

Literature Review

Update on blood group terminology and molecular polymorphisms

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Blood group antigens are surface markers on the outside of the red cell membrane that contain many anchored surface proteins; many of these are polymorphic and carry the different blood groups. Aside from the sugar (glycan or carbohydrate) antigens, e.g., ABO, P1PK, Lewis, H and I, the red cell membrane contains three types of proteins that carry blood group antigens: single-pass proteins (MNS, Lutheran, Kell, Scianna, Landsteiner- Wiener, Gerbich, Knops, Indian and OK), multi-pass proteins (Rh, Duffy, Kidd, Diego, Colton, Kx, Raph, Gill and Rh-associated glycoprotein) and glycosylphosphatidylinositol (GPI)-linked proteins (Yt, Dombrock, Cromer and John Milton Hagen).¹ Presently, blood group antigens have been numbered by the International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology. To assign a number, the blood group antigen is defined serologically by antibodies made by humans. Currently, a total of 346 authenticated blood group antigens are recognized, of which 308 are clustered within 36 blood groups systems. Each system has been shown to be genetically discrete from every other system. Collections consist of serologically, biochemically, or genetically related antigens, which do not suit the criteria required for system status. Moreover, other antigens cannot be included in a system or collection were classified as the 700 series of low-incidence antigens with an incidence of less than 1% and the 901 series of high-incidence antigens with an incidence of greater than 90%.² In this article, we describe the update blood group terminology and molecular polymorphisms. In addition, we summarize the related studies of new alleles and antigens reported in Thailand.

Blood group systems

Blood group systems consist of one or more antigens controlled at a single gene locus, or by two or more very closely linked homologous genes with little or no observable recombination between them. They follow Mendelian inheritance (independent inheritance) and most blood group genes are co-dominant. An overview of blood group systems with gene name and chromosome location is shown in Table 1.^{1,2} According to the ISBT terminology; the first three digits represent the system ordering in the discovery (e.g. 001 for ABO).

Blood group collections

Blood group collections (200 series) consist of serologically, biochemically or genetically related antigens, which do not fit the criteria required for system status and do not follow independent inheritance. Currently, 15 antigens are joined in six blood group collections: 205 Cost; 207 li; 208 Er; 209; 210 and 213; however, 201 Gerbich, 202 Cromer, 203 Indian, 204 Auberger, 206 Gregory, 211 Wright and 212 Vel are outdated owing to reallocation in blood group systems.

700 series, low-incidence antigens

The criteria of 700 series includes an incidence of < 1% in most populations tested, distinction from all other numbered in the 700 series and those of the blood group systems and collections and demonstration of inheritance through at least two generations. Currently, 17 antigens are included in the 700 series (Table 2).

901 series, high-incidence antigens

The criteria of 901 series includes an incidence of > 90% in most populations tested, distinction from all

Table 1 Overview of blood group systems with gene name and chromosome location^{1,2}

ISBT numbers	System name	System symbol	Gene name(s)*	Chromosomal location	CD numbers	Number of antigens
001	ABO	ABO	<i>ABO</i>	9q34.2		4
002	MNS	MNS	<i>GYPA, GYPB, (GYPE)</i>	4q31.21	CD235a CD235b	49
003	P1PK	P1PK	<i>A4GALT</i>	22q13.2	CD77	3
004	Rh	RH	<i>RHD, RHCE</i>	1p36.11	CD240	55
005	Lutheran	LU	<i>LU</i>	19q13.32	CD239	24
006	Kell	KEL	<i>KEL</i>	7q33	CD238	36
007	Lewis	LE	<i>FUT3</i>	19p13.3		6
008	Duffy	FY	<i>ACKR1</i>	1q21-q22	CD234	5
009	Kidd	JK	<i>SLC14A1</i>	18q11-q12		3
010	Diego	DI	<i>SLC4A1</i>	17q21.31	CD233	22
011	Yt	YT	<i>ACHE</i>	7q22.1		3
012	Xg	XG	<i>XG, MIC2</i>	Xp22.32	CD99 [†]	2
013	Scianna	SC	<i>ERMAP</i>	1p34.2		7
014	Dombrock	DO	<i>ART4</i>	12p13-p12	CD297	10
015	Colton	CO	<i>AQP1</i>	7p14		4
016	Landsteiner-Wiener	LW	<i>ICAM4</i>	19p13.2	CD242	3
017	Chido/Rodgers	CH/RG	<i>C4A, C4B</i>	6p21.3		9
018	H	H	<i>FUT1</i>	19q13.33	CD173	1
019	Kx	XK	<i>XK</i>	Xp21.1		1
020	Gerbich	GE	<i>GYPC</i>	2q14-q21	CD236	11
021	Cromer	CROM	<i>CD55</i>	1q32	CD55	19
022	Knops	KN	<i>CR1</i>	1q32.2	CD35	9
023	Indian	IN	<i>CD44</i>	11p13	CD44	5
024	Ok	OK	<i>BSG</i>	19p13.3	CD147	3
025	Raph	RAPH	<i>CD151</i>	11p15.5	CD151	1
026	John Milton Hagen	JMH	<i>SEMA7A</i>	15q22.3-q23	CD108	6
027	I	I	<i>GCNT2</i>	6p24.2		1
028	Globoside	GLOB	<i>B3GALT3</i>	3q25		2
029	Gill	GIL	<i>AQP3</i>	9p13		1
030	Rh-associated glycoprotein	RHAG	<i>RHAG</i>	6p12.3	CD241	4
031	FORS	FORS	<i>GBGT1</i>	9q34.13-q34.3		1
032	JR	JR	<i>ABCG2</i>	4q22.1	CD338	1
033	LAN	LAN	<i>ABCB6</i>	2q36		1
034	VEL	VEL	<i>SMIM1</i>	1p36.32		1
035	CD59	CD59	<i>CD59</i>	11p13	CD59	1
036	Augustine	AUG	<i>SLC29A1</i>	6p21.1		2

*As recognized by the HUGO Gene Nomenclature Committee <http://www.genenames.org/>

[†]*MIC2* product

Table 2 The 700 series of low-incidence antigens and the 901 series of high-incidence antigens²

Low-incidence antigens (700 series)			High-incidence antigens (901 series)		
Number	Name	Symbol	Number	Name	Symbol
700002	Batty	By	901008		Emm
700003	Christiansen	Chr ^a	901009	Anton	AnWj
700005	Biles	Bi	901012	Sid	Sd ^a
700006	Box	Bx ^a	901014		PEL
700017	Torkildsen	To ^a	901015		ABTI
700018	Peters	Pt ^a	901016		MAM
700019	Reid	Re ^a			
700021	Jensen	Je ^a			
700028	Livesay	Li ^a			
700039	Milne				
700040	Rasmussen	RASM			
700044		JFV			
700045	Katagiri	Kg			
700047	Jones	JONES			
700049		HJK			
700050		HOFM			
700054		REIT			

other numbered high-incidence specificities, and demonstration that the antigen is lacking from the red cells of at least two siblings, i.e., that the negative phenotype is genetically determined. Currently, 6 antigens are included in the 901 series (Table 2).

Molecular genetics of blood group polymorphisms

Genetic basis is responsible for blood group polymorphisms. Each blood group system shows either a single or a cluster of two or three closely-linked homologous genes. The different genetic basis are taken into account for blood group polymorphisms including single-nucleotide polymorphisms (SNPs), gene deletion, single nucleotide deletion, sequence duplication plus a nonsense mutation and intergenic recombination between closely-linked homologous genes, hybrid genes and hybrid proteins.

SNPs

Most blood group polymorphisms are caused by one or more SNPs, which encode amino acid substitutions

of a glycosyltransferase or an extracellular membrane protein. The examples of those SNPs of blood group polymorphisms are shown in Table 3.^{1,3}

Gene deletion

The deletion of the whole gene occurs in the D polymorphism of the Rh system. The common D-negative phenotype among Caucasians results from homozygosity of *RHD* gene deletion, leading to an absence of RhD protein on the red cell membrane.⁴

Single nucleotide deletion

The single nucleotide deletion is introduced among common blood group O individuals. The common sequence of the O allele (*O*¹ or *OO1*) is actually identical to that of the sequence of the A allele, but apart from the deletion of a single nucleotide in exon 6 that is responsible for a reading-frame shift generating a translation stop signal codon 117. This allele encodes a truncated protein lacking the catalytic site, so the group O red cell membrane remains unconverted to the H antigens and to A and/or B antigens.⁵ Moreover, for A₂ blood group

Table 3 List of blood group polymorphisms resulting from SNPs^{1,3}

System	Gene	Polymorphism	SNPs	Amino acid change
ABO	<i>ABO</i>	A/B	526C>G, 703G>A, 796C>A, 803G>C	R176G, G235S, L266M, G268A
MNS	<i>GYPA</i>	M/N	59C>T, 71G>A, 72T>G 143C>T	S1L, G5E
	<i>GYPB</i>	s/S		T29M
RH	<i>RHCE</i>	C/c	48C>G, 178A>C, 203G>A, 307T>C	C16W, I16L, S68N, S103P
		e/E	676G>C	A226P
LU	<i>LU</i>	Lu ^a /Lu ^b	230G>A	R77H
		Au ^a /Au ^b	1615A>G	T539A
KEL	<i>KEL</i>	k/K	578C>T	T193M
		Kp ^b /Kp ^a	841C>T	R281W
		Js ^b /Js ^a	1790T>C	L597P
FY	<i>FY</i>	Fy ^a /Fy ^b	125G>A	G42D
		Fy ^b /Fy	-67T>C	Not coding
JK	<i>SLC14A1</i>	Jk ^a /Jk ^b	838G>A	D280N
DI	<i>SLC4A1</i>	Di ^b /Di ^a	2561C>T	P854L
YT	<i>ACHE</i>	Yt ^a /Yt ^b	1057C>A	H353N
SC	<i>ERMAP</i>	Sc1/Sc2	169G>A	G57R
DO	<i>DO</i>	Do ^b /Do ^a	793G>A	D265N
CO	<i>AQP1</i>	Co ^a /Co ^b	134C>T	A45V
LW	<i>ICAM4</i>	LW ^a /LW ^b	299A>G	Q70R
CROM	<i>DAF</i>	Tc ^a /Tc ^b	155G>T	R18L
KN	<i>CR1</i>	Kn ^a /Kn ^b	4681G>A	V1561M
		MsC ^a /McC ^b	4768A>G	K1590E
		Sl ^a /Vil	4801A>G	R1601G
IN	<i>CD44</i>	In ^b /In ^a	137G>C	R46P
AUG	<i>SLC29A1</i>	AUG1/ AUG2	1171G>A	E391K

individuals, the A^2 ($A201$) allele has the same sequence as the A^1 ($A101$) allele, but the single nucleotide deletion occurs in the codon of A^2 before the usual translation stop codon. The result of this polymorphism abolishes the loss of the stop codon so an extra 21 amino acid residues are found at the carboxyl terminal.⁶

Sequence duplication plus a nonsense mutation

Sequence duplication plus a nonsense mutation is found in the *RHD* pseudogene, inactive form of *RHD* genes (*RHDY*) of the Rh blood group system showing RhD negative phenotype, common among Africans. The *RHDY* appears completely identical to the *RHD* gene but a 37 bp duplication of the last 19 nucleotides of

intron 3 and the first 18 nucleotides of exon 4 can be observed. This might result from a reading-frame shift and premature stop codon plus a nonsense mutation (Y269stop) in exon 6.⁷

The intergenic recombination between closely-linked homologous genes, hybrid genes and hybrid proteins

Intergenic recombinations between closely-linked homologous genes, leading to hybrid genes and hybrid proteins occur in the Rh and MNS blood group systems. For the Rh blood group system, the *RH* gene contains 10 exons of *RHD* and *RHCE* in opposite orientations on chromosome 1. To give an example of some partial D antigens results, the DVI is the most commonly accounted

partial D phenotype, for which exons 1 to 3 and 7 to 10 of the variant gene contain the *RHD* sequence, but exons 4 to 6 contain the *RHCE* sequence. This results in a hybrid protein that can appear similar to the stable expressed RhD protein on the red cell membrane but lacking some D epitopes.⁸ Additionally, for the MNS system, the highly polymorphic system contains several low-incidence antigens. These antigens are carried on glycophorin A (GPA), glycophorin B (GPB) or hybrid proteins that give rise from gene conversion within the *glycophorin* (*GYP*) gene families, especially in the Miltenberger subsystem. The three types of organization of MNS glycophorin hybrid genes include *GYP(A-B)*, *GYP(A-B-A)* and *GYP(B-A-B)* hybrids. These 11 classes or glycophorin variants (GP.) of the Miltenberger subsystem are classified by the organization of MNS glycophorin hybrid genes: GP.Hil and GP.JL encode *GYP(A-B-A)* hybrids, GP.Vw, GP.Hut, GP.Nob, GP.Joh and GP.Dane encode *GYP(A-B)* hybrids and GP.Mur, GP.Hop, GP.Bun and GP.HF encode *GYP(B-A-B)* hybrids.^{1,9}

Reports of new alleles or antigens in Thailand

Phenotyping and genotyping discrepancies

Several types of homozygosity for a silent gene at the JK locus resulting in the null Jk(a-b-) phenotype could not differentiate the identified *JK*A* and *JK*B* carrying mutations using genotyping results.¹ Four Thai blood donors were previously reported as Jk(a-b-) phenotype by conventional tube test and urea lysis test. Three donors were genotyped as *JK*B/JK*B*, of which two donors carried the homozygous allele of *JK*02N.01* (c.342-1G>A; p.Arg114_Thr156del), alternative splicing of intron 5 of the *JK* gene for common mutation among Asian and Polynesian populations. Moreover, one donor was homozygous for a *JK*02N.07* (c.896G>A; p.Gly299Glu) mutation at exon 9, as commonly found among Polynesians. Notably, one other donor of the *JK*A/JK*A* genotype was heterozygous to *JK*01N.04* (c.956C>T; p.Thr319Met) of exon 10 and *JK*01W.01* (c.130G>A; p.Glu44Lys) of exon 4, commonly

found among American-African and Caucasian populations, respectively.¹⁰

For the Lutheran blood group system, a rare Lu(a-b-) phenotype results from recessive inheritance of variants in the Lutheran glycoprotein-encoding *LU* gene. Additionally, variations in the *KLF1* gene encode a nonfunctional erythroid Krüppel-like factor (EKLF) transcription factor, leading to a dominant effect to the inhibitor of Lutheran, In(Lu) phenotype. This factor could reduce antigen expression of the LU, P1, In^b and AnWj antigens.¹¹ A recent study reported regarding a Thai blood donor who had Lu(a-b-) phenotype tested by conventional tube test using commercial polyclonal anti-Lu^a and anti-Lu^b. On the contrary, a genotyping on ID CORE XT detected an apparent *LU*B/LU*B* genotype. The confirmation tests demonstrated as a new allele; *KLF1*BGM15*, c.304C (Ser102Pro) in *cis* with frameshift variant of c.484insC (p.Lys162Glnfs*352).¹²

Genotyping approach to confirm phenotyping results

The rare P^k phenotype was identified from the two Thai sisters who had underwent 8 and 11 pregnancy losses, respectively, within the first 24 weeks of gestation. Anti-P and anti-PX2 were identified in their plasma using conventional tube test and the red cell phenotype was P-, PX2- and P^k+ using flow cytometry. They constituted a rare P^k phenotype, lacking the P and PX2 antigens. The gene responsible for P and PX2 antigen expression is *B3GALNT1* that encodes 3-β-N-acetylgalactosaminyltransferase, which catalyses the addition of N-acetylgalactosamine (GalNAc) in the β1-3 linkage to P^k, the precursor of P reactions. Consequently, anti-P of IgG3 type can cause recurrent miscarriages following the cytotoxic attack of the globoside-rich fetal portion of the placenta.¹³ Their sequencing reported homozygosity for a nonsense mutation, c.420T>G in *B3GALNT1*, which introduces a premature stop codon of p.Tyr140Ter.¹⁴

The Augustine (At^a) antigen was formerly one of the high incidence antigens in the 900 series (901003)¹ and previously proposed by ISBT as the 36th blood group

system (ISBT036).² The prevalence of At(a+) or AUG1 is > 99% in both African and Caucasian ancestries; the At(a-) or AUG2 phenotype, caused by recessive inheritance, is therefore very rare.¹ In the fifth pregnancy of a Guinean woman, serological investigations confirmed that the mother was At(a-) with anti-At^a and was provided optimal management of suspected fetomaternal incompatibility and risk of bleeding at delivery in Thailand.¹⁵ The reagent of human anti-At^a for serological analysis required to identify the At(a-), is available in only a few reference laboratories. According to nonsynonymous SNPs in *SLC29A1*, encoding the equilibrative nucleoside transporter 1 (ENT1) is caused by a missense point mutation at c.1171G>A resulting in p.Glu391Lys substitution. Polymerase chain reaction with sequence-specific primer (PCR-SSP) was performed to predict the AUG blood type. The At(a+) individuals were c.1171G homozygote or c.1171G/A heterozygote; while, the At(a-) individual was c.1171A homozygote.¹⁶

For JENU (MNS49), a new high-frequency antigen on GPB, a thalassemic Thai patient, who developed multiple alloantibodies, showed panreactive alloantibody of unknown specificities. Two compatible units were screened among 3,600 blood donors and genotyping of the patient and compatible donors were homozygous *GYP*Mur* of *GYP(B-A-B)* hybrid. Serological typing of patient and donors were Mi(a+), GP.MUT(+), GP.Mur(+), GP.Hil(+) and S(-), suggesting GP.Mur. However, their red cells were negative with anti-s monoclonal antibody but were positive with anti-s polyclonal antibody. Then the patient's plasma was defined by epitope mapping and the results provided evidence for a high-frequency antigen on GPB, JENU (MNS49). Therefore, GP.Mur homozygote lacking normal GPB individual are JENU- and are at risk of alloimmunization when exposed to red cells carrying normal GPB (JENU+).¹⁷

Regarding the findings of new alleles or antigens, red cell genotyping is currently used in reference laboratories in Thailand. These would be helpful in solving, especially in complicated cases that could not

be identified by serological testing due to low-incidence antigens, unavailability of antisera and positive direct antiglobulin tests. The probability of finding extended-matched donors in Thai populations is unlikely for these new alleles and antigens' individuals. In this case, the autologous or family-related donation is recommended. In addition, the appropriate molecular techniques from the basis of blood group polymorphisms for mass screening in blood donors should be implemented in blood bank laboratories to provide safe blood transfusions.

Conclusion

To date, more than 300 red cell antigens are recognized in 36 blood group systems, collections, low-incidence antigens and high-incidence antigens. Blood group polymorphisms have been continuously identified and reported in various populations. In cases of unidentified antibody specificity or unavailable typing reagents, the study of the molecular basis of blood group polymorphisms is suggested to overcome any ambiguity.

References

1. Reid ME, Lomas-Francis C, Olsson ML. *The blood group antigen factsbook*. 3rd ed. London: Academic Press; 2012.
2. Storry JR, Castillo L, Chen Q, Daniels G, Denomme G, Flegel WA, 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.
3. Daniels G. *The molecular genetics of blood group polymorphism*. *Transpl Immunol*. 2005;14:143-53.
4. Wagner FF, Flegel WA. *RHD gene deletion occurred in the Rhesus box*. *Blood*. 2000;95:3662-8.
5. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. *Molecular genetic basis of the histo-blood group ABO system*. *Nature*. 1990;345:229-33.
6. Yamamoto F, McNeill PD, Hakomori S. *Human histo-blood group A2 transferase coded by A2 allele, one of the A subtypes, is characterized by a single base deletion in the coding sequence, which results in an additional domain at the carboxyl terminal*. *Biochem Biophys Res Commun*. 1992;187:366-74.
7. Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, et al. *The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in africans with the Rh D-negative blood group phenotype*. *Blood*. 2000;95:12-8.

8. Wagner FF, Gassner C, Muller TH, Schonitzer D, Schunter F, Flegel WA. Three molecular structures cause rhesus D category VI phenotypes with distinct immunohematologic features. *Blood*. 1998;91:2157-68.
9. Palacajornsuk P, Nathalang O, Tantimavanich S, Bejrachandra S, Reid ME. Detection of MNS hybrid molecules in the Thai population using PCR-SSP technique. *Transfus Med*. 2007;17:169-74.
10. Sriwanitchrak P, Sriwanitchrak K, Tubrod J, Kupatawintu P, Kaset C, Nathalang O. Genomic characterisation of the Jk(a-b-) phenotype in Thai blood donors. *Blood Transfus*. 2012;10:181-5.
11. Singleton BK, Burton NM, Green C, Brady RL, Anstee DJ. Mutations in *EKLF/KLF1* form the molecular basis of the rare blood group In(Lu) phenotype. *Blood*. 2008;112:2081-8.
12. Garcia-Sanchez F, Pardi C, Kupatawintu P, Thornton N, Rodriguez MA, Lucea I, et al. Identification of new *KLF1* and *LU* alleles during the resolution of Lutheran typing discrepancies. *Transfusion*. 2016;56:1413-8.
13. Hellberg A, Ringressi A, Yahalom V, Säfwenberg J, Reid ME, Olsson ML. Genetic heterogeneity at the glycosyltransferase loci underlying the GLOB blood group system and collection. *Br J Haematol*. 2004;125:528-36.
14. Ricci Hagman J, Hult AK, Westman JS, Hosseini-Maaf B, Jongru-amklang P, Saipin J, et al. Multiple miscarriages in two sisters of Thai origin with the rare P^k phenotype caused by a novel nonsense mutation at the *B3GALNT1* locus. *Transfus Med*. 2018. doi:10.1111/tme.12544. Epub 2018 Jun 6.
15. McBean R, Liew YW, Wilson B, Kupatawintu P, Emthip M, Hyland C, et al. Genotyping confirms inheritance of the rare At(a-) type in a case of haemolytic disease of the newborn. *J Pathol Clin Res*. 2015;2:53-5.
16. Nathalang O, Intharanut K, Sasikarn W, Nathalang S, Kupatawintu P. A new polymerase chain reaction: sequence-specific primer method for the Augustine blood type. *Blood Transfus*. 2016;14:577-9.
17. Lopez GH, Wilson B, Liew YW, Kupatawintu P, Emthip M, Hyland CA, et al. An alloantibody in a homozygous *GYP**Mur individual defines JENU (MNS49), a new high-frequency antigen on glycoporphin B. *Transfusion*. 2017;57:716-7.

