REVIEW ARTICLE

The MNS blood group system: a review

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ABSTRACT

In transfusion science, blood group systems are one of the significant factors that must be considered throughout the donation and transfusion processes. One of these blood group systems is the MNS system, which was discovered second only to the ABO system, in 1927. The system is comprised of a large group of polymorphic, high-frequency and low-frequency antigens, which have formed from genetic recombination, unequal crossing over, gene conversion, and single nucleotide polymorphisms (SNPs). Besides the genetic mechanisms, the phenotypic frequencies among population groups are interesting characteristics of this blood group system. The antigens are found on single pass sialoglycoproteins A and B on the red blood cell (RBC) membrane as well as the renal endothelium and epithelium. They are involved in many roles such as preventing RBC agglutination in blood vessels, complement regulation, transmembrane signalling, anion exchange, and binding cytokines. They are even thought to bind bacteria and malarial parasites, meaning that individuals with null phenotypes have resistance to some infectious diseases. The clinical significance of this blood group system in transfusion practice comes from the alloantibodies that can cause transfusion reactions or haemolytic disease of the fetus and newborn (HDFN).

This review aims to provide an informative and critical summary of the MNS blood group system in the context of transfusion science.

Keywords: MNS blood group system, sialoglycoproteins, red blood cell, transfusion reactions.

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INTRODUCTION

In transfusion science, many of the human blood group systems have alloantibodies that are clinically significant, with the potential to cause adverse events during blood transfusion (1). It is therefore important to understand the potential for adverse immunological events for each of the blood group systems and in this review the MNS blood group system will be considered.

Discovery

In the MNS blood group system, the major antigens are M, N, S, s, and U (2, 3). After the discovery of the ABO system, Landsteiner and Levine later discovered the M and N antigens in 1927. The letters of these antigens were taken from the word 'immune', as they discovered that rabbits became immunised to produce anti-M and anti-N, following exposure to human red blood cells (RBCs) (3-5). Later in 1947, Walsh and Montgomery discovered an antibody in a patient against a new antigen which they called S, named after 'Sydney ' in Australia where it was first found. The antithetical antigen of S was named s, following its discovery in 1951 (3-5). Genetic studies into several families showed that the loci of M/N and S/s were closely linked and that there was a high degree of polymorphism of this blood group system (6). The U antigen of the MNS system was later discovered in 1953 by Wiener, Unger, and Gordon, and stands for 'universal' based on its universal distribution as it is expressed in almost all Caucasians and Africans (3-5, 7, 8).

According to the most recent classifications by the International Society of Blood Transfusion (ISBT), there are a total of 50 antigens in the MNS blood group system (ISBT 002) (9). The frequencies of different MNS phenotypes in Caucasian and African populations are displayed in Table 1 (1, 4, 7, 10). Other than the main antigens, there are others of high prevalence, existing in over 90% of the population, or low prevalence, seen in less than 1% of the population (6).

Genetics

The genes encoding the MNS proteins are found on the long arm of chromosome 4 (2, 4, 5, 11). The two codominant alleles for the M (MNS1) and N (MNS2) antigens are found on the *GYPA* gene which encodes the glycophorin A (GPA) protein (2, 3, 11). It is thought that the *GYPA* gene duplicated and underwent cross-over between two chromatid strands, resulting in progenitor *GYPB* and *GYPE* segments that became independent genes (3, 4). This makes the *GYPA*, *GYPB*, and *GYPE* genes very similar (>95% homology) and closely linked, allowing further recombination to occur (3, 4). This confers the formation of a highly diverse group of antigens in this system that is much larger than in other blood group systems such as ABO, Kell, Duffy, and Kidd (2, 4, 9 12). The *GYPB* gene encodes for glycophorin B (GPB) and has an MNS3 allele for the S antigen, and an MNS4 allele for the s antigen, which are also co-dominantly expressed (2, 3, 11). The M and N alleles differ by three single nucleotide polymorphisms (SNPs) on the *GYPA* gene, leading to serine and glycine in the M antigen and leucine and glutamic acid in the N antigen at amino acid positions 1 and 5, respectively (Figure 1) (2-4, 7). On the other hand, the S and s alleles only have one SNP difference in the *GYPB* gene, leading to methionine on the S antigen and threonine on the s antigen at position 29 (Figure 1) (2-4, 7). The role of the *GYPE* gene is not well understood as its protein is not found on the RBC surface; however, it is thought to contribute to other MNS variants through gene rearrangement with *GYPB*. It has an identical genetic sequence to *GYPA*, so expression would result in M antigens on the RBC surface (3-5, 11).

Proteins and antigens

The M/N and S/s antigens are found on single pass sialoglycoproteins A and B, respectively, as displayed in Figure 1. They are found in the glycocalyx of the RBC membrane as well as the renal endothelium and epithelium (2-5). GPA is glycated by N-linked and O-linked oligosaccharides, while GPB only has N-glycosylation (4, 6). The sialic acid contributes to the negative charge on the RBC surface, preventing RBC agglutination for good blood flow in small blood vessels (2-5, 14). This glycation is also essential for the insertion and expression of GPA and GPB on the RBC surface (6). Other major functions include complement regulation, transmembrane signalling, facilitating anion exchange by band 3, and binding cytokines (2, 5, 6, 14). By interacting with band 3, the glycophorins provide physical support to the RBC cytoskeleton and assist with CO_2 exchange between tissues. This is reinforced by the fact that individuals with the Mur glycophorin variant with high band 3 expression were found to have a higher rate of CO_2 respiration (15). They also bind bacteria, viruses, and the *Plasmodium falciparum* parasite, due to the high abundance of glycophorins on RBCs and other cells (2, 3, 5). The MNS null phenotype of the system provides a selective advantage for resistance to some infections with some of the populations from African malaria-endemic countries having a relatively higher frequency of the S-s- and U- phenotypes (Table 1) (2, 4, 14, 16). It is thought that *P. falciparum* binds to band 3 and the sialic acid on GPA or some GPB to form a tight complex and invade the RBCs (17). Despite the overall low prevalence of S-s- phenotypes indicated by Table 1, some select African populations were found to have a S-s- frequency of up to 35%. Furthermore, a vast majority of these individuals also had the U- phenotype (18). A more recent study by Leffler *et al.* revealed that a rare MNS hybrid variant called Dantu, is present in East African populations which also confers

protection against severe malaria (16). This variant is thought to result in a hybrid between the extracellular domain of GPB and the transmembrane and intracellular domains of GPA, reducing the ability of *P. falciparum* to bind to band 3 (16, 17). Not only does the Dantu variant reduce the risk of severe malaria, it also reduces the morbidity of individuals once infected (17).

The GPA structure is thought to act as a receptor for bacteria such as pathogenic strains of *Escherichia coli*, causing urinary tract infections and possibly meningitis and septicaemia in neonates (19, 20). Several viruses have been reported to also use glycophorin structures for RBC invasion, including hepatitis A virus, reovirus, Influenza virus, and rotavirus. In contrast to *P. falciparum* infection, the presence of glycophorins on the RBC surface helps to protect individuals against these viruses, bringing viruses in the blood to sites of immune processing away from important target tissues via RBCs (21, 22).

As discussed earlier, the genetic linkage and high similarity between the genes result in a highly polymorphic and diverse group of antigens (5, 11). The main antigens M, N, S, s, and U are expressed on the cord RBC surface at birth (7). The U antigen is also present on the GPB structure and is distributed in high frequency along with other antigens such as En^a, as listed in Table 2 (2, 4, 11). Most U- individuals have associated S-s- phenotype due to the absence or alteration of the GPB protein overall (1, 5, 8). Similarly, En^a- is associated with the absence of the M and N antigens on GPA (14). The En^a antigen is present on GPA, close to the RBC surface and is part of the RBC envelope, where its name comes from (7).

Another interesting property of the MNS blood group antigens is their sensitivity to certain laboratory enzymes. For example, M and N antigens are cleaved from the glycophorin structures using ficin, papain, trypsin, and pronase, while the S and s antigens are sensitive to a-chymotrypsin and pronase with some sensitivity to both ficin and papain (2, 10). Their cleavage sites in the GPA and GPB structures are shown in Figure 1 (12). This characteristic is useful in the diagnostic identification of alloantibodies, as laboratory enzymes can destroy specific antigens and enhance the activity of others (e.g., ABO, Rh, Kidd, Lewis, P) (23). This fact also allows the differentiation of antibodies in a mixture of antibodies that respond differently to enzyme treatment (24). Some of the low frequency MNS antigens are enzyme resistant with the M variant antigen resistant to trypsin, papain, ficin, and bromelin (25).

Alloantibodies

Alloantibodies against the MNS blood group antigens have different characteristics and properties. Anti-M and anti-N are usually IgM and show a dosage effect, and some anti-M can have a partial IgG component. Alloantibodies can directly agglutinate M or N-positive RBCs due to the high expression of GPA (2, 3, 5, 11) in saline (8). Both anti-M and anti-N are more reactive and specific at a slightly acidic pH, around 6.5 (6). As they are cold-reactive, they can be detected at lower temperatures meaning they can often interfere in ABO grouping reactions, necessitating ABO grouping to be performed at 37°C (5, 11). Anti-M & N are naturally occurring alloantibodies, having been found in children without exposure to M or N antigens. As they are mostly reactive at body temperature, most are not clinically significant in blood transfusion (2-5, 11). Rare examples of anti-M and anti-N that are reactive at 37°C have been reported and have led to both immediate and delayed-type haemolytic transfusion reactions (HTR) bringing about extravascular red cell destruction (2, 4, 5, 8, 11). Anti-M rarely causes haemolytic disease of the fetus and newborn (HDFN) and neonatal RBC aplasia (2, 4-6, 8, 11). Anti-M is more common in young children, patients with bacterial infections, and pregnant women with an M+ fetus. Generally, anti-N is less often seen due to the structurally similar "N" antigen on GPB, preventing N- individuals from producing anti-N (10). On the other hand, anti-En^a are usually IgG and have caused severe HTR and HDFN reacting with the high-frequency epitopes on GPA. Because of the high prevalence of En^a epitopes in the population, it is difficult to find compatible donor units for En^a- patients with anti-En^a, who are usually also M-N- (2, 3, 5).

In contrast, antibodies against the S, s, and U antigens are generally IgG, though rare cases of IgM anti-S have been reported (2, 5, 10, 11). IgG class antibodies require the use of anti-human antiglobulin in the indirect antiglobulin test (IAT) alloantibody screen. Agglutination by anti-s can be enhanced by slightly reducing the pH to 6.0 from the buffered saline pH of 7.0 (5, 8, 10). The presence of these alloantibodies is more clinically significant than anti-M and anti-N, as they are reactive at body temperature and can cross the placenta. Anti-S and anti-s have been reported to cause delayed HTR and HDFN, in higher frequencies than anti-M or anti-N (1, 8, 14). Anti-U is rare but is known to be more severe or fatal in the event of transfusion reactions and must be considered in S-s- individuals due to the GPB-null phenotype (1, 3, 8). This means that S-, s-, or U- patients require transfusion with null phenotype blood to avoid adverse transfusion reactions (13).

Autoantibodies in this blood group system are rare, but some autoimmune diseases have been reported in the past (2, 14, 26). This includes auto anti-N in a dialysis patient using equipment that was disinfected by formaldehyde. Other examples of auto anti-N, auto anti-S, and auto anti-U have been reported to cause autoimmune haemolytic anaemia as well as warm autoantibodies against M, N, S, s, U, and En^a antigens (2, 6, 7, 26).

Genetics, Antigens, and Antibodies of Hybrid Variants

Many hybrid variants are formed through unequal crossing over, gene conversion, missense mutations, and splicing variants (4, 22, 27). New hybrid glycophorins are expressed following a repair of a defective splice site in exon III of the *GYPB* gene and this is the 'hotspot' of recombination between the homologous sequences (22, 27).The different hybrid types are named GYP(A-B) and GYP(B-A) caused by unequal crossing over; and GYP(A-B-A), GYP(B-A-B), GYP(A-E-A), and GYP(B-E-B) caused by gene conversion (4, 27, 28). Unequal crossing over between two chromatin strands results in different hybrid variant types based on the recombination site and arrangements as demonstrated in Figure 2. For instance, the GYP(A-B) strand is missing *GYPA* and *GYPB,* but the GYP(B-A) strand has both *GYPA* and *GYPB.* GYP(A-B-A) only has *GYPB* and GYP(B-A-B) has *GYPA*, by the replacement of defective splice sites (4, 27). Missense mutations or splicing variants are caused by SNPs present in the introns or exons and lead to a change in the amino acid sequence or splice site (4). Unequal crossing over as well as gene deletion are also responsible for null phenotypes such as M-N-En^a-, S-s-U-, and M^kM^k (lack of both M/N and S/s antigens) (5, 26).

MNS hybrid variants are more commonly seen in Asian populations (4). Most of the hybrid variants are low-prevalence antigens (Table 2); however, corresponding alloantibodies may be clinically significant for causing both immediate and delayed HTR and HDFN (4, 5). For example, the Mur glycophorin is common in China, Thailand, Taiwan, Singapore, Malaysia, and Vietnam, and exposure triggers an antibody response to Mi^a, Mur, MUT, and Hil antigens. The IgG class alloantibodies formed against these antigens have caused HTR and HDFN; however, some are naturally occurring and harmless (27). Alloantibodies against the Vw and Mi^a antigens on the Vw glycophorin are also common however they are clinically insignificant unless they are IgG (22, 27). Clinically significant IgG class anti-Vw examples are more common in European countries such as Switzerland, causing HDFN (22). In transfusion medicine, the identification of irrelevant IgM antibodies is avoided by using alloantibody screening cells without corresponding antigens (22). Conversely, new alloantibody screening cells or kodecytes have been developed to include specific blood group variant antigens for which antibodies may be more common and clinically significant in certain populations. For example, the epitopes of Mur and MUT peptides were successfully attached onto RBCs by KODE™ technology, without affecting the qualitiy of alloantibody screening or identification tests (30).

Table 1: MNS Phenotype Frequencies in Caucasians and Africans (1, 4, 7, 10)

MNS Phenotype	Caucasians (%)	Africans (%)
M+N-	30	25
M+N+	49	49
M-N+	21	26
S+s-	10	6
S+s+	42	24
S-s+	48	68
S-s-	0	2
U+	100	99
En^a+	100	100

Table 2. Other antigens in the MNS Blood Group System (4, 7)

Classification	Antigen (n=50)
Polymorphic	M, N, S, s
High prevalence	U, En^a, ENKT, "N", ENEP, ENEH, ENAV, ENDA, ENEV, JENU
Low prevalence	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, MNTD, SARA, KIPP, SUMI

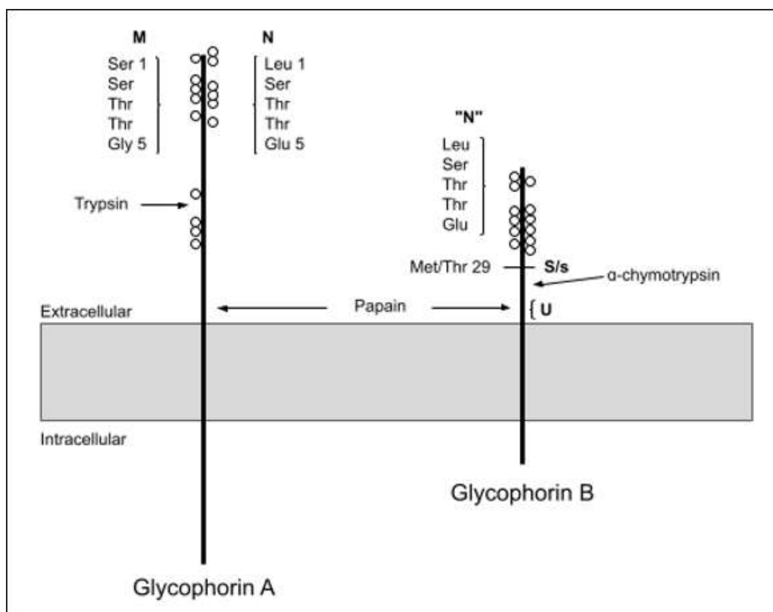

Figure 1. Structure of glycophorin A and glycophorin B adapted from (12)

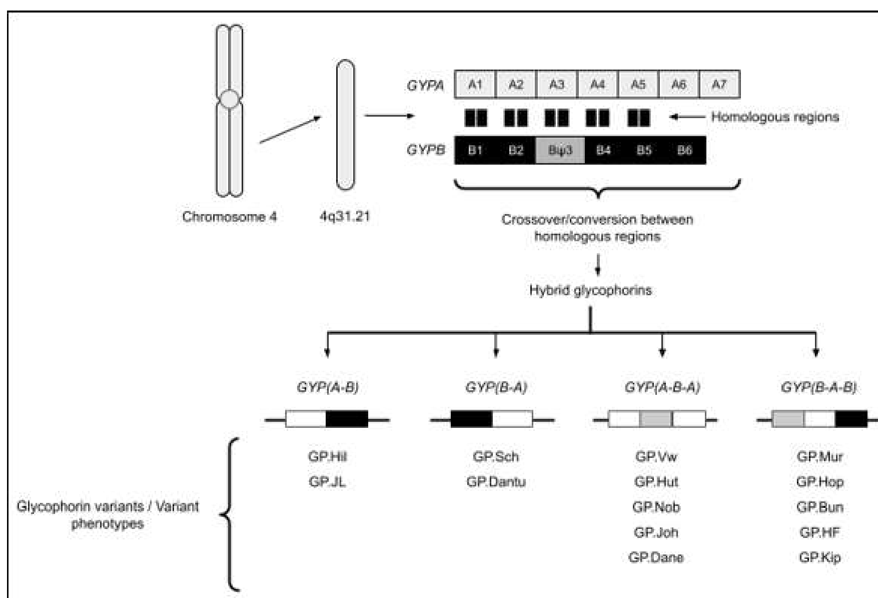

Figure 2. Genetic mechanism of variant glycophorins A and B adapted from (29)

CONCLUSION

The MNS blood group system exhibits diverse molecular and immunological properties. The genes, proteins, antigens, and corresponding alloantibodies can contribute to an individual's unique MNS blood group. This review emphasizes the importance of matching MNS blood groups for patients requiring blood transfusion in whom atypical clinically significant alloantibodies of the MNS system have been encountered and threaten HTR or HDFN. While some MNS blood group variations are rare or less clinically significant, each should be considered, as clinical consequences can be severe.

The understanding of the MNS system has been evolving since its discovery in 1927 and today, 50 antigens have been identified in this highly polymorphic system.

AUTHOR INFORMATION

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