Review Article

Molecular genetic narrative review of the novel blood group systems KANNO, SID, CTL2, PEL, and MAM

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Abstract: Review of the strategies used to define this set of new blood group genes demonstrates that characterization of the remaining and potentially new blood group systems will require the sophisticated molecular tools outlined in these five blood group systems. Large nucleotide arrays using unrelated cases are surprisingly powerful in finding the molecular basis of blood group antigens (KANNO). So too is knowledge of the biochemical nature of the antigen. Glycosyltransferase gene sequencing, again among unrelated samples, was the focus to define the molecular basis of SID. Other strategies are combined, such as whole genome or targeted sequencing (KANNO, SID), gene expression using *in vitro* transfection (CTL2, PEL, MAM), show how thorough the molecular basis for blood group antigens has become. The number of antigens defined in each system is low, which is a measure of the high prevalence antigens that each of these systems represent. The KANNO antigen is represented by one SNV; SID has four null alleles; CTL2 has two antigens (RIF being an single nucleotide variant (SNV) and VER), PEL has one null allele and MAM five null alleles. The discovery of the genes responsible for these antigens has not provided insights into any selective pressures that could suggest the presence of antigen-negative red blood cells (RBCs). Also, none of the null alleles seem to affect RBC structure or function.

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Introduction

Ten years ago, the number of blood groups systems had reached the 30 mark with the addition of RHAG as the latest system (1). It was easy to recite the fact that of the 3 billion bases that make up the human haploid genome, from the roughly 30,000 genes (at that time), there were 30 blood group systems expressing approximately 300 antigens (the actual number was 308), many of which changed the three- letter codon by one nucleotide. Now ten years later, we have departed from that mnemonic as the 40th and 41st systems were confirmed by the International Society Of Blood Transfusion (ISBT) Working Party on Blood Group Immunogenetics and Blood Group Terminology (2) [It's worth noting that, at the time of writing, the ISBT recognized 41 blood group system genes; in December 2020, the ISBT Red Blood Cell (RBC) Immunogenetics and Blood Group Terminology working party add the 42nd (EMM) and 43rd (ABCC1) systems].

Many of these more recent blood group systems are associated with extremely rare antigen-negative phenotypes and null alleles requiring international searches for compatible blood (3). Equally important, in-depth analyses in the past have attempted to elucidate structural elements and guide our understanding of the function of some red blood cell (RBC) surface moieties (4). However, it is the latter information that can be lacking. We might know the function of the moieties that carry blood group antigens, but the reasons underlying blood group antigen variation is seldom confirmed to be due to selective pressure from the environment: bacteria, viruses, and parasites (4-6). There are very few examples how the environment

impinges on the evolution of blood group antigen diversity, with the Duffy system the clearest example. The Duffy-null phenotype has evolved twice in malarial infested regions through two separate evolutionary events (7,8). Genetic changes to some systems can be attributed with morphologic changes to the RBC (e.g., Diego blood group system and Southeast Asian Ovalocytosis), or disease (e.g., XK-null and neuroacanthocytosis), and indirectly associated with disease susceptibility (e.g., ABO and Norwalk viral infection) although association does not necessarily mean causality; still left unanswered is the effect of ABO expression on SARS-CoV-2 infection outcome.

The discovery of blood group systems is by-and-large due to clinical cases, although the genes that express some blood group antigens are found by using molecular tools in creative ways. There is no exception with the latest members: KANNO, SID, CTL2, PEL, and MAM. This review summarizes these latest members to the ISBT blood group systems including genetic, biological, and structural information that is available. General background on the genes can be obtained by visiting Online Mendelian Inheritance in Man (OMIM) at the National Center for Biotechnology Information website. I present the following article in accordance with the Narrative Review reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-21-25/rc).

KANNO (ISBT 037)

The KANNO blood group system is comprised of one antigen, KANNO1. KANNO is carried on the glycosyl-phosphatidyl-inositol-linked moiety known as the prion protein (PrP: CD230). The protein is the expressed product of *PRNP*; a 15 kb-gene located at 20p13. The nascent protein is comprised of 253 amino acids, of which a 22-amino acid leader sequence and the 23-amino acid GPI-link motif are cleaved from expressed membrane protein. PrP is associated with transmissible forms of neurodegenerative spongiform encephalopathies. Inherited mutations are associated with Creutzfeldt-Jakob and Gerstmann-Staussler diseases and a severe form of insomnia. The antigenicity of inherited mutations is uncertain.

The discovery of gene responsible for the expression of KANNO was no easy task (9). Initially, the observed antigen-negative phenotype is very low. It is classified as a high-prevalence antigen. A small collection of individuals with KANNO RBC antibodies were initially chosen to identify the gene responsible for their serologic signature. In total, the study examined KANNO-negative individuals from 4 families along with 415 healthy controls. First, 22 samples from the four families (four KANNO-negative individuals with anti-KANNO, two individuals without antibody, and 16 KANNO-positive relatives) were tested using the Affymetrix single-nucleotide polymorphism array. Four KANNO-negative cases and 415 controls comprised the dataset for further analysis using genome-wide association studies. The result of 642,203 autosomal single-nucleotide variants (SNVs) were used for linkage analysis. A single statistically significant association of an intronic SNV with a high P value was identified as the candidate gene with a chromosomal location of 20p13. Whole exome sequencing (WES) was used to confirm a c.655G>A (p.Glu219Lys) missense in exon 2 was only present in the four KANNO-negative individuals. Additional studies confirmed the missense in the remaining KANNO-negative individuals. The investigators confirmed that the RBC membrane protein carried KANNO using monoclonal antibody-specific immobilization of erythrocyte antigens (MAIEA) and *in vitro* expression studies to confirm the molecular assignment of KANNO to *PRNP*.

The SNV associated with the KANNO-negative phenotype (rs1800014) frequency varies between the 1,000 Genomes and gnomAD databases. Frequencies vary in East Asians (4.1% *vs.* 5.6%) and South Asians (2.5% *vs.* 4.1%). It is reportedly <0.5% in other populations. The amino acid substitution was predicted to be structurally benign (PolyPhen 2 analysis), and the pathogenicity of the amino acid substitution was inconclusive; ClinVar data interpretations were conflicting. The variant has been reported to be protective against sporadic Creutzfeldt-Jacob disease (10).

SID (ISBT 038)

The gene encoding SID is *B4GALNT2*; a 56.5 kb gene located at 17q21.32 (11). Post-transcription modification of *B4GALNT2* includes a natural variant comprised of an alternatively spliced exon 1. It encodes a transferase responsible for the synthesis of the single antigen, termed Sd^a, of the SID blood group system. The transferase adds an N-acetylgalactosamine to glycans on glycoproteins and glycosphingolipids (12), and specifically on the Tamm-Horsfall urine glycoprotein (13). Nucleotide changes that abolish the transferase enzymatic activity (null alleles) result in the true Sd(a-) negative phenotype, which has a worldwide frequency of approximately 4% regardless of ethnicity. The clinical significance of SID and the Sd(a-)

phenotype is uncertain. Anti-Sd[a] was first described in 1967, and the antigen was identified in human and guinea pig urine. Anti-Sd[a] have a classic RBC hemagglutination signature that remains unexplained; small agglutinates, often describes as shiny in a sea of unagglutinated cells. The molecular basis for the Sd(a-) phenotype remained elusive until 2019, and was accomplished using nine blood and three plasma-derived DNA samples from individuals with an Sd(a-) phenotype.

Again, the molecular basis for the Sd[a] antigen used a molecular strategy first followed by serologic confirmation (14). Sequencing the gene was followed by extensive annotation of SNVs. The data was compared with the 1,000 Genomes. SN*Vs*. with a frequency of at least 0.05% were selected on the basis that it altered the amino acid sequence. In total, 18 missense variants, four variants predicted to affect splice sites, one insertion and two large structural variants or 'blocks' that would affect coding information were identified. Four variants listed on the ISBT working party SID table are associated with an Sd(a-) phenotype. Protein structure modeling using a relatively unrelated transferase provided circumstantial information on transferase activity. Domain and protein sequence homology studies focused on the region containing a candidate SNV (rs7224888). Linkage disequilibrium and splice-site prediction also provided evidence for the Sd(a-) phenotype.

Three exon changes and one splice site mutation are attributed to the Sd(a-) phenotype, and compound heterozygotes have been described. Three missense mutations, i.e., c.1307A>G (Glu436Arg), c. 1396T>C (Cys466Arg), and c.1567C>T (Arg523Trp), along with the splice-altering SNV c.1134+5G>A, all result in null alleles (a nonfunctional β1,4 N-acecytgalactose transferase) leading to the Sd(a-) phenotype. The c.1396T>C SNV has a frequency of 12% in Europeans, while c.1307A>G and c.1567C>T are rarely observed. The splice site SNV has a frequency of 11% in Swedish blood donors. One of the large blocks correlated with a non-coding RNA gene (*RP11-708H21.4*) proximal to *B4GALNT2* that is associated with colon cancer tumorigenesis and poor prognosis (15).

CTL2 (039)

The protein expressing a high frequency antigen termed CTL2 (choline transporter-like 2) protein is the product of *SLC44A2*, a 42 kb-gene located at 19p13.2. Recently confirmed to be expressed on erythrocytes (16), CTL2 contains ten transmembrane domains with crucial cysteine residues conserved among the other family members, CTL1 and CTL4. Hydropathy plot of the multi-transmembrane passing protein places the codon change (p.Pro398Thr) responsible for the loss of the high-frequency antigen on the third exofacial loop. Interestingly, *SLC44A2* is also expressed on granulocytes with immunoprecipitation studies identifying the CTL2 protein as HNA-3a (17). The relationship if any of HNA-3 with the CTL2 expressed on RBCs has yet to be determined.

Individuals expressing the wild-type gene are deemed VER+. A total of six individuals with CTL2 variation have been characterized (18,19). The initial VER- propositus is Moroccan and was defined using a whole genome sequencing approach with the gene responsible confirmed by transfections and immunoprecipitation studies. Cross-compatibility studies have identified five unrelated Moroccans. Using transfected L-929 cells, a c.1192C>A SNV has been associated with the loss of the RIF high frequency antigen. A single European was identified with a large 37 kb-deletion, defines the VER- phenotype, and is associated with hearing loss in the high-frequency range (20).

PEL (040)

The molecular basis for the PEL blood group system took nearly 40 years to identify. The gene encoding PEL is *ABCC4/MRP4*; a 281 kb-gene located at 13q32.1 It joins LAN (*ABCB6*), JR (*ABCG2*), and the unrelated Augustine (*ENT1*) gene, as ATP-binding cassette transporter molecules expressed on RBCs (21). The PEL-negative (null) phenotype is due to a 67,528 bp-deletion in the distal portion of the gene. The PEL-negative phenotype was first described by Daniels *et al.* with the proband from Quebec, Canada (22), and four unrelated persons from the region make up the known worldwide null phenotype. Monocyte-monolayer assays have shown that PEL is clinically important in transfusion, but no perinatal cases have been reported. Additional mutations are associated with a loss of transporter function, and altered platelet aggregation can be shown with low-dose platelet activation agents. Transporter redundancy might be a reason for the failure to show any RBC defect. The loss of MRP4 function is observed in leukemia (23,24).

Whole-exome sequencing using one PEL-negative member from four unrelated families was unsuccessful in identifying the gene. However, SDS-PAGE comparative proteomics among a single PEL-negative and wild-type controls and the WES data identified *ABCC4* as

the candidate gene. Extensive molecular, biochemical, and serologic (flow cytometry) analyses including SDS-PAGE western blotting with a murine monoclonal antibody, mass-spectroscopy, CRISPR/Cas9 editing (gene knockout), transfection with a *ABCC4* construct, and immunoprecipitation were used to confirm the moiety carried the PEL antigen (23).

Lesson learned from the identification of the moiety expressing PEL (ABCC4) underscores the fact that the identify of blood group genes from alloimmunization does not end with the search for rare blood. It has clinical implications beyond transfusion medicine. The expression of ATP-binding cassette transporters is affected by the sum total of RBC transporter members, which has implication in the prognostic value of testing in blood cancers and possibly platelet function (21,23).

MAM (041)

MAM was allocated to the 901 series of high prevalence antigens after its identification in a perinatal case (25). The antigen is expressed on the epithelial membrane 3 protein (EMP3) moiety that was confirmed to be expressed on RBCs and platelets. *EMP3* is a 5 kb-gene located at 19q.13.31. Along with *EMP1*, *EMP2*, *PMP22* (peripheral myelin protein 22), claudin family of genes, and the gamma subunit of voltage-gated calcium channels, they are all members of a superfamily specializing in cell-to-cell contact, and tumor suppression (26).

The MAIEA assay and antibodies targeting CD44 (Indian blood group) demonstrated the non-covalent interaction of EMP3 and CD44 (27). In addition, flow cytometry and confocal microscopy showed that CD44 is poorly expressed on MAM-deficient erythrocytes. That *EMP3* is responsible for MAM expression was confirmed using whole-exome sequence analysis to identify candidate genes followed by Sanger sequencing. Five *EMP3* variant alleles affecting MAM antigen expression include gene deletion, exon deletions, and nonsense mutations. Thus, all nucleotide changes result in the lack of expression of any gene product. Among the changes, the Genome Aggregation Database (gnomAD) listed the nonsense c.123C>G variant as the most frequent at 0.017% (26) Additional strategies to confirm the gene included transduction with short hairpin RNA used to transiently disrupt EMP3 expression, CRISPR/Cas9 gene editing (knockout) of the MAM expressing BEL-A2 cell line, and transfection with wild-type *EMP3* construct to overexpress MAM. Hemagglutination using a single anti-EMP3 murine monoclonal antibody could distinguish between papain/Peptide:N-glycanase F treated wild-type *vs.* MAM-RBCs, and anti-MAM could be inhibited (absorbed) from binding to RBCs with wild-type RBCs, but not MAM- RBCs. Functionally, EMP3 may play a role in early erythropoiesis. Erythropoietic capability from EMP3-deficient peripheral blood exceeded that of wild-type, suggesting an avenue to improve the *in vitro* cultivation of RBCs (27).

Fetal and neonatal hemolytic disease varies with anti-MAM alloimmunization. Severe cases have been reported with or without thrombocytopenia with the absence of disease in one case (25,28). MAM expression was confirmed on platelets, and the significance in the antenatal management of anti-MAM alloimmunization has not been firmly established since thrombocytopenia is commonly observed in other several fetal hemolytic anemias (29,30).

Conclusions

The clinical importance of blood group antigens lies largely in pregnancy and allogenic transfusion, but also in transplantation. Red cell alloimmunization drives the development of serologic and molecular reagents tools used to unravel blood group antibody specificities to help manage clinical decision making for the delivery of babies at a safe gestational age and for safe compatible transfusions. Soon, the complex molecular studies used to identify novel blood group genes will be commonplace and the pace of the discovery of additional blood group systems will increase once again.

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References

1. Tilley L, Green C, Poole J, et al. A new blood group system, RHAG: three antigens resulting from amino acid substitutions in the Rh-associated glycoprotein. Vox Sang 2010;98:151-9.
2. Storry JR, Clausen FB, Castilho L, et al. International Society of Blood Transfusion Working Party on Red Cell Immunogenetics and Blood Group Terminology: Report of the Dubai, Copenhagen and Toronto meetings. Vox Sang 2019;114:95-102.
3. Flickinger C. REGGI and the American Rare Donor Program. Transfus Med Hemother 2014;41:342-5.
4. Cartron JP, Bailly P, Le Van Kim C, et al. Insights into the structure and function of membrane polypeptides carrying blood group antigens. Vox Sang 1998;74 Suppl 2:29-64.
5. Doughty BR. The changes in ABO blood group frequency within a mediaeval English population. Med Lab Sci 1977;34:351-4.
6. Flegel WA. COVID-19 insights from transfusion medicine. Br J Haematol 2020;190:715-7.
7. Miller LH, Mason SJ, Dvorak JA, et al. Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. Science 1975;189:561-3.
8. Zimmerman PA, Woolley I, Masinde GL, et al. Emergence of FY*A(null) in a Plasmodium vivax-endemic region of Papua New Guinea. Proc Natl Acad Sci U S A 1999;96:13973-7.
9. Omae Y, Ito S, Takeuchi M, et al. Integrative genome analysis identified the KANNO blood group antigen as prion protein. Transfusion 2019;59:2429-35.
10. Shibuya S, Higuchi J, Shin RW, et al. Protective prion protein polymorphisms against sporadic Creutzfeldt-Jakob disease. Lancet 1998;351:419.
11. Montiel MD, Krzewinski-Recchi MA, Delannoy P, et al. Molecular cloning, gene organization and expression of the human UDP-GalNAc:Neu5Acalpha2-3Galbeta-R beta1,4-N-acetylgalactosaminyltransferase responsible for the biosynthesis of the blood group Sda/Cad antigen: evidence for an unusual extended cytoplasmic domain. Biochem J 2003;373:369-79.
12. Blanchard D, Capon C, Leroy Y, et al. Comparative study of glycophorin A derived O-glycans from human Cad, Sd(a+) and Sd(a-) erythrocytes. Biochem J 1985;232:813-8.
13. Donald AS, Yates AD, Soh CP, et al. A blood group Sda-active pentasaccharide isolated from Tamm-Horsfall urinary glycoprotein. Biochem Biophys Res Commun 1983;115:625-31.
14. Stenfelt L, Hellberg Å, Möller M, et al. Missense mutations in the C-terminal portion of the B4GALNT2-encoded glycosyltransferase underlying the Sd(a-) phenotype. Biochem Biophys Rep 2019;19:100659.
15. Sun L, Jiang C, Xu C, et al. Down-regulation of long non-coding RNA RP11-708H21.4 is associated with poor prognosis for colorectal cancer and promotes tumorigenesis through regulating AKT/mTOR pathway. Oncotarget 2017;8:27929-42.
16. Bryk AH, Wiśniewski JR. Quantitative Analysis of Human Red Blood Cell Proteome. J Proteome Res 2017;16:2752-61.
17. Greinacher A, Wesche J, Hammer E, et al. Characterization of the human neutrophil alloantigen-3a. Nat Med 2010;16:45-8.
18. Gassner C, Hyland C. ISBT Working party on red cell immunogenetics and blood group terminology. Transfusion Today 2020;125:18-9.
19. Vrignaud C, Mikdar M, Koehl B, et al. Alloantibodies directed to the SLC44A2/CTL2 transporter define two new red cell antigens and a novel human blood group system. Transfusion 2019;59:18A.

20. Nair TS, Kommareddi PK, Galano MM, et al. SLC44A2 single nucleotide polymorphisms, isoforms, and expression: Association with severity of Meniere's disease? Genomics 2016;108:201-8.
21. Flegel WA, Srivastava K, Sissung TM, et al. Pharmacogenomics with red cells: a model to study protein variants of drug transporter genes. Vox Sang 2021;116:141-54.
22. Daniels GL, Simard H, Goldman M, et al. PEL, a 'new' high-frequency red cell surface antigen. Vox Sang 1996;70:31-3.
23. Azouzi S, Mikdar M, Hermand P, et al. Lack of the multidrug transporter MRP4/ABCC4 defines the PEL-negative blood group and impairs platelet aggregation. Blood 2020;135:441-8.
24. Schuetz JD. "OMICs" reveal the molecular basis of a rare blood group. Blood 2020;135:396-7.
25. Montgomery WM Jr, Nance SJ, Donnelly SF, et al. MAM: a "new" high-incidence antigen found on multiple cell lines. Transfusion 2000;40:1132-9.
26. Alaminos M, Dávalos V, Ropero S, et al. EMP3, a myelin-related gene located in the critical 19q13.3 region, is epigenetically silenced and exhibits features of a candidate tumor suppressor in glioma and neuroblastoma. Cancer Res 2005;65:2565-71.
27. Thornton N, Karamatic Crew V, Tilley L, et al. Disruption of the tumour-associated EMP3 enhances erythroid proliferation and causes the MAM-negative phenotype. Nat Commun 2020;11:3569.
28. Li W, Fernandes BJ, Denomme GA. MAM is an N-glycan linked carbohydrate antigen expresse on all blood cells. Transfusion 2002;42:10S.
29. Macher S, Wagner T, Rosskopf K, et al. Severe case of fetal hemolytic disease caused by anti-C(w) requiring serial intrauterine transfusions complicated by pancytopenia and cholestasis. Transfusion 2016;56:80-3.
30. van den Akker ES, Klumper FJ, Brand A, et al. Kell alloimmunization in pregnancy: associated with fetal thrombocytopenia? Vox Sang 2008;95:66-9.

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