondered how folks learned to write papers and found out most of it happened because of generous editors like you.

It has always been a pleasure working with Mary, since my first days at the American Red Cross in Rockville. Over the years, I have been awed by her dedication to the journal, not only in its existence but in maintaining its scientific integrity while at the same time, really guiding those authors who needed a little help. I have been one of those authors! Furthermore, as I review the blood group literature for one project or another, I realize what an enormous contribution Mary made to the field on Immunohematology. I have come across a couple of instances where "new" discoveries turned out to be not so new but published by Mary and her colleagues at NIH several years previously.

Managing Editor for the American Red Cross

Journal *Immunohematology* Receives the National Institutes of Health

Richard J. Davey Lectureship Award

PHILADELPHIA — American Red Cross managing editor, Mary McGinniss, was recently selected to receive the Richard J. Davey Lectureship award, which will be presented at the Immunohematology and Blood Transfusion Symposium on October 2 and 3, hosted by both the National Institutes of Health and the American Red Cross.

The award is selected by the symposium committee annually to recognize individuals whose contributions have significantly advanced the field of transfusion medicine. It specifically recognizes outstanding achievements in research or clinical practice, and commitment to education.

University of BRISTOL

Masters (MSc) in Transfusion and Transplantation Sciences at The University of Bristol, England

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2016 and will last for 1 year. A part-time option lasting 2 or 3 years is also available. There may also be opportunities to continue studies for PhD or MD following the MSc. The syllabus is organized jointly by The Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Pathology and Microbiology. It includes:

- Scientific principles of transfusion and transplantation
- Clinical applications of these principles
- Practical techniques in transfusion and transplantation
- Principles of study design and biostatistics
- An original research project

Application can also be made for Diploma in Transfusion and Transplantation Science or a Certificate in Transfusion and Transplantation Science.

The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site:
http://ibgrl.blood.co.uk/MSc/MscHome.htm

For further details and application forms please **contact**:

Dr Patricia Denning-Kendall
University of Bristol
Paul O'Gorman Lifeline Centre
Department of Pathology and Microbiology
Southmead Hospital
Westbury-on-Trym, Bristol BS10 5NB, England
Fax +44 1179 595 342, Telephone +44 1779 595 455, e-mail: p.a.denning-kendall@bristol.ac.uk.

Online Specialist in Blood Bank (SBB) Certificate and Masters in Clinical Laboratory Management Program
Rush University
College of Health Sciences

Continue to work and earn graduate credit while the Rush University SBB/MS program prepares you fo the SBB exam and the Diplomat in Laboratory Management (DLM) exam given by ASCP Board of Certification! (Please note acceptable clinical experience is required for these exams.)

Rush University offers online graduate level courses to help you achieve your career goals. Several curricular options are available. The SBB/MS program at Rush University is currently accepting applications for Fall 2016. For additional information and requirements, please visit our website at: **www.rushu.rush.edu/cls/**

Rush University is fully accredited by the Higher Learning Commission (HLC) of the North Central Association of Colleges and Schools and the SBB Certificate Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP).

Applications for the SBB/MS Program can be submitted online at the folowing website: **http://www.rushu.rush.edu/admiss/hlthadm.html**

Contact: Yolanda Sanchez, MS, MLS(ASCP)^{CM}SBB, Director, by e-mail at Yolanda_Sanchez@rush.edu or by phone at 312-942-2402 or Denise Harmening, PhD, MT(ASCP), Director of Curriculum by e-mail at Denise_Harmening@rush.edu

RUSH UNIVERSITY MEDICAL CENTER

The Johns Hopkins Hospital Specialist in Blood Bank Technology Program

The Johns Hopkins Hospital was founded in 1889. It is located in Baltimore, Maryland, on the original founding site, just 45 minutes from Washington, DC. There are approximately 1,000 inpatient beds and another 1,200 outpatient visits daily; nearly 600,000 patients are treated each year.

The Johns Hopkins Hospital Transfusion Medicine Division is one of the busiest in the country and can provide opportunities to perform tasks that represent the entire spectrum of Immunohematology and Transfusion Medicine practice. It provides comprehensive support to all routine and specialized areas of care for surgery, oncology, cardiac, obstetrics, neonatal and pediatric, solid organ and bone marrow transplant, therapeutic apheresis, and patients with hematological disorders to name a few. Our intradepartment Immunohematology Reference Laboratory provides resolution of complex serologic problems, transfusion management, platelet antibody, and molecular genotype testing.

The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is an onsite work-study, graduate-level training program for certified Medical Technologists, Medical Laboratory Scientists, and Technologists in Blood Banking with at least 2 years of full-time Blood Bank experience.

The variety of patients, the size, and the general intellectual environment of the hospital provide excellent opportunities for training in Blood Banking. It is a challenging program that will prepare competent and knowledgeable graduates who will be able to effectively apply practical and theoretical skills in a variety of employment settings. The Johns Hopkins Hospital Specialist in Blood Bank Technology Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP). Please visit our website at http://pathology.jhu.edu/department/divisions/transfusion/sbb.cfm for additional information.

Contact: Lorraine N. Blagg, MA, MLS(ASCP)^{CM}SBB
Program Director
E-mail: lblagg1@jhmi.edu
Phone: (410) 502-9584

The Johns Hopkins Hospital
Department of Pathology
Division of Transfusion Medicine
Sheikh Zayed Tower, Room 3100
1800 Orleans Street
Baltimore, Maryland 21287

Phone (410) 955-6580
Fax (410) 955-0618
Web site: http://pathology.jhu.edu/department/divisions/transfusion/index.cfm

Becoming a Specialist in Blood Banking (SBB)

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 registry (BOR) 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 issues
- 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	Managers of Blood Centers	LIS Coordinators	Educators
Supervisors of Reference Laboratories	Research Scientists	Consumer Safety Officers	
Quality Assurance Officers	Technical Representatives	Reference Lab Specialists	

Why become an SBB?

Professional growth Job placement Job satisfaction Career advancement

How does one become an SBB?

- Attend a CAAHEP-accredited SBB Technology program ***OR***
- Sit forthe examination based on criteria established by ASCP for education and experience.

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

Facilities with CAAHEP-accredited programs, onsite or online, are listed below.
Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org

California	American Red Cross Blood Services	Pomona, CA
Florida	Academic Center at OneBlood	St. Petersburg, FL
Illinois	Rush University	Chicago, IL
Indiana	Indiana Blood Center	Indianapolis, IN
Louisiana	University Medical Center New Orleans	New Orleans, LA
Maryland	National Institutes of Health Clinical Center	Bethesda, MD
	The Johns Hopkins Hospital	Baltimore, MD
	Walter Reed National Military Medical Center	Bethesda, MD
Texas	University Health System and Affiliates School of Blood Bank Technology	San Antonio, TX
	University of Texas Medical Branch	Galveston, TX
Wisconsin	Blood Center of Wisconsin	Milwaukee, WI

Revised October 2016

Instructions for Authors | New Blood Group Allele Reports

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:

Phenotype	Allele Name	Nucleotide(s)	Exon(s)	Amino Acid(s)	Allele Detail	References
e weak	*RHCE*01.01*	48G>C	1	Trp16Cys	*RHCE*ce48C*	1

   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.

Immunohematology

Instructions for Authors

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
   a. List under abstract
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)

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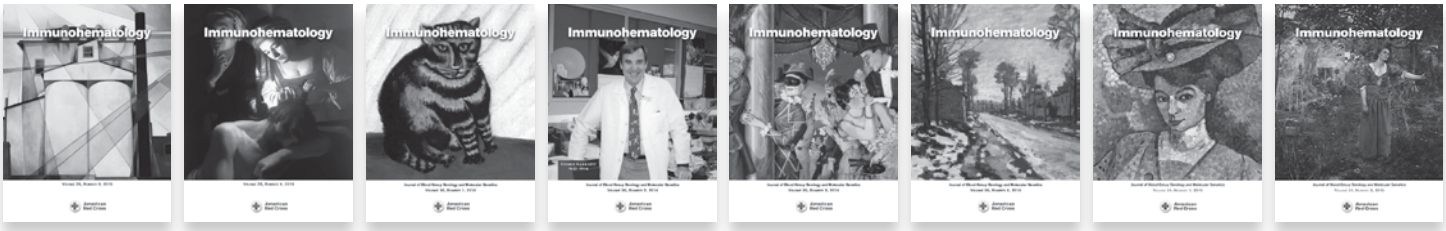

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