Review: molecular basis of MNS blood group variants

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The MNS blood group antigens are expressed in the RBC membrane on glycophorin A (GPA), glycophorin B (GPB), or combinations of both. GPA expresses the M or N antigen, whereas GPB expresses the S or s antigen and the N antigen ('N'). Both glycophorin genes (*GYPA* and *GYPB*) are located on the long arm of chromosome 4 and share 95 percent sequence identity. This high degree of sequence identity, together with the rare involvement of a third homologous gene (*GYPE*), provides an increased chance of recombination, resulting in hybrid molecules that often carry one or more novel antigens. Some of the antigens in the MNS system result from a single nucleotide substitution. The MNS blood group system now consists of more than 40 distinct antigens. This review summarizes the molecular basis associated with some of the antigens in the MNS blood group system. ***Immunohematology*** **2006;22:171–182.**

Key Words: MNS blood group, MNS antigens, glycophorin genes, hybrid glycophorins, genetic mechanism, molecular basis

MNS Blood Group System

The MNS blood group system was the second blood group system to be discovered.[1] The MNS antigens are carried on glycophorin A (GPA), glycophorin B (GPB), or hybrids of GPA and GPB, and are fully developed at birth. Currently, this blood group system consists of more than 40 distinct antigens (Table 1), and is second only to the Rh blood group system in its complexity.[2] The antigens of the MNS system arise from single nucleotide substitution, unequal crossing over, or gene conversion between the glycophorin genes (Table 2).

Glycophorin A and Glycophorin B

GPA is the most abundant sialoglycoprotein in the RBC membrane with an estimated 1×10^6 copies per RBC.[3-5] GPA consists of 131 amino acids with an approximate molecular weight of 43 kDa [by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)], organized into three domains: an extracellular N-terminal domain of 72 amino acids, a hydrophobic membrane-spanning domain of 23 amino acids, and a C-terminal cytoplasmic domain of 36 amino acids.

GPB is similar to GPA, but consists of 72 amino acids, has an approximate molecular weight of 25 kDa (by SDS-PAGE) and is also organized into three domains: an extracellular N-terminal domain of 44 amino acids, a hydrophobic membrane-spanning domain of 20 amino acids, and a short C-terminal cytoplasmic domain of 8 amino acids. There are an estimated 2×10^5 copies of GPB present on the RBC membrane.[4,5]

On intact RBCs, GPA is susceptible to cleavage by trypsin at amino acid residue 31 and 39 but is resistant to α-chymotrypsin cleavage whereas GPB is resistant to trypsin cleavage but sensitive to α-chymotrypsin at amino acid residue 32.[3,6] Thus, these proteolytic enzymes are useful in laboratory testing to identify antibodies in the MNS blood group system.

Function of Glycophorin A and Glycophorin B

GPA and GPB contribute most of the carbohydrate on the RBC membrane. The amino-terminal domains of GPA and GPB carry O-glycans while only GPA carries an asparagine-linked-glycan (N-glycan).[5] The O-glycans are smaller molecules than N-glycans and are attached to serine or threonine. In glycophorins, it is the O-glycans that carry most of the sialic acid and contribute to the net negative charge of the RBC through the high sialic acid content of each glycoprotein. The negative charge keeps RBCs from sticking to each other and to the endothelial cells of the blood vessels.[5,7] The negatively charged glycocalyx also protects the RBC from invasion by bacteria and other pathogens.[8,9] GPA-deficient RBCs are more resistant to invasion by *Plasmodium falciparum* merozoites due to the reduction of sialic acid on the RBCs because sialic acid appears to be essential for adhesion of the parasite to the RBC.[10-14] The sialic acid attached to GPA and GPB has been reported to be the target of the influenza virus[8] and the encephalomyocarditis virus.[15] Moreover, GPA plays the role of chaperone for band 3 transport to the RBC membrane.[16,17]

Table 1. Antigens of the MNS blood group system[2]

Antigen	Historical name	ISBT number
M		MNS1
N		MNS2
S		MNS3
s		MNS4
U		MNS5
He	Henshaw	MNS6
Mi^a	Miltenberger	MNS7
M^c		MNS8
Vw	Gr, Verweyst	MNS9
Mur	Murrell	MNS10
M^g	Gilfeather	MNS11
Vr	Verdegaal	MNS12
M^e		MNS13
Mt^a	Martin	MNS14
St^a	Stones	MNS15
Ri^a	Ridley	MNS16
Cl^a	Caldwell	MNS17
Ny^a	Nyberg	MNS18
Hut	Hutchinson	MNS19
Hil	Hill	MNS20
M^v	Armstrong	MNS21
Far	Kam	MNS22
s^D	Dreyer	MNS23
Mit	Mitchell	MNS24
Dantu		MNS25
Hop	Hopper	MNS26
Nob	Noble	MNS27
En^a		MNS28
ENKT	En^aKT	MNS29
'N'		MNS30
Or	Orriss	MNS31
DANE		MNS32
TSEN		MNS33
MINY		MNS34
MUT		MNS35
SAT		MNS36
ERIK		MNS37
Os^a		MNS38
ENEP		MNS39
ENEH		MNS40
HAG		MNS41
ENAV	AVIS	MNS42
MARS		MNS43
ENDA	High – GPA-B-A	MNS44
ENEV	High – V62G	MNS45
MNTD	Low – T17R	MNS46

Table 2. Molecular mechanisms and associated antigens

Molecular mechanism	Associated antigens
Single nucleotide substitution	GPA: Vr, Mt^a, Ri^a, Ny^a, Or, ERIK, Os^a, ENEP/HAG, ENAV/MARS
	GPB: S/s, M^v, s^D, Mit
Two or more nucleotide substitution	M/N
Unequal crossing over	St^a, Dantu, Hil, TSEN, MINY, SAT
Gene conversion	He, Mi^a, Vw/Hut/ENEH, Mur, M^g, M^c, St^a, Hil, Hop, Nop, DANE, MINY, MUT

GYP Gene Family

The genes encoding GPA (*GYPA*) and GPB (*GYPB*) are homologous and are located on the long arm of chromosome 4.[18] Although it may not encode a RBC product, a third homologous gene, *GYPE,* which is adjacent to *GYPB,* can contribute to hybrid genes. The *GYPA* has seven exons, *GYPB* has six exons (of which exon 3 is a pseudoexon or non-coding exon), and *GYPE* has six exons (of which exons 3 and 4 are pseudoexons).[19–22] For each of the *GYP* genes, exon 1 and part of the 5′ end of exon 2 encode the leader sequence for the corresponding glycophorin, exons 2 to 4 encode the extracellular domains, exon 5 encodes the transmembrane domains of each glycophorin, and exon 6 and part of the 5′ end of exon 7 of *GYPA* encode the cytoplasmic domain of GPA (Fig. 1: Modified from Reid ME, 1994).[23]

Polymorphic Antigens

M, N, S, and s antigens

GPA carries M and N antigens. M has serine and glycine while N has leucine and glutamic acid at position 1 and 5, respectively.[24,25] The first 26 amino acids of GPB are identical to GPA carrying the N

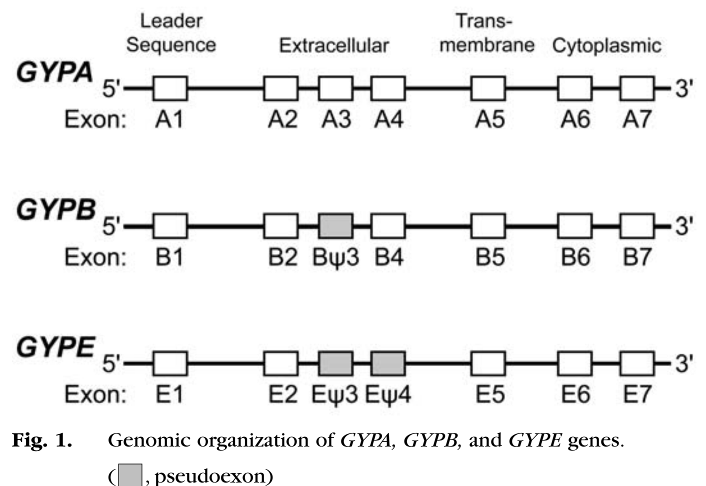

Fig. 1. Genomic organization of *GYPA, GYPB,* and *GYPE* genes. (, pseudoexon)

antigen.[19,24,26] GPB carries S or s antigens. The Ss polymorphism of GPB depends on a single amino acid substitution at position 29; S has methionine and s has threonine[27] (Table 3).

Table 3. Amino acid polymorphisms of GPA and GPB

Glycophorin	Gene	Common variants	Amino acid polymorphisms
GPA	*GYPA*	GPA^M	1 Ser, 5 Gly
		GPA^N	1 Leu, 5 Glu
GPB	*GYPB*	GPB^S	29 Met
		GPB^s	29 Thr

High-Prevalence Antigens

U antigen

The U antigen is commonly found in all populations. The U– phenotype is found among Blacks and its prevalence in certain regions of Africa is as high as 35 percent.[28] For expression, the U determinant requires the presence of GPB amino acids 33 to 39[29] and possibly an interaction with another membrane protein, the Rh associated glycoprotein (RhAG).[30] The U– phenotype is associated with an absence of GPB or with certain altered forms of GPB.[31] U– RBCs (except those that are Dantu+ and some Rh_{null}/Rh_{mod} RBCs) are S–s–. Of S–s– RBCs, approximately 16 percent have weak expression of U antigen ($U+^{var}$) encoded by a hybrid *GYP* gene. Of these $U+^{var}$ samples approximately 23 percent are He+.[32,33]

En^a antigen

Anti-En^a is a global term used for antibodies that detect high prevalence antigens on GPA. The sensitivity of these antigens to enzyme treatment is dependent on their location on GPA. Thus, En^a antigens are classified as trypsin sensitive (TS), ficin sensitive (FS), and ficin resistant (FR). En^a antigens are absent from GPA-deficient RBCs and those carrying certain variants of GPA.[3,5,7,34]

Genetic Mechanisms Giving Rise to Variant Phenotypes

Variant phenotypes may occur as a consequence of a single amino acid substitution, crossing over, gene conversion, or gene deletion (Table 2). A novel sequence of amino acids exposed on the outside surface of the RBC can result in the expression of novel antigens.

GYP *gene deletions giving rise to null phenotype RBCs*

A deletion of *GYPA* (exon 2 to 7) and *GYPB* (exon 1) gives rise to the rare En(a–)Fin phenotype and RBCs from these individuals lack GPA and, thus, antigenic determinants associated with GPA.[31,35] Deletion of *GYPB* (exon 2 to 6) and *GYPE* (exon 1) precludes production of GPB and RBCs from these individuals have the S–s–U– phenotype.[36] A deletion of *GYPA* (exon 2 to 7), *GYPB* (exon 1 to 6) and *GYPE* (exon 1) results in the M^kM^k genotype and RBCs from these individuals lack both GPA and GPB and, thus lack all MNS blood group antigens[20,37] (Table 4).

Table 4. Molecular basis of null phenotype RBCs

Null phenotype RBCs	Molecular basis
En(a–)Fin	Deletion of *GYPA* (exon 2 to 7) and *GYPB* (exon 1)
S–s–U– (deletion type)	Deletion of *GYPB* (exon 2 to 6) and *GYPE* (exon 1)
M^kM^k	Deletion of *GYPA* (exon 2 to 7), *GYPB* (exon 1 to 6), and *GYPE* (exon 1)

Nucleotide substitution

Those antigens in the MNS system that result from a single nucleotide substitution within *GYPA* or *GYPB*[31] are listed in Table 5.

Crossing over and gene conversion

The *GYPA, GYPB,* and *GYPE* share more than 95 percent sequence identity, span at least 150 kb of DNA,

Table 5. Molecular basis of antigens in the MNS system involving single nucleotide substitution[38]

Antigen	Exon	Nucleotide change	Amino acid change
GPA			
ENEH/Vw/Hut	3	140C>T>A	Thr28Met/Lys
Vr	3	197C>A	Ser47Tyr
Mt^a	3	230C>T	Thr58Ile
Ri^a	3	226G>A	Glu57Lys
Ny^a	3	138T>A	Asp27Glu
Or	3	148C>T	Arg31Trp
ERIK	4	232G>A	Gly59Arg
Os^a	3	217C>T	Pro54Ser
ENEP/HAG	4	250G>C	Ala65Pro
ENAV/MARS	4	244C>A	Glu63Lys
GPB			
S/s	4	143T>C	Met29Thr
M^v	2	65C>G	Thr3Ser
s^D	4	173C>G	Pro39Arg
Mit	4	161G>A	Arg35His

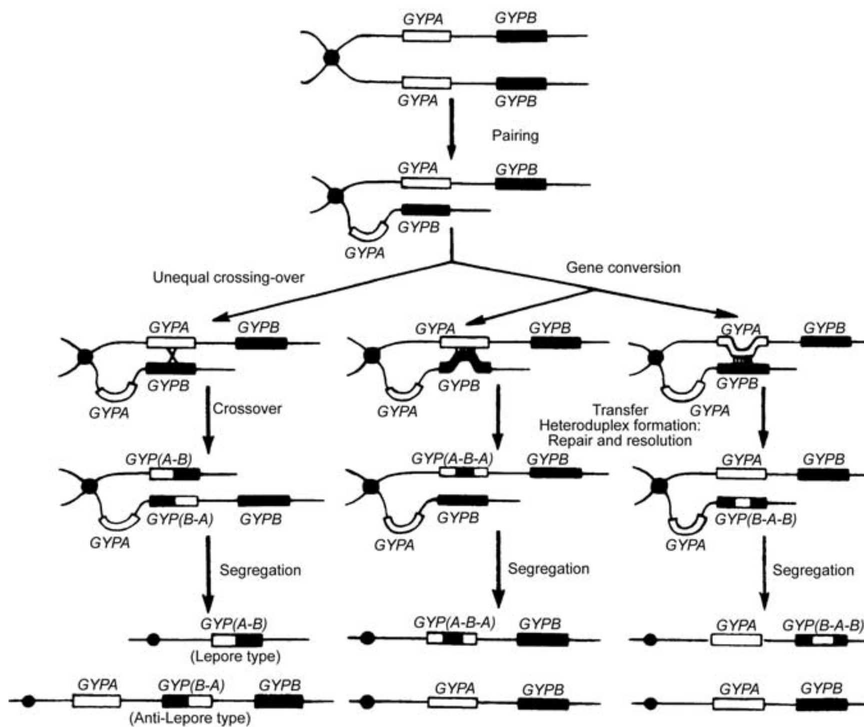

Fig. 2. An unequal crossing-over event and gene conversion events between homologous glycophorin genes on the long arm of chromosome 4 during meiosis.

[Figure is adapted from C.-H. Huang (Huang C-H, Blumenfeld OO. MNSs blood groups and major glycophorins: Molecular basis for allelic variation. In: Molecular Basis of Human Blood Group Antigens. Cartron J-P, Rouger P (eds). New York: Plenum Press, 1995; p.160.), with permission.]

and are adjacent at the MNS locus in the order of 5′-*GYPA-GYPB-GYPE*-3′. *GYPE* probably does not encode a RBC membrane component but it does participate in gene rearrangements resulting in variant alleles. Sequence homology between the glycophorin genes increases the chance of recombination because of unequal crossing over or gene conversion events (Figure 2: Adapted with permission from Cheng-Han Huang).[31,39]

Unequal crossing over is a mutual exchange of nucleotides between misaligned homologous genes during meiosis; this mechanism occurs between regions of homology with generation of two recombinants in reciprocal arrangement. A chromosome carrying the hybrid *GYP(A-B)* does not have *GYPA* and *GPYB* (Lepore type). In contrast, a chromosome carrying a hybrid *GYP(B-A)* also has both *GYPA* and *GYPB* (Anti-Lepore type).

Gene conversion can occur during the process of DNA repair between homologous genes during meiosis.[31,40] Gene conversion is the transfer of nucleotides from one gene to another gene and does not result in a reciprocal product. Gene conversion can cause insertion of nucleotides from *GYPA* into the *GYPB* or *GYPB* into the *GYPA*. As a result of this mechanism, the chromosome carrying a *GYP(B-A-B)* also carries a *GYPA* but not *GYPB,* whereas the chromosome carrying a *GYP(A-B-A)* also carries a *GYPB* but not *GYPA.* During gene conversion, a consensus splice sequence may be altered and a number of recombinant products have been described. These alterations sometimes cause transcription of part of the *GYPB* pseudoexon 3 and hence allow translation of a novel sequence of amino acids.[31] Gene conversion gives rise to hybrid genes that encode novel glycophorin molecules carrying certain low-prevalence antigens in the MNS blood group system (Table 6).

Low-Prevalence Antigens

He

The He antigen is found in about 3 percent of African Americans but is very rare in Caucasians.[41] Protein sequencing showed that GP.He is identical to GPB except for an alteration at the amino-terminus; the amino acids leucine, threonine, and glutamic acid found in positions 1, 4, and 5 of GPB are replaced by threonine, serine, and glycine, respectively.[42] In GP.He, the presence of a glycine residue at position 5 is recognized by some anti-M. DNA analysis has shown that He antigen is due to gene conversion resulting in a *GYP(B-A-B)* or a *GYP(B-A-ΨB-A)* hybrid. In addition to the classic He+S+ and He+s+ variants, four other *GYPB* variants of GP.He have been described as follows.

GP.He(P2) is characterized by two mutations in *GYPB:* a C>G change at the 3′ end of exon 5, which creates a cryptic acceptor splice site, and a G>T change at position +5 of intron 5, which alters the consensus donor splice site.[43] These two mutations cause skipping of exon 5 and a shift in the open reading frame with chain elongation. Consequently, a portion of the 3′ untranslated region is read during protein synthesis, causing GP.He(P2) to have a new transmembrane hydrophobic sequence. This variant does not express the S antigen or the U antigen. GP.He(P2) has not been detected in the RBC membrane; however, these S–s–RBCs are $He+^{w}$ because of the expression of low levels

of GPB.He. GP.He(NY) is characterized by a partial deletion of exon 5 that alters the open reading frame and is predicted to encode a protein of 43 amino acids, which has not been demonstrated in the RBC membrane. The S–s– RBCs are He+^{w} caused by the expression of low levels of GPB.He.[33]

GP.He(GL) is characterized by two nucleotide changes: a T>G mutation at nucleotide -6 of the acceptor splice in intron 3, which leads to skipping of exon 4, and a C>G mutation in exon 5. The latter point mutation results not only in a predicted Thr>Ser substitution at position 65 but in the creation of a new acceptor splice site; partial inactivation of the normal splice site results in four different cDNAs. The full-length transcript GP.He-1, which is equivalent to GPB in molecular size, contains a Thr>Ser substitution at position 65 and encodes He, S, and U antigens. Transcript GP.He-2 codes for a polypeptide with an intact transmembrane segment but, because of the deficiency of exon 4, the encoded protein lacks the sequence defining the S and U antigens as well as the cleavage sites of α-chymotrypsin.[3] Transcripts GP.He-3 and GP.He-4 are low-level transcripts with major deletions which probably prevent insertion of their putative protein products into the membrane.[44]

GP.Cal is an example of a gene conversion event resulting in a GP(B-A-ΨB-A) hybrid that carries both He and St^a antigens. The *GYPA* recombination site is in exon 2; the mature protein, after cleavage of the leader peptide, is GP(A-A). *GYPB* also contributes the pseudo-exon, which is out-spliced.[45] The 5′ portion of GP.Cal is similar to GP.He with a GP(B-A-B) arrangement, whereas its 3′ portion has a GP(B-A) configuration identical to the GP.St^a.[46]

SAT

The SAT antigen is associated with two different glycophorin isoforms.[47] GP.TK is a GP(A-B) hybrid composed of exons 1 to 4 of *GYPA*^{N} and exons 5 to 6 of *GYPB.*[48] This transcript encodes 104 amino acids with a hexapeptide sequence "Ser-Glu-Pro-Ala-Pro-Val" at positions 69 to 74 produced by the junction between GPA and GPB, and encodes the SAT antigen. GP.SAT is associated with a *GYPA-B-A* hybrid gene which encodes a GPA-B-A hybrid (Table 6). This variant is characterized by an insert, between exon 4 of *GYPA* and exon 5 of *GYPB,* of nine bases (three amino acids) originating from the 5′ end of exon 5 of *GYPB.* The tri-peptide sequence "Ala-Pro-Val" inserted into the GPA molecule creates the SAT-specific sequence.[49]

Table 6. Hybrid glycophorin molecules, phenotypes, and associated low prevalence antigen[38]

Molecular basis	Glycophorin	Phenotype symbol	Antigens associated with hybrid
GYP(A-B)	GP(A-B)	GP.Hil (Mi.V)	Hil, MINY
		GP.JL (Mi.XI)	TSEN, MINY
		GP.TK	SAT
GYP(B-A)	GP(B-A)	GP.Sch (M^r)	St^a
		GP.Dantu	Dantu (see Table 7)
GYP(A-B-A)	GP(A-B-A)	GP.M^g	M^g
		GP.KI	Hil
	GP(A-B)	GP.SAT	SAT
GYP(B-A-B)	GP(B-A-B)	GP.Mur (Mi.III)	Mi^a, Mur, MUT, Hil, MINY
		GP.Bun (Mi.VI)	Mi^a, Mur, MUT, Hop, Hil, MINY
		GP.HF (Mi.X)	Mi^a, MUT, Hil, MINY
		GP.Hop (Mi.IV)	Mi^a, Mur, MUT, Hop, TSEN, MINY
	GP(B-A-B)	GP.He; (P2, GL)	He
GYP(B-A-ΨB-A)	GP(A-A)	GP.Cal	He, St^a
GYP(A-ΨB-A)	GP(A-B-A)	GP.Vw (Mi.I)	Mi^a, Vw
		GP.Hut (Mi.II)	Mi^a, Hut, MUT
		GP.Nob (Mi.VII)	Nob
		GP.Joh (Mi.VIII)	Nob, Hop
		GP.Dane (Mi.IX)	Mur, DANE
	GP(A-A)	GP.Zan (M^Z)	St^a
GYPA 179G>A	GPA	GP.EBH	ERIK (from transcript 1) See text
	GP(A-A)	GP.EBH	St^a (from transcript 2) See text
GYP(A-ΨE-A)	GP(A-A)	GP.Mar	St^a

Dantu

GP.Dantu is encoded by a *GYP(B-A)* hybrid. This hybrid gene consists of exon 1, exon 2, pseudoexon 3, exon 4 of *GYPB,* and exons 5 to 7 of *GYPA.* There are four types of Dantu phenotype, designated NE, MD, Ph, and JO variants (Table 7). The MD hybrid gene is flanked by *GYPA* and *GYPB,* suggesting that this type originated from a single unequal crossing-over event.[50] The NE and Ph variants contain a *cis GYPA* but lack a *GYPB.*[51] In the case of NE, the GYP(B-A) hybrid gene is duplicated; thus NE and Ph variants can be distinguished by the ratio of GP.Dantu [GP(B-A) hybrid] to GPA.[52] RBCs with the JO variant have only one-half the normal levels of GPA, thus leading to the assumption that this variant may contain a GP(A-B-A) hybrid molecule and an unchanged *GYPB* rather than an unchanged *GYPA* and GP(B-A) hybrid.[53]

Table 7. Mechanism, ethnicity, and occurrence of Dantu phenotypes

Dantu phenotypes	Mechanism	Ethnicity	Occurrence (relative)
NE	*GYPA-GYP(B-A)-GYP(B-A)*	Black	Most (common phenotype)
MD	*GYPA-GYP(B-A)-GYPB*	White	Rare (1 proband)
Ph	*GYPA-GYP(B-A)*	Black	Rare (1 proband)
Jo	*GYP(B-A)-GYPB*	White	Rare (1 proband)

M^g

The M^g antigen is associated with a *GYP(A-B-A)* hybrid; it has the same amino acids at positions 1 (leucine) and 5 (glutamic acid) as *GYPA^N* but threonine in position 4 is substituted by an asparagine.[54] RBCs with GP.M^g do not react with anti-M and anti-N but do react with anti-M^g.[55] M^g+ RBCs react with anti-DANE because of the amino acids Asn-Glu-Val at positions 4, 5, and 6 are thought to be part of the DANE epitope.[56,57]

St^a (Stones)

The St^a antigen is most commonly associated with a *GYP(B-A)* hybrid but also can be associated with *GYP(A-B-A)* or *GYP(A-E-A)* hybrid genes.[38]

GP.Sch (M^r) is encoded by a *GYP(B-A)* hybrid. The hybrid gene arose from a single unequal crossing over between misaligned *GYPA* and *GYPB.* This misalignment led to *GYPB* exons 1 to pseudoexon 3 being joined to exons 4 to 7 of *GYPA.* The St^a epitope is determined by the amino acid sequence of the junction of exon 2 to exon 4 of either GPB or GPA. The molecule is composed of amino acids 1 to 26 of GPB and amino acids 59 to 131 of GPA.

GP.Zan (M^Z) is encoded by a *GYP(A-ΨB-A)* hybrid. The GP.Zan phenotype is characterized by the co-transmission of M and St^a.[58] The homologous segment of *GYPB* pseudoexon 3 replaces exon 3 and the 5′ end of intron 3 of *GYPA,* introducing the defective donor splice site of the pseudoexon. Thus, the encoded glycophorin consists of GPA lacking the amino acids encoded by exon 3.

GP.EBH is another St^a carrying glycophorin, which arises by a single nucleotide substitution at position 179 in *GYPA.* This variant is caused by a G>A mutation in the 3′ end of exon 3,[39] which creates a Gly>Arg substitution at amino acid position 59. The mutation also affects pre-mRNA splicing because of the partial inactivation of the adjacent 5′ donor splice site. The full-length transcript (transcript 1) encodes a variant GPA molecule with the arginine substitution at amino acid position 59 defining the ERIK antigen, whereas the shorter transcript (transcript 2) lacks exon 3 and carries the St^a antigen.[58,59]

GP.Mar is encoded by a *GYP(A-ΨE-A)* hybrid that arose from a homologous DNA transfer from *GYPE* to *GYPA.* This *GYPE* segment covers pseudoexon 3 and extends to the defective donor splice site mutated by the G>A transition at +1 position of GT dinucleotide, which abolishes a donor splice site as well as the expression of exon 3 of GPA.[60] *GYP.Mar* gene is apparently identical to the *GYP.Zan* in the mode of gene conversion and in the resulting glycophorin.

The so-called or obsolete Miltenberger subsystem

A number of low-prevalence antigens in the MNS blood group system were for many years grouped together in the Miltenberger (Mi.) subsystem.[61] Originally, RBCs reactive with the anti-Mi^a serum were classified into four classes on the basis of their different reactions with four type sera called Verweyst (Vw), Miltenberger (Mi^a), Murrell (Mur), and Hill (Hil).[62] Some of the classes of Miltenberger did not react with anti-Mi^a but reacted with one or more of the other three specific antisera, e.g., GP.Hil (Mi.V) RBCs reacted with anti-Mi^a but did not react with anti-Hil.[63] The Miltenberger subsystem grew to 11 classes, which were defined by one or more determinants reacting with type-specific antisera (Table 8).[38,64]

As the complexity of the Miltenberger subsystem increased, further expansion no longer seemed feasible, desirable, or relevant. A notation was introduced to replace the classification of MNS variants into the increasingly complicated Miltenberger subsystem. In this notation, the serologically specified phenotypes are defined by characteristic glycophorin variants (GP. for the glycoprotein and GYP. for the gene) with the abbreviated name of the propositus in whom the variant has been described, e.g., Mi.V becomes GP.Hil and the encoding gene is referred to as *GYP.Hil.* The Miltenberger subsystem will not be expanded further and is now considered obsolete.[65] The molecular basis of antigens in the obsolete Miltenberger subsystem can be placed into the following categories.

1. Glycophorin A-B hybrids: GP(A-B)

GP.Hil (Mi.V) and GP.JL (Mi.XI) are each encoded by a *GYP(A-B)* hybrid gene (Fig. 3-I). The *GYPA* to *GYPB* junction in the *GYP.Hil* gene is located at the 5′ end of the intron 3 of *GYPA,* whereas in *GYP.JL* the junction occurs at the 3′ end of intron 3 and includes 7 nucleotides (nts) of exon 4 of *GYPB.* The allele responsible for GP.Hil encodes s antigen, travels with either Ms or Ns, and expresses Hil and MINY

Table 8. GP classification and associated antigens of the obsolete Miltenberger subsystem[38]

GP.	Mi.Reaction of RBCs with antiserum										
	Classes	**Mi^a**	**Vw**	**Mur**	**Hil**	**Hut**	**MUT**	**Hop**	**Nob**	**DANE**	**MINY**	**TSEN**
GP.Vw	Mi.I	+	+	–	–	–	–	–	–	–	–	–
GP.Hut	Mi.II	+	–	–	–	+	+	–	–	–	–	–
GP.Mur	Mi.III	+	–	+	+	–	+	–	–	–	+	–
GP.Hop	Mi.IV	+	–	+	–	–	+	+	–	–	+	+
GP.Hil	Mi.V	–	–	–	+	–	–	–	–	–	+	–
GP.Bun	Mi.VI	+	–	+	+	–	+	+	–	–	+	–
GP.Nob	Mi.VII	–	–	–	–	–	–	–	+	–	–	–
GP.Joh	Mi.VIII	–	–	–	–	–	–	+	+	–	–	–
GP.Dane	Mi.IX	–	–	+	–	–	–	–	–	+	–	–
GP.HF	Mi.X	+	–	–	+	–	+	–	–	–	+	–
GP.JL	Mi.XI	–	–	–	–	–	–	–	–	–	+	+

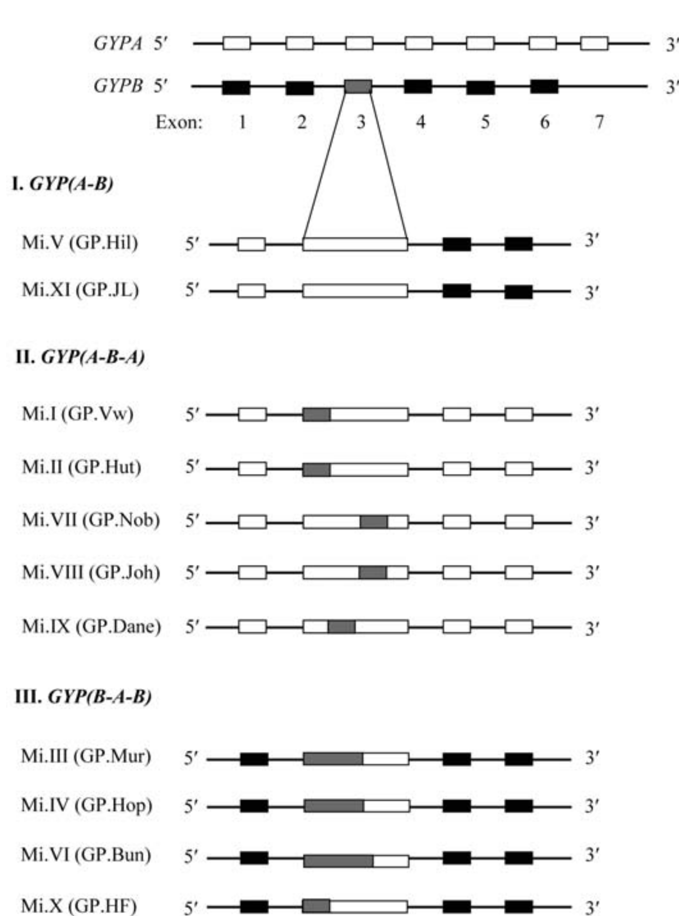

Fig. 3. Genomic organization of some glycophorin hybrids. (, GPA; , GPB; and , pseudoexon B)

antigens,[22,66,67] whereas GP.JL expresses an altered S, a weak M, TSEN, and MINY antigens.[67–71] No example of GP.Hil phenotype was found in testing 50,000 English donors, but GP.Hil was found with a frequency of 1 in 2000 in one survey of Swiss blood donors.[72]

2. Glycophorin A-B-A hybrids: GP(A-B-A)

GP.Vw (Mi.I), GP.Hut (Mi.II), GP.Nob (Mi.VII), GP.Joh (Mi.VIII), and GP.Dane (Mi.IX) are each encoded by a *GYP(A-B-A)* hybrid (Fig. 3-II). In these hybrid genes, inserts of different short portions of the pseudoexon of *GYPB* replace the same number of nucleotides in exon 3 of *GYPA*. The small inserts range from 1 to 16 bp and do not alter the open reading frame or disrupt the splice sites. Thus, the short part of the pseudoexon is translated in this hybrid.

The insert encoding GP.Vw and GP.Hut variants results in an amino acid polymorphism at position 28; the threonine present in GPA is changed to methionine in the case of GP.Vw[73] and to lysine in the case of GP.Hut. GPVw and GP.Hut phenotype RBCs are recognized by anti-Vw and anti-Hut, respectively. The allele responsible for GP.Vw usually travels with Ns, NS, or MS whereas GP.Hut travels with MS or Ns. The highest prevalence of GP.Vw phenotype, 1.43 percent, was found in southeastern Switzerland.[41]

RBCs with GP.Nob express the Nob antigen. Structural analysis of GP.Nob showed that it differs from GPA at amino acid positions 49 and 52; the arginine at position 49 in GPA is substituted by threonine and the tyrosine at position 52 is replaced by serine because of ten nucleotides in exon 3 of *GYPA* (nt 67–76) that have been replaced by the corresponding sequence of the GPB pseudoexon.[74,75] The GP.Nob phenotype has been only found in white donors; three positive reactors with the Raddon serum were found in tests on 4929 random group O blood donors at Bristol, England, a frequency of 0.06 percent.[76] GP.Nob is associated with Ms and MS.

GP.Joh closely resembles GP.Nob but has the Hop antigen as well as the Nob antigen. The altered GPA of

GP.Joh differs from GP.Nob by having only the arginine to threonine substitution at amino acid position 49.[77] The frequency of GP.Joh is unknown. The *GYP.Joh* traveled with Ns in the families of the two known propositi.

RBCs carrying GP.Dane express Mur and DANE antigens. In GP.Dane, exon 3 of *GYPA* (codons 35–41) is replaced by the corresponding sequence of the *GYPB* pseudoexon.[78] As a consequence of this gene conversion event, the hepta peptide sequence of GPA, ^{35}Ala-Ala-Thr-Pro-Arg-Ala-His^{41}, is changed to the hexapeptide sequence ^{35}Pro-Ala-His-Thr-Ala-Asn^{40}. GP.Dane has a prevalence of 0.43 percent in Danes. In the four Danish propositi, *GYP.Dane* was inherited with *MS*.[56]

3. Glycophorin B-A-B hybrids: GP(B-A-B)

GP.Mur (Mi.III), GP.Hop (Mi.IV), GP.Bun (Mi.VI), and GP.HF (Mi.X) are each encoded by a *GYP(B-A-B)* hybrid (Fig. 3-III).

GP.Mur RBCs are Mur+, Hil+ and MINY+ and the allele responsible for GP.Mur always travels with s antigen, either as Ms or Ns. GP.Bun is almost identical to GP.Mur but GP.Bun cells are Hop+ and the allele responsible for GP.Bun was aligned with Ms. Both GP.Mur and GP.Bun are encoded by a *GYPB^s* but differ in the length of the GPB pseudoexon insert (55 bp for GP.Mur and 131 bp for GP.Bun).[79] Because this segment comprises a portion of both exon 3 and intron 3, which carries a functional 5′ splicing signal, the rearrangement results in the expression of a normally unexpressed *GYPB* pseudoexon sequence. The *GYP.Bun* gene differs from the *GYP.Mur* gene by only one nucleotide in the coding sequence. This results in a predicted arginine (GP.Mur) or threonine (GP.Bun) at position 48. GP.Mur and GP.Bun are rare in Caucasians but GP.Mur has a prevalence between 5 and 10 percent in some Asian populations,[40,80,81] 9.6 percent in Thais, and 7.3 percent in Taiwanese.[82]

GP.Hop, which expresses TSEN but not Hil, is identical to GP.Bun. The allele responsible for GP.Hop always travels with S, whereas the allele for GP.Bun always carries the s antigen.[61,83]

GP.HF is characterized by M and an unusually strong s antigen as well as by its reactivity with anti-Hil and anti-MINY. This glycophorin hybrid is similar to GP.Mur and GP.Bun.[84] In GP.HF, a 98-bp insert from exon 3 of *GYPA* creates a *GYP(B-A-B)* hybrid, which encodes a peptide differing from GP.Mur by five amino acid residues and from GP.Bun by six amino acid residues.

Conclusion

The MNS system is a complex blood group system consisting of more than 40 antigens on GPA and GPB, or on hybrid glycophorin molecules. It is second only to the Rh blood group system in its complexity. The antigens of the MNS blood group system arise from single nucleotide substitution, unequal crossing over, gene conversion, or both between the glycophorin genes. Some of these molecular mechanisms occur as a consequence of misalignment of the chromosomes carrying the glycophorin genes during meiosis. This is possible because the glycophorin family of genes is homologous and adjacent on the chromosome.

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