



Glycophorins and the MNS blood group system: a narrative review

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Abstract: The MNS blood group system, International Society of Blood Transfusion (ISBT) 002, is second after the ABO system. *GYP A* and *GYP B* genes encode MNS blood group antigens carried on glycophorin A (GPA), glycophorin B (GPB), or on variant glycophorins. A third gene, *GYP E*, produce glycophorin E (GPE) but is not expressed. MNS antigens arise from several genetic mechanisms. Single nucleotide variants (SNVs) contribute to the diversity of the MNS system. A new antigen SUMI (MNS50), p.Thr31Pro on GPA has been described in the Japanese population. Unequal crossing-over and gene conversion are the mechanisms forming hybrid glycophorins, usually from parent genes *GYP A* and *GYP B*. *GYP E* also contributes to gene recombination previously only described with *GYP A*. Recently, however, *GYP E* was shown to recombine with *GYP B* to form a *GYP(B-E-B)* hybrid. A *GYP(B-E-B)* hybrid allele encodes a mature GP(E-B) molecule expressing a trypsin-resistant M antigen but no S/s. Another novel glycophorin GP.MOT has been described carrying Mi^a, Mur, MUT, and KIPP antigens. GP.MOT is encoded by a *GYP(B-A)* hybrid allele. Newly reported cases of haemolytic transfusion reaction (HTR) or haemolytic disease of the fetus and newborn (HDFN) due to antibodies to MNS antigens is a constant reminder of the clinical significance of the MNS system. In one HDFN case, anti-U and anti-D were detected in an Indian D⁻, S⁻s-U⁻ mother. The S⁻s-U⁻ phenotype is rare in Asians and Caucasians but it is more commonly found in the African populations. Several types of novel *GYP B* deletion alleles that drive the S⁻s-U⁻ phenotype have been recently described. Two large *GYP B* deletion alleles, over 100 kb, were identified as the predominant alleles in the African population. The use of advanced DNA sequencing techniques and bioinformatic analysis has helped uncover these large gene-deletion variants. Molecular typing platforms used for MNS genotyping are also discussed in this review. In conclusion, this review considers currently recognised MNS antigens and variants, new hybrid alleles and *GYP B* gene deletion alleles as well as clinical case studies. These new discoveries contribute to our understanding of the complexity of the MNS system to guide decision-making in genetic analysis and transfusion medicine.

Keywords: MNS blood group system; blood group antigens; hybrid glycophorins; variant glycophorins; Mi^a antigen (MNS7); anti-Mi^a antibodies; blood group genetics; blood type

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Introduction

After the discovery of the ABO blood group system in 1900, Landsteiner and Levine searched for more human blood groups (1). In 1927, rabbits injected with human red blood cells (RBCs) produced antibodies against M (MNS1) and N (MNS2) blood group antigens (1,2). The names of these antigens came from the word “immune” and this discovery created the second blood system now known as the MNS blood group system (2). Examples of human anti-M and anti-N were reported in later years (3).

In 1947, Walsh and Montgomery reported a female patient with puerperal fever who developed an antibody recognising the S antigen (MNS3) (4). The S antigen was named after Sydney, the capital city of New South Wales, Australia. The s antigen (MNS4), antithetical to S, was described in 1951 (4,5). Two years later, the fifth antigen U (stands for universal, MNS5) in this system was discovered (6,7). Currently, 50 antigens in the MNS system are recognised by the International Society of Blood Transfusion (ISBT) Working Party (WP) on Red Cell Immunogenetics and Blood Group Terminology (RCIBGT), *Table 1* (8). These antigens are carried on glycoprotein A (GPA), glycoprotein B (GPB) or variant glycoproteins. Amongst antibodies to MNS antigens, many are regarded as clinically significant with reported cases of haemolytic disease of the fetus and newborn (HDFN) and haemolytic transfusion reactions (HTRs). The history of the MNS system has been detailed in reference textbooks (1,2,9-11) and review articles (12-17). We present the following article in accordance with the Narrative Review reporting checklist (available at <http://dx.doi.org/10.21037/aob-21-9>).

GPA, GPB, and glycoprotein E (GPE)

GYP A and *GYP B* genes encode GPA, 150 amino acids, and GPB, 91 amino acids, respectively. *GYP A* and *GYP B* Exon 1 up to the 5' end of Exon 2 encode a 19-amino acid leader sequence, from the 3' end of Exon 2 up to Exon 4 encode amino acids residing in the extracellular region, Exon 5 in the transmembrane and Exon 6–7 in the intracellular domain. The leader sequence is cleaved after the protein is inserted into the cell membrane (1). Therefore, the mature GPA and GPB molecule has 131 and 72 amino acid residues, respectively, *Figure 1* (10). *GYPE* encodes GPE which is a 78-amino acid molecule that includes a 19-residue leader sequence (18). The mRNA transcript produced by *GYPE*

was shown to be very unstable affecting GPE expression (18). GPE has not been detected on the surface of RBC. However if expressed, GPE would be a 59-amino-acid molecule carrying an M antigen. The Exon 2 sequence coding for M antigen in *GYPE* is identical to *GYP A* (14,19).

GPA and GPB are single-pass transmembrane sialoglycoproteins heavily glycosylated with abundant *O*-glycans (10). Only GPA carries *N*-glycan (10). These carbohydrate molecules contribute a strong net negative charge to the surface of RBCs preventing RBC aggregation thus maintaining blood flow in the circulation (10). It is estimated that there are 1×10^6 copies of GPA and 2.5×10^5 copies of GPB per RBC (15,20).

Genes of the MNS blood group system

GYP A, *GYP B* and *GYPE* genes form a 350-kb gene cluster on chromosome 4q31.21, *Figure 2A* (21,22). Analysis of this region suggests that *GYP A* was the ancestral gene and that a series of molecular events formed *GYP B* and *GYPE* genes (23). Firstly, ancestral *GYP A* is duplicated. Two chromatid strands, each carrying a duplicated *GYP A*, misaligned. This was followed by unequal crossing-over occurring within the *Alu* sequences present in each strand producing a progenitor *GYP B*/*GYPE* genomic segment (23). Subsequent duplication of this segment gave rise to independent *GYP B* and *GYPE* genes. The 3' sequences for *GYP B* and *GYPE* were acquired from an unrelated genomic segment (23).

GYP A, *GYP B* and *GYPE* genes show a high degree of homology, over 95%, from the 5' flanking sequences to the *Alu* sequence located in Intron 5 which is approximately 1 kb downstream from Exon 5 (21,23). Sequence homology and the intron-exon gene structure organisation (*Figure 2B*) is thought to facilitate the numerous gene recombination events occurring in these three glycoprotein genes (14). Furthermore, an approximately 1 kb region was identified as a major recombination hotspot spanning between Intron 2–Exon 3 junction and Intron 3–Exon 4 junction (10). The presence of multiple direct repeat sequences and palindromic sequences, particularly the 35-bp complex palindrome within Exon 3 of *GYP A* and pseudoexon 3 of *GYP B*, are distinct features of these genes (10). Reference sequences associated with these genes are listed in *Table 2* (8).

Variant glycoproteins: genetic mechanisms

Several genetic mechanisms contribute to the diversity

Table 1 Classification of MNS antigens in the general population

	n	Antigens
Polymorphic antigens	n=4	M, N, S, s
High-frequency antigens	n=10	U, En ^a , ENKT, 'N', ENEP, ENEH, ENAV, ENDA, ENEV, JENU
Low-frequency antigens	n=36	He, Mi ^a , M ^c , Vw, Mur, M ^g , Vr, M ^e , Mt ^a , St ^a , Ri ^a , Cl ^a , Ny ^a , Hut, Hil, M ^v , Far, s ^D , Mit, Dantu, Hop, Nob, Or, DANE, TSEN, MINY, MUT, SAT, ERIK, Os ^a , HAG, MARS, MNTE, SARA, KIPP, SUMI

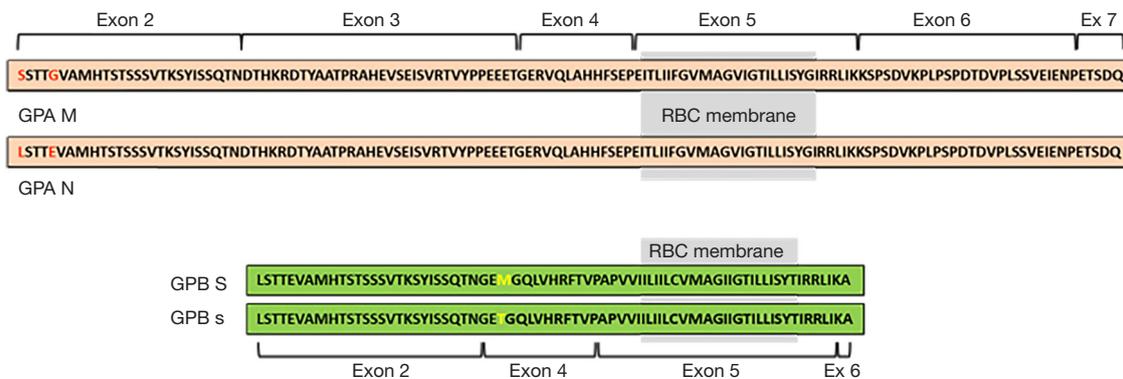


Figure 1 Amino acid sequences for GPA and GPB. The red texts on GPA show the amino acid differences for M (p.Ser20; p.Gly24) and N (p.Leu20; p.Glu24). In GPB, yellow texts indicate S (p.Met48) and s (p.Thr48). The regions of GPA and GPB predicted to be in the transmembrane domain are indicated with a gray background (1). GPA, glycophorin A; GPB, glycophorin B.

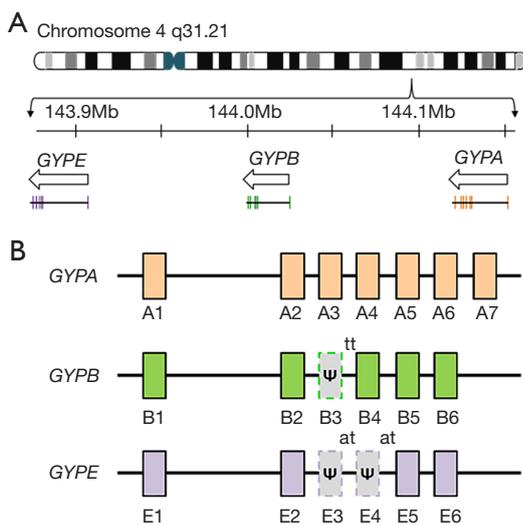


Figure 2 Location of MNS glycoprotein genes on chromosome 4. (A) Schematic diagram of the glycoprotein gene cluster. (B) *GYPA*, has 7 exons, *GYPB* has 5 exons and 1 pseudoexon (B3), and *GYPE* has 4 exons and 2 pseudoexons (E3 and E4). Instead of a functional splice site “gt”, *GYPB* and *GYPE* pseudoexons carry defective donor splice sites “tt” and “at”, respectively (1,2).

of variant glycoproteins (10,12). Unequal crossing-over and gene conversion are the two main mechanisms forming hybrid glycoprotein variants (10,12). Currently, there are over 30 hybrid genes in the MNS system (8). Examples of these hybrid alleles are shown in *Table 3*. The third mechanism is single nucleotide polymorphism (10,12).

Unequal crossing-over

In this mechanism, *GYPA* and *GYPB* genes misaligns during meiosis (13). The two sister chromatid strands exchange genetic material of unequal length generating two hybrid genes in reciprocal arrangements. As a result, one strand received less (Lepore type) and the other received more (anti-Lepore type) genetic material than what each initially gave (14). Unequal crossing-over forms *GYP(A-B)* and *GYP(B-A)* hybrid alleles, *Table 3*.

Gene conversion

This mechanism also occurs during meiosis when nucleotide

Table 2 Reference sequences for MNS glycophorin genes

Source	<i>GYP A</i>	<i>GYP B</i>	<i>GYP E</i>
Genomic	NG_007470.3	NG_007483.3	NG_009173.1
Transcript	NM_002099.8	NM_002100.6	NM_002102.4
Protein	NP_002090.4	NP_002091.4	NP_941391.2

Table 3 Mechanisms forming variant glycoporphins

Mechanisms	Variant alleles
Unequal crossing-over	
<i>GYP(A-B)</i>	e.g., <i>GYP*Hil</i> , <i>GYP*JL</i>
<i>GYP(B-A)</i>	e.g., <i>GYP*Dantu</i> , <i>GYP*Sch</i> , <i>GYP*MOT</i>
Gene conversion	
<i>GYP(A-B-A)</i>	e.g., <i>GYP*Vw</i> , <i>GYP*Hut</i> , <i>GYP*Zan</i>
<i>GYP(B-A-B)</i>	e.g., <i>GYP*Mur</i> , <i>GYP*Bun</i> , <i>GYP*Hop</i> , <i>GYP*HF</i> , <i>GYP*Kip</i>
<i>GYP(A-E-A)</i>	e.g., <i>GYP*Mar</i>
<i>GYP(B-E-B)</i>	e.g., <i>GYP*Man</i> , <i>GYP*Ros</i> , <i>GYP*Dia</i> , <i>GYPB-E(2-4)-B</i>
Single nucleotide polymorphism causing missense mutations or splicing variants	
<i>GYP A</i> c.91A>C	e.g., <i>GYP A*SUMI</i>
<i>GYP B</i> c.161G>A	e.g., <i>GYP B*Mit</i>
<i>GYP A</i> c.232G>A	e.g., <i>GYP A*Erik</i> or <i>GYP*EBH</i>

The four examples of *GYP(B-E-B)* hybrid alleles listed above do not encode S or s (24,25).

sequences from a donor chromatid strand replace a homologous sequence in the acceptor chromatid strand (10). This transfer of genetic material is non-reciprocal. Gene conversion between *GYP A* and *GYP B* forms *GYP(A-B-A)* and *GYP(B-A-B)* hybrid genes (13,14). Examples of *GYP(B-A-B)* hybrid genes are formed when the defective donor splice site “tt” in *GYP B* pseudoexon 3 is replaced by the functional splice site “gt” from *GYP A* Exon 3 (10), *Table 3*. Conversely, an active splice site on *GYP A* Exon 3 replaced by the inactive splice site from *GYP B* pseudoexon 3 produces a variant glycoporphin called GP.Zan (26). *GYP E* also recombines with *GYP A* forming *GYP(A-E-A)*—encoding GP.Mar—and with *GYP B* to form *GYP(B-E-B)* (24,25). Few examples of *GYP(B-E-B)* alleles have been identified and they differ from each other based on the position and length of the *GYP E* gene insert, *Table 3* (24,25).

Recently, a trypsin-resistant M antigen was identified in 0.05% of the Japanese population (24). This antigen was

encoded by a novel variant glycoporphin *GYPB-E(2-4)-B* (24). Exons 3 and 4 of this allele are pseudoexons. The final protein is a 59-amino-acid GP(E-B) molecule expressing M antigen. To our knowledge, this was the first report of a glycoporphin molecule expressing a *GYP E* product. *GYPB-E(2-4)-B* has a similar structure to *GYPB-E-B.Ros* allele reported by Willemetz *et al.* in a Caucasian individual from Portugal (25). However, the 5' gene breakpoint for *GYPB-E-B.Ros* has not been fully defined (24,25). Two other examples of *GYPB-E-B* alleles were identified in African individuals—*GYPB-E-B.Man* (Gambia) and *GYPB-E-B.Dia* (Mali) (25).

Single nucleotide variants (SNVs)

SNVs in the exon or intron regions of *GYP A* and *GYP B* genes produce variant glycoporphins either by an amino acid change, or disrupting the normal splicing mechanisms if the nucleotide is adjacent to or near the splice site, *Table 3*.

Table 4 *GYPB* deletion types

ISBT allele name	<i>GYP</i> deletion size described by Gassner <i>et al.</i> (30) [2020]	Gassner <i>et al.</i> (30) [2020]	Lane <i>et al.</i> (31) [2020]	Leffler <i>et al.</i> (32) [2017]
<i>GYPB*05N.01</i>	110.24 kb deletion includes the entire <i>GYPB</i>	110-kb deletion	DEL_B_LEFT	DEL1
<i>GYPB*05N.02</i>	103.26 kb deletion includes the entire <i>GYPB</i>	103-kb deletion	DEL_B_RIGHT	DEL2
<i>GYPB*05N.03</i>	18.61 kb deletion includes <i>GYPB</i> exon 2–6	19-kb deletion	DEL_PART_B	DEL8

Based on the similarity in size and location of the deletion within the *GYP* locus, the authors of this manuscript assigned DEL_PART_B allele as *GYPB*05N.03*. ISBT, International Society of Blood Transfusion.

Splice sites are important markers during splicing, when introns are removed and exons are fused together, by spliceosomes. Single base substitutions in the donor splice site (gt) will cause skipping of the preceding exon (27). Examples of glycophorin variants formed by this mechanism include GPEBH and GP(P2).

- ❖ SNV in the exon—the GPEBH phenotype arose from a c.232G>A (p.Gly59Arg) in *GYPB* Exon 3. This SNV is located adjacent to the “gt” splice site in Intron 3 and produces several transcripts. One transcript forms GPA carrying the ERIK antigen (p.Gly59Arg). Another forms a GPA molecule, lacking the Exon 3 product, expressing St^a (MNS15) antigen (28).
- ❖ SNV in the intron—the *GYPB*P2* allele has a c.270+5G>T polymorphism located in Intron 5. This base change results in skipping of Exon 5 forming GP(P2) expressing a S–s–U^{var} phenotype (29).

Loss of *GYPB* gene (S–s–U– phenotype)

Homozygous deletion of glycoprotein genes generate null phenotypes such as M^kM^k (deletion of *GYPB* and *GYPE*), En(a–) (a deletion of *GYPB*) and S–s–U– (deletion of *GYPB*) (8). The S–s–U– phenotype is present in approximately 1% of individuals of African heritage and the predominant *GYPB* deletion alleles have been identified (30–32). However, the major alleles responsible for the S–s–U– in Asians, Caucasians and other population groups are yet to be determined.

S–s–U– phenotype in Africans

Studies by Leffler *et al.* (32), Gassner *et al.* (30), and Lane *et al.* (31) identified several examples of *GYPB* deletion alleles (whole and partial gene deletion) that give rise

to S–s–U– phenotype (Table 4). The two most common alleles identified in these studies were *GYPB*05N.01* and *GYPB*05N.02* alleles (30–32). Both alleles have a deletion span of over 100 kb in the *GYP* locus which includes the entire *GYPB* gene. These were observed in individuals from West Africa (The Gambia, Sierra Leone, Nigeria, Burkina Faso and Cameroon) and East Africa (Tanzania and Kenya) (30,32). In addition, *GYPB*05N.01* was identified in a sample from North Africa (Algeria), Central Africa (DR Congo) and Southern Africa (South Africa) (30). A 19-kb deletion within the *GYPB* gene, *GYPB*05N.03*, was identified in an African Barbadian individual (31).

S–s–U– phenotype in Asians

Rare examples of S–s–U– have been reported in Asians (33). In 1972, a pregnant woman of Indian heritage was typed as U– (33). She had post-partum transfusion after her first pregnancy. In her third pregnancy, she delivered a U+ baby showing signs of mild HDFN (33). Anti-U and anti-c antibodies were detected in the mother and were eluted from the baby’s RBCs. The mother has two siblings who were also S–s–U– (33). Another case of HDFN due to anti-D and anti-U antibodies in a pregnant D–, S–s–U– woman in India was described (34).

Three *GYPB* deletion types were identified in Asian individuals: (I) *GYPB*05N.03* in a Bengali individual (31,32), (II) a 112-kb deletion which includes *GYPB* Exons 4–7 to *GYPB* Exon 1 identified in a Gujarati Indian (31), and (III) a 224-kb deletion, DEL_EB-1c, in the *GYP* locus that includes the whole *GYPB* and *GYPE* genes in a Sri Lankan Tamil individual (31,32). Three DEL_EB types described by Lane *et al.* (31) resembles the DEL6 variant identified by Leffler *et al.* (32). The predominant *GYPB* deletion allele in Asians is not known.

S-s-U- phenotype in Caucasians

The S-s-U- phenotype was reported in a Caucasian blood donor during routine phenotyping (35). The proband was identified as “Fav.”. Three family members of “Fav.” were also S-s-U- (35). DNA analysis for “Fav.” showed that *GYPB* Exon 2–5 and *GYPE* Exon 1 were deleted (18). This allele is designated as *GYPB*01N* (18,31,35). *GYPB*01N* allele was not described in the cohort of African and Asian population groups in recently published studies (30–32). Other examples of S-s-U- in Caucasians (Finland) have been reported (1).

S-s-U- phenotype in the Americas

Lane *et al.* identified two other 224-kb *GYPB* deletion types: DEL_EB-1a in an African from Barbados and DEL_EB-1b in a Peruvian individual (31). S-s- phenotype was found in two Central American Indians (Honduras) (1).

MNS antigens recently recognised by ISBT

Review articles on the MNS system published before 2014 included 46 blood group antigens. Since then, four new blood group antigens have been added (1,2,13,14).

SARA (MNS47)

A regular blood donor whose cells were used as a reagent RBC for antibody identification reacted to a serum from a patient (36). Serological studies showed the antigen is novel and inherited (36). This antigen was originally named “SARAH” but is now called SARA (8,36). A whole-exome sequencing study on SARA+ individuals, from two independent families, showed that *GYPB* c.240C>T was the genetic basis for the SARA antigen (37). In 2015, the ISBT WP on RCIBGT assigned SARA as MNS47 (37). At least two cases of HDFN due to antibodies against SARA antigen have been reported (38,39).

KIPP (MNS48)

RBCs from a blood donor of German origin showed a unique reactivity profile using antibodies with known specificity to low-frequency MNS antigens (40). This phenotype was called GP.Kip (40). A laboratory report (J.

Poole, International Blood Group Reference Laboratory, personal communication, 27 October 1988) stated that ‘Kip’ is the short form for the name Kippenhahn, the German proband. GP.Kip was also described in an Australian blood donor (40). DNA sequencing for the Australian example revealed *GYP*Kip* as a hybrid *GYP(B-A-B)* gene (41). GP.Kip is Mi^a+ and carries p.Ser51 which is distinct from other Mi^a+ *GYP(B-A-B)* hybrid glycoporphins (41). Several Japanese individuals have been identified as GP.Kip (42–44). The KIPP antigen on GP.Kip is recognised by two anti-Hop(+Nob) antisera, Anek and Raddon (40). Another Mi^a+ hybrid glycoporphin called GP.MOT also express KIPP antigen (45,46).

JENU (MNS49)

A Thai individual with thalassemia was transfused with RBCs (47). Following transfusion, anti-E, anti-c, anti- Jk^b , anti-S and an antibody to a high-frequency antigen on GPB were identified in the patient’s serum (47). Epitope mapping analysis using 12-mer peptides, representing the extracellular domain of GPB, showed that an antibody in the patient’s plasma recognised an epitope with the sequence SYISSQTNGETG (47). This sequence is encoded by *GYPB* Exon 2 and Exon 4 producing $^{38}SYISSQTN^{45}$ and $^{46}GETG^{49}$, respectively. This epitope is called JENU (47). The name JENU is a combination of ‘JE’—the first two letters from surname of the antibody producer, and ‘NU’ from the high-frequency antigens ‘N’ (MNS30) and U (MNS5) on GPB. Phenotyping and genotyping showed the patient was GP.Mur homozygote (GP.Mur/GP.Mur) (47). GP.Mur/GP.Mur individuals do not express normal GPB and are, therefore, JENU-negative (47). These individuals can produce antibodies to GPB including anti-JENU (47,48).

SUMI (MNS50)

A patient’s serum was found reactive to RBCs from a blood donor during compatibility testing but non-reactive to the antibody identification panel of RBCs (49). Subsequent serological investigations were performed and called this antigen—SUMI. An anti-SUMI monoclonal-antibody producing cell line was created. Anti-SUMI was used to screen 541,522 blood donors and identified 23 were SUMI-positive (49). Molecular analysis showed all 23 individuals carried a single nucleotide change *GYPB* c.91A>C

Table 5 Serological profile of Mi^a+ hybrid glycoporphorins

GP	Mi ^a	Vw	Hut	Mur	MUT	Hop	Hil	TSEN	MINY	KIPP
GP.Vw	+	+	-	-	-	-	-	-	-	-
GP.Hut	+	-	+	-	+	-	-	-	-	-
GP.Mur	+	-	-	+	+	-	+	-	+	-
GP.Hop	+	-	-	+	+	+	-	+	+	+
GP.Bun	+	-	-	+	+	+	+	-	+	+
GP.HF	+	-	-	-	+	-	+	-	+	-
GP.Kip	+	-	-	+	+	-	+	-	+	+
GP.MOT	+	-	NT	+	+	-	-	NT	-	+

The serological profile for GP.Kip was assembled from several published sources (40,42,50-52). Anek antiserum was used to detect the presence or absence of KIPP antigen on Mi^a+ hybrid glycoporphorins (52,53). NT, not tested.

Table 6 Frequency of Mi^a in blood donor

Country	moAb used	Total tested	Mi ^a + donors	Frequency
Japan [2019] (44)	CBC-172	826,379	831	0.1%
Australia [2020] (57)	CBC-172	5,098	11	0.22%
USA [2019] (58)	GAMA210	4,600 [#]	103	ND
India [2016] (59)	GAMA210	1,000	1	0.1%

[#], number of blood donations from Asian American blood donors. ND, not determined.

(p.Thr31Pro). SUMI is a low-frequency antigen on GPA with a prevalence of 0.0042% in blood donors in Japan (49). SUMI antigen was designated as MNS50 by the ISBT WP on RCIBGT.

Mi^a and its associated hybrid glycoporphorins

Mi^a (MNS7) is immunogenic and its clinical significance is widely reported (16). Currently, eight hybrid glycoporphorins express Mi^a, *Table 5*. A new Mi^a+ hybrid glycoporphorin called GP.MOT was recently described in a Japanese blood donor (45,46). GP.MOT is encoded by *GYP*MOT*—a *GYP(B-A)* hybrid gene. *GYP*MOT* is formed when a section of *GYP A* is replaced by homologous sequences from *GYP B* (45,46). The resulting structure for *GYP*MOT* is *GYPB(1-2)-BA(3)-A(4-7)*. *GYP*MOT* was previously reported as *GYP(A-B-A)* hybrid gene (45,46). The Exon 3 sequence, predicting amino acid sequence DKHKRDT YPAHTANEVSEISVTTVSPPEEET, for *GYP*MOT* is identical to *GYP*Kip* (41,46). Both GP.MOT and GP.Kip are KIPP+, *Table 5*.

The Mi^a epitope is recognised by two murine monoclonal antibodies—CBC-172, binding to epitope 48-HKRDTYAA-55, and GAMA210, binding to epitopes 44-TNDKHKRD-51, and 43-QTNDMHKR-50 (54,55). These moAb equally gave strong agglutination reactions (3+ to 4+ in a “0–4” scale) against a panel Mi^a positive RBCs (56). CBC-172 and GAMA210 monoclonal antibodies have been used to screen for Mi^a in large blood donor populations, *Table 6* (44,57-59).

Generally, Mi^a is rare in Caucasian (9) and African (60,61) population groups and is more commonly found in Asian populations (62-65). In the 1960s, studies on the indigenous population in the American continents reported Mi^a in Seneca Indians in North America, Quecha Indians in Ecuador (66) and in a Mataco individual in Argentina (67). Of all the Mi^a+ hybrid glycoporphorins, GP.Mur is most commonly encountered while others are geographically or ethnically-specific. The availability of anti-Mi^a typing reagents and in combination with molecular typing allowed identification of Mi^a+ hybrid glycoporphorins in other population groups, *Table 7*.

Table 7 Population groups where Mi^a+ hybrid glycoporphins have been identified

GP	Initial reports	Current reports
GP.Vw	Reported in Caucasians (9) and Thais (63). One Kekchi individual from Guatemala was Vw+ (68)	One Vw+ blood donor was reported in India (69) and 17 in Japan (44)
GP.Hut	Previously reported in Caucasians (9) and Thais (63)	GP.Hut was described in two African American blood donors (61) and in 182 Japanese blood donors (44)
GP.Bun	GP.Bun is usually found in the Thai population (53,70)	GP.Bun blood donors were identified in China (65,71), the US (58), Japan (44), and Australia (57)
GP.Hop	Two GP.Hop blood donors were reported. One in the UK, GP.Hop (MH) (72), and the other is from Australia, GP.Hop (ES) (72,73)	Nine blood donors in Thailand were phenotyped as GP.Hop (74)
GP.HF	Identified in the Japanese population (44,52,75)	First report of <i>GYP*HF</i> in a Chinese blood donor (65)
GP.Kip	GP.Kip was reported in a German individual and in an Australian blood donor (40)	Several GP.Kip blood donors were identified in Japan (42,44)
GP.MOT	No prior reports	Japanese blood donor (45,46)

Table 8 Reactivity profile of hybrid glycoporphins to anti-s reagents (47,76,77)

GP	Monoclonal IgG 771002 Lorne	Polyclonal Z186 Quotient	Monoclonal IgM P3BER	Polyclonal IgG bioCSL	Monoclonal IgG P3YAN3
GP.Mur	+	+	-	+	+
GP.Bun	+	+	-	Not tested	Not tested
GP.Hil	+	+	-	Not tested	Not tested

Variant glycoporphins with altered antigen expression

Qualitative expression of *s* (MNS4) in GP.Mur, GP.Bun and GP.Hil

The products of *GYPB* Exon 2 (B2) and Exon 4 (B4) form the extracellular region of GPB. The *s* antigen, found on the B4 segment of GPB, resides near the B2-B4 junction site (10). In *s*+ hybrid glycoporphins, the B2-B4 junction site does not exist. This is either due to the insertion of an Exon 3 product between B2 and B4 (e.g., GP.Mur or GP.Bun) or that the B2 segment was replaced by products of *GYP A* (e.g., GP.Hil). Studies have shown that structural changes adjacent to B4 alter the *s* presentation and may not be recognised by some anti-*s* typing reagents, *Table 8* (10,47,76,77).

Cleghorn reported that in one GP.Mur+ Chinese family, one in five potent anti-*s* antisera failed to react with the *s*/(Hil) antigen (78). This is consistent with recent studies showing GP.Mur homozygote RBCs reacted variably

to a panel of anti-*s* typing reagents (47,76,77,79). Anti-*s* monoclonal antibody P3BER does not recognise the *s* antigen on GP.Mur, GP.Bun, and GP.Hil RBCs suggesting these hybrid glycoporphins express a variant *s* antigen (47,76,77). Anti-*s* has been reported in a *s*+ GP.Mur individual (1).

In one study, plasma from an alloimmunised GP.Mur/GP.Mur individual was tested against synthetic 12-mer peptides representing the extracellular domain of GPB (47). Peptide mapping analysis showed three distinct reactivity domains (47). The first domain (peptides 4–7) represents the JENU epitope. The second (peptide 9) and third (peptides 11–13) domains represent epitopes for *s* and *U*, respectively (47). Data suggests that three antibodies were present: anti-JENU, anti-*s*, and anti-*U*. However, at the time of publication, only anti-JENU was reported (47).

Qualitative *U* (MNS5) expression in GP.Mur

GP.Mur homozygote individuals do not possess normal

GPB and are at risk of alloimmunisation when exposed to RBCs carrying normal GPB (47). A case was reported in a pregnant GP.Mur/GP.Mur individual of Thai ethnicity (48). Plasma from the patient reacted positive with all routine screening panel cells. Antibody identification investigations showed that the patient's plasma failed to reactive with S–s–U– and M^kM^k RBCs but were weakly positive with S–s–U+ cells identifying an anti-U antibody (48). This suggests that GP.Mur/GP.Mur individuals express a variant form of U antigen and can form anti-U antibody when exposed to normal GPB.

Altered S (MNS3) expression in GPB.Mit

The Mit (MNS24) antigen, *GYPB* c.161G>A, is carried on GPB.Mit (1). Mit+ RBCs are usually associated with reduced S expression (1). In one case report, an apparent alloanti-S was detected in a S+s+ male, Caucasian patient (80). The patient's RBCs were tested using multiple anti-S reagents and consistently gave positive results. Molecular typing by SNP-microarray predicted S–s+ while DNA sequencing predicted S+s+ (80). In addition, a c.161G>A (p.Arg54His) was detected predicting Mit antigen. This is the first report demonstrating GPB.Mit RBCs express an altered S and Mit+ individuals are at risk of alloimmunisation producing alloanti-S antibody (80).

Antibodies to hybrid glycoporphins in patients and blood donors

Mi³+ screening cells are used to detect antibodies against antigens carried on hybrid glycoporphins. Antibodies to these antigens are commonly reported in several patient groups in Asia. The incidence of anti-Mi³ antibodies was reported at 0.08% in 20,283 patients of Guangxi, China (81) and 2.07% in 143 thalassemia patients in Thailand (82). In Malaysia, anti-MUT, anti-Mur, and anti-Mur + MUT was detected in 0.60% (n=70,543) patients in a tertiary care hospital (83). In a study in Brazil, Nakasone *et al.* reported the prevalence of anti-Mi³ in 7,119 patients was 0.41% (84). Nakasone *et al.* recommended the use of Mi³+ screening cells in Brazil in areas with a significant Asian population (84).

In Japan, the frequency of GP.Hil in blood donors is 0.03% (4/13,546) (44). GP.Hil and GP.JL RBCs were used to screen sera from 137,340 blood donors (44). Anti-Hil and anti-MINY antibodies were detected in 10 and 3 blood

donors, respectively (44).

Cases demonstrating the clinical significance of hybrid glycoporphins

Antibodies to MNS antigens are frequently naturally occurring and can be ignored unless these antibodies are reactive at 37 °C (14). There are rare examples of anti-M and anti-N antibodies reactive at 37 °C causing immediate and delayed HTR (85). Antibodies to low-frequency MNS antigens Mi³, Hut, and Mur are also clinically significant and have been implicated in immediate and delayed HTR and HDFN (15,16,85). The case studies presented below are consistent with previous reports (16).

HDFN due to anti-Mur [2016]

A baby exhibited jaundice 24 hours post-delivery and was treated with phototherapy. One week later, jaundice was still evident and another round of phototherapy was given (86). The mother, father and baby were all Group A, D+ (86). Mother's plasma failed to react with standard antibody screening cells, although reactive positive with RBCs from the baby and father. Serological investigations showed that antibody from the mother reacted with Mur+ RBCs. DNA typing showed the father was homozygous for *GYP*Mur* (86). Based on serological and molecular data, the authors concluded that the antibody most likely caused HDFN was anti-Mur. The ancestry of the mother is Chinese and the father is Vietnamese (86). The authors signalled that in the United States, it is prudent to consider antibodies to variant glycoporphins in patients of Asian ancestry when investigating for HDFN (86).

HTR due to anti-Mur antibodies in a patient with leukemia [2017]

A 41-year-old male Hispanic individual was diagnosed with leukemia (87). The patient is transfusion-dependent requiring 1–2 units of RBCs every 1–2 weeks. After one such blood transfusion event, the patient's haemoglobin dropped to 6.6 g/dL. Two months post-transfusion, anti-Jk^a and anti-Mur antibodies were detected in the patient's plasma (87). A lookback study was undertaken to determine the ethnicity of blood donors linked to the RBCs received by the patient. Of the 30 blood donors, four had Asian ancestry (87). In regions where a significant population of blood donors are of Asian ancestry, screening for anti-Mur in chronically-transfused

patients could help prevent HTR.

Suspected HTR due to anti-Mi^a and anti-Vw antibodies in a sickle cell disease patient [2019]

A regularly-transfused African American patient with sickle cell anemia received a unit of packed RBCs. Following-transfusion, the patient experienced severe back pain (88). Post-transfusion heart rate and blood pressure readings were higher compared to pre-transfusion (88). Pre- and post-transfusion samples from the patient did not react with an antibody screening RBC panel. Antibody screening using RBCs expressing low-prevalence antigens showed the patient had multiple antibodies: anti-Vw, anti-Mi^a and anti-Go^a (88).

HTR due to anti-Hut in a geriatric patient [2020]

A 74-year-old female individual presented with rigors, tachycardia, and fever during transfusion. Post-transfusion sample from the patient indicated hyperbilirubinemia (89). The patient's pre- and post-transfusion samples were negative with the reagent RBC panel and but reacted with one of two donor units of RBCs. RBC from the donor was M⁺ N⁻ Mi^{a+}. Patient's plasma was positive with GP.Hut (Mi^{a+} Mur⁻ Hut⁺ MUT⁺) cells and negative with GP.Mur cells (Mi^{a+} Mur⁺ Hut⁻ MUT⁺). This is the first report on anti-Hut causing HTR (89).

HDFN due to anti-Mi^a [2020]

The Mur antigen (MNS10), expressed by hybrid glycoporphins such as GP.Mur (Table 6), is considered low-frequency in Caucasian and African populations but is more commonly found in Southeast Asian and East Asian populations (90). A clinical case involving a pregnant individual of Chinese and Filipino ethnicity delivered a baby showing signs of HDFN. The infant's RBC were DAT positive. Serological studies showed anti-Mi^a was present in the maternal plasma (90). The father's RBC were Mi^{a+} Hil⁺ and MINY⁺. DNA sequencing analysis showed the father was *GYPB/GYP*Mur*. Anti-Mi^a antibodies are not routinely detected in North America because screening cells used in the laboratory are not Mi^{a+}. The authors advocate the use of Mi^{a+} screening cells to improve detection of anti-Mi^a especially in individuals of Asian background suspected with antibodies to low-frequency antigens (90).

Genotyping for hybrid glycoporphins

Hemagglutination technique is the conventional method to identify blood group antigens (91). However, serological typing has limitations. It is difficult to accurately phenotype recently transfused patients or when reliable typing reagents are not available (91). Commercial monoclonal antibody GAMA210, a typing reagent for Mi^a, has lately become available (58). However, characterising hybrid glycoporphins requires more than one typing reagent and is performed only in specialised laboratories who have access to rare antisera. Molecular typing can overcome these challenges. A brief description of genotyping techniques used to type for hybrid glycoporphins is described below. This list is non-exhaustive.

Polymerase chain reaction-sequence specific primer (PCR-SSP)

Palacajornsuk *et al.* designed two sets of primers to detect six hybrid glycoporphin genes (92). The first primer set produces two types of amplicons depending on the gene present—148-bp band for *GYP*Mur*, *GYP*Hop*, and *GYP*Bun* and a 151-bp for *GYP*Hut* and *GYP*HF*. The second set of primers targets *GYP*Vw* producing a 296-bp band (92). A 434-bp human-growth hormone band was used as an internal DNA control. While one primer set was specific for only one hybrid glycoporphin, *GYP*Vw*, the other targets five hybrid glycoporphins and would require DNA sequencing to define the specific hybrid glycoporphin gene present (92).

High-resolution melting (HRM) analysis

HRM analysis is a powerful screening tool to detect polymorphisms based on the melting property of double-stranded DNA (dsDNA) (93). HRM requires a real-time PCR equipment to perform and uses an intercalating dye. This dye emits fluorescence only when bound to dsDNA (93). Fluorescence is monitored throughout the testing process. HRM is a two-step process. The first step is a standard PCR procedure. As more amplicons are generated, fluorescence is increased. The second is the HRM analysis step. At this stage, heat is gradually increased to promote denaturation of dsDNA. As amplicons dissociate, fluorescence is decreased. Fragment length, GC content, sequence and heterozygosity influence the unique DNA melting profile of amplicons (93). This signature melt profile is the basis for genotyping assignments in comparison to

known DNA controls. HRM have been successfully applied to genotype for *GYP*^{Mur}*, *GYP*^{Bun}*, and *GYP*^{HF}* and to determine zygosity for hybrid glycoporphin genes (47,65).

Matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry (MALDI-TOF MS)

The MassARRAY (Agena Bioscience) system combines PCR and MALDI-TOF MS technologies to detect single nucleotide polymorphisms (94). After PCR amplification, custom-designed primers hybridise to target regions (94,95). Annealed primers are extended by a single base, mass-modified dideoxynucleotide terminators, specific to the complementary nucleotide on the template (95). Products are spotted onto a chip and then shot with a laser beam to desorb and ionise. The time it takes for the ionised molecules to travel towards the detector—TOF—is calculated. TOF is proportional to mass of the extended product (95). MALDI-TOF MS is powerful in detecting SNV alleles and hybrid glycoporphin alleles (73,94). MALDI-TOF MS phenotype predictions for M/N and S/s antigens have been shown to be highly concordant with serology (94).

Multiplex ligation-dependent probe amplification (MLPA)

The MLPA genotyping platform was developed by MRC Holland, The Netherlands (96,97). This technique uses two oligonucleotide probes to interrogate a particular target sequence. One of two probes carry a unique length of nucleotide sequences making it distinct with other probes in a multiplex PCR set-up (96). Once the two adjacent probes annealed to their target sequences, ligation occurs creating a single fragment (96). The ligated probes are then amplified and PCR products are size-separated by capillary electrophoresis. MLPA analysis software converts these fragments as peaks and are analysed to predict phenotype and zygosity (96). MLPA has been evaluated for RBC genotyping and can detect several types of hybrid glycoporphins including *GYP*^{Mur}* (96,97).

Fluidic microarray

The ID Core XT genotyping platform (Progenika/Grifols) is a multiplex PCR, hybridisation-based assay to detect multiple alleles encoding blood group antigens belonging to 10 blood group systems (98,99). This platform uses fluorochrome-labelled microspheres coupled with an allele-specific oligonucleotide probe (98,99). DNA is amplified using

nucleotides labelled with biotin. Biotin-labelled amplicons hybridises to oligonucleotide probes. Following this step, a fluorescent molecule called streptavidin-phycoerythrin is added that will bind to biotin. Fluorescence signal is then detected by Luminex flow analyser (98). Data is analysed by ID Core XT analysis software (98). ID Core XT platform targets nucleotide at *GYP* c.140 to predict Mi^a (MNS7) (99).

Next-generation sequencing (NGS)

NGS also called Massively Parallel Sequencing (MPS) is a high throughput DNA sequencing platform (91). MPS-based whole-genome sequencing (WGS) and whole-exome sequencing using Illumina HiSeq and MiSeq, respectively, have been used to predict multiple RBC antigens (100-102). Briefly in these MPS platforms, DNA is fragmented and adapters are attached to the end of the fragments in preparation for DNA amplification. After amplification, dsDNA fragments are denatured forming single-stranded templates. Sequencing by synthesis begins when primers are extended with fluorescent-labelled dNTPs. Laser is applied and the fluorescent signal is detected. MiSeq sequencing platform, in particular, can generate sequence reads up to 300 bp. Sequence reads are then aligned to the reference sequence and a variant call file is generated. Data is interpreted to predict blood group antigens. Short-read sequences produced by MPS is powerful in detecting SNVs (100-102). However, using short-read sequences to characterise large structural variants, especially those formed by *RHD/RHCE* genes, and *GYP^A/GYP^B* genes can be challenging (101). Hybrid alleles, exon/intron deletions, and exon/intron duplications are examples of these structural variants. Long-read sequences, over 5 kb, can overcome this limitation (103).

Discussion

The cumulative reports of new gene *GYP^B* deletion alleles, hybrid alleles, and blood group antigens demonstrate the diversity and complexity of the MNS blood group system. Case reports of HTR and HDFN highlight the immunogenic potential and clinical significance of MNS blood group antigens. Gene conversion and unequal crossing-over are genetic mechanisms frequently associated with the MNS system. These molecular mechanisms that produce novel blood group antigens are the same genetic mechanisms that disrupt them. For example, in GP.Mur, gene conversion facilitated expression of Mi^a , Mur, MUT, Hil, MINY antigens but also disrupted the expression of

JENU antigen. Newly reported case studies associated with antibodies to MNS antigens were an enduring reminder of the clinical significance of this system. The identification of Mi^3+ hybrid glycoporphins in other population groups suggests these variant glycoporphins may not be as geographically or ethnically exclusive as first thought. The introduction of DNA sequencing technology has allowed us to identify variant glycoporphins when antisera is not available. Recent history suggests that the MNS story is not finished. With the utilisation of current and emerging typing platforms, discovery of new alleles and gene variants is almost inevitable.

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