

Editorial

Mi^a and its associated hybrid glyophorins: no longer neglected

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Introduction

The International Society of Blood Transfusion (ISBT) Working Party (ISBT WP) for Red Cell Immunogenetics and Blood Group Terminology currently recognizes 50 antigens in the MNS blood group system (ISBT002).¹ The polymorphic-, high- and low-frequency red cell antigens associated with the system are carried on glyophorin A (GPA), glyophorin B (GPB) or variant glyophorins. Provided that some MNS antigens are known to arise from substitutions of amino acids and/or alterations of glycosylation in GPA and GPB, many are considered abnormal hybrid GP molecules, consisting partially of GPA and partially of GPB. These hybrid GPs are thought to have formed from the unequal crossing-over or gene conversion involving *GYP A* and *GYP B*.² These molecular mechanisms that can generate novel blood group antigens are similar to the genetic mechanisms that disrupt their expression - s and JENU antigens.³

The immunogenic Mi^a (MNS7) antigen has been reported to have clinical significance in cases involving hemolytic transfusion reactions (HTRs),⁴ hemolytic disease of the fetus and newborn (HDFN),⁵ and hydrops fetalis.⁶ Despite the anti-Mi^a being initially classified as the phenotype of the Miltenberger (Mi) subsystem, it has subsequently been determined to represent a mixture of antibodies to low-frequency antigens, encompassing anti-Vw, -Mur, -Hut, -MUT, -Hop, -Hil, -TSEN, -MINY and -KIPP. To date, eight hybrid GPs - GP.Vw, GP.Hut, GP.Mur, GP.Hop, GP.Bun, GP.HF, GP.Kip, and GP.MOT - express the Mi^a antigen.^{2,3} Additionally, recently published case reports related to antibodies to MNS antigens, particularly the Mi^a-positive hybrid GPs, provide a persistent reminder of the clinical significance of this system.^{7,8} The review series in this issue focuses

on introducing DNA sequencing technology as an alternative method of identifying Mi^a and its associated hybrid GPs on occasions where an array of antisera is unavailable, and the expression of some MNS antigens has been disrupted with these hybrid GPs.

Identification of Mi^a-positive hybrid glyophorins

Among Mi^a-positive hybrid GPs, unequal crossing-over and gene conversion are the two main mechanisms forming eight hybrid GP variants. An anti-Mi^a could be reactive to red cells from a GP.MOT encoded by *GYP*MOT* - a *GYP(B-A)* hybrid gene. GP.Vw and GP.Hut are encoded by *GYP*Vw* and *GYP*Hut*, respectively - *GYP(A-B-A)* hybrid genes; GP.Mur, GP.Bun, GP.Hop, GP.HF, and GP.Kip are encoded by *GYP*Mur*, *GYP*Bun*, *GYP*Hop*, *GYP*HF* and *GYP*Kip*, respectively - *GYP(B-A-B)* hybrid genes.^{2,3} In the first investigation conducted in 2007 on 94 Thai blood donors exhibiting the Mi^a-positive phenotype, GP.Mur (83/94, 88.3%) was the most frequently observed variant, followed by GP.Bun (11/94, 11.7%), which had been determined using DNA sequencing.⁹

In 2020, 63 Thai blood donor samples that screened positive for a *GYP(B-A-B)* hybrid gene using polymerase chain reaction (PCR) were sequenced with primers encompassing *GYP B* exons 3 to 4. DNA sequencing revealed that *GYP*Mur* was the predominant hybrid allele with 56/63 samples (88.9%) containing 51 *GYP*Mur/GYP B* heterozygotes, three *GYP*Mur/GYP*Mur* homozygotes, and two compound heterozygote of *GYP*Mur* and a distinct *GYP*Bun*-like allele. Following the findings in the study of Thai blood donors, two *GYP*Bun*-like alleles were subsequently affiliated to the *GYP*Thai* and *GYP*Thai II* alleles. The *GYP A* segment insertion in *GYP*Bun*-like alleles, *GYP*Thai* (22 or 33 bp) and

*GYP*Thai II* (46 or 117 bp), has shorter breakpoints in intron 3 than those of the *GYP*Bun* allele (131 bp); however, the encoded amino acid sequence, is anticipated to be identical to that of GP.Bun. Additionally, in the remaining seven (7/63, 11.11%) samples, the heterozygosity for the *GYP*Thai* allele was identified.¹⁰ According to the current study, the *GYP*Mur* allele is confirmed as the most common allele among Thai blood donors. This study initially screened donors with Mi^a-positive phenotypes using PCR with sequence-specific primer (PCR-SSP) to differentiate between *GYP*Vw* and groups of *GYP*Hut*, *GYP*Mur*, *GYP*Hop*, *GYP*Bun*, and *GYP*HF* alleles. DNA sequencing was later used to characterize hybrid variants of *GYP(B-A-B)*. Of a total of 127 Mi^a-positive *GYP(B-A-B)* hybrid donors, 115/127 donors (90.55%) had the *GYP*Mur* allele; 111 of those donors were *GYP*Mur/GYPB* heterozygotes, and the remaining 4 donors were *GYP*Mur/GYP*Mur* homozygotes. *GYP*Bun*-like alleles were also identified in the study, which was similar to the study that Jongruamklang and colleagues reported. Those alleles were identified in 12/127 donors (9.45%); 11 were *GYP*Thai/GYPB* heterozygotes and 1 was *GYP*Thai II/GYPB* heterozygotes.¹¹

Hybrid glycoporphins with altered antigen expression

In general, the s antigen can be found nearby to the GPB Exon B2/B4 junction site and resides on Exon B4 of the extracellular region of GPB. The GPB Exon B2/B4 junction site does not exist in s+ hybrid GPs due to a *GYP A* insertion repairing the GPB ψ 3 nonfunctional splice site, e.g., GP.Mur or GP.Bun.¹² Related studies indicated that structural changes adjacent to the GPB Exon B4 modify the s expression and could be undetectable through specific anti-s typing reagents. The s antigen on GP.Mur and GP.Bun red cells, for instance, fails to be detected by anti-s monoclonal antibody P3BER, indicating that these hybrid GPs express an alternative variant of the s antigen.^{7,10,12,13}

JENU (MNS49), a high-frequency antigen on GPB spans across the GPB Exon B2/B4 junction. An individual who is JENU negative either has lost the entire (or

almost entire) GPB or contains two *GYP(B-A-B)* hybrid variant alleles disrupting the JENU antigen, such as among individuals who have the *GYP*Mur/GYP*Mur* homozygote.⁷ As a result, when GP.Mur homozygote individuals, who do not have normal GPB, are exposed to red blood cells carrying normal GPB, they are capable of developing alloimmunization - anti-JENU.^{7,8}

Conclusion

The cumulative reviews of Mi^a and its associated hybrid glycoporphins demonstrate the diversity and complexity of the MNS blood group system. The establishment of DNA sequencing technology has rendered it possible to detect glycoporphin variants in the absence of an antisera profile. The identification of Mi^a-positive hybrid GPs in a Thai population suggests these variant GPs may be as geographically or ethnically exclusive as first thought and serve as an ongoing reminder of their clinical significance due to their disrupting some antigen expressions.

References

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