

Case report

Effect of the s^D allele on s antigen phenotyping

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Abstract:

The MNS blood group system is considered clinically significant in the Thai population, which differs from that observed in the Caucasian populations. It is governed by the highly homologous GYPA and GYPB genes, with high polymorphism from various single nucleotide polymorphisms (SNPs) or gene conversion (hybrid genes). Antigen testing for the MNS blood group system is increasingly intricate and requires vigilance in monitoring for abnormal results, followed by molecular confirmation to ensure accuracy. The s^D antigen, designated as the MNS23 antigen encoded by c.173C > G of the GYPB gene is classified as a low-incidence antigen with a frequency below 1%. However, serological studies of Thai individuals revealed an s^D antigen frequency of approximately 2% among random group O blood donors. Notably, s^D antigen has been reported to cause severe hemolytic disease of the fetus and newborn (HDFN). In addition, s^D+ red blood cells are associated with altered s antigen expression compared to standard s+ red blood cells. It is crucial to confirm that blood donors do not possess antigens corresponding to the patient's antibodies before transfusion. A Thai blood donor was observed to have discrepancy s phenotyping using different monoclonal anti-s and molecular technique using commercial kit showed s+ phenotype. Sanger sequencing was performed and revealed this donor has homozygous GYPB*sD allele as the cause of discrepancy results. This report is the first to describe the homozygous GYPB*sD allele causing false negative s phenotyping using monoclonal anti-s clone P3YAN3 in the Thai population.

Keywords : ● MNS blood group system ● s^D antigen ● s antigen

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รายงานผู้ป่วย

ผลของยีน s^D ต่อการแสดงออกของแอนติเจน s

พลอยมณี สุวรรณวุฒิชัย ปัญญา รัตน์กาญจกิจ ศิวาพร นามแดง มรกต เอมทิพย์ และ ศิริลักษณ์ เพ็ญเจริญ
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บทคัดย่อ

หมู่โลหิต MNS มีความสำคัญทางคลินิกในประเทศไทย ซึ่งแตกต่างจากชาวผิวขาว ซึ่งแอนติเจนควบคุมโดยยีน GYPA และ GYPB ที่มีความใกล้เคียงกันมาก ทำให้เกิดลักษณะ gene conversion จากการเปลี่ยนแปลงลำดับเบส (single nucleotide polymorphism; SNPs) หรือการแลกเปลี่ยนระหว่างยีน (hybrid gene) ส่งผลให้มีความหลากหลายของแอนติเจนสูง การตรวจแอนติเจนหมู่โลหิต MNS จึงมีความซับซ้อนเพิ่มขึ้น จำเป็นต้องเฝ้าระวังผลที่ผิดปกติ และตรวจยืนยันด้วยเทคนิคระดับโมเลกุลเพิ่มเติม เพื่อให้ได้ผลที่ถูกต้อง s^D เป็นแอนติเจนในหมู่โลหิต MNS ลำดับที่ 23 ซึ่งเกิดจาก SNPs c.173C > G บนยีน GYPB โดยจัดอยู่ในกลุ่ม low-incidence antigen คือ มีความถี่น้อยกว่าร้อยละ 1 ในประชากร อย่างไรก็ตามการศึกษาความถี่ในคนไทย โดยใช้เทคนิคทางซีโรโลยีพบว่า ความถี่ของแอนติเจน s^D ในตัวอย่างผู้บริจาคโลหิตหมู่ O คือร้อยละ 2 และมีรายงานแอนติบอดีสามารถทำให้เกิดโรคเม็ดเลือดแดงแตกในทารกแรกเกิดอย่างรุนแรงอีกด้วย นอกจากนี้พบว่า เซลล์เม็ดเลือดแดงที่เป็น s^D+ ทำให้เกิดการแสดงออกของแอนติเจน s อ่อนลงกว่าการแสดงออกในเซลล์เม็ดเลือดแดงทั่วไป ผู้บริจาคโลหิตชาวไทยรายหนึ่งพบผลการตรวจแอนติเจน s ให้ผลไม่สอดคล้องกันระหว่างการตรวจด้วยน้ำยาส่งรูปที่ต่างโคลนกัน ผลการทดสอบด้วยเทคนิคอนุชีววิทยาด้วยน้ำยาส่งรูปแปลผลแอนติเจน $s+$ จึงทำการทดสอบ Sanger sequencing พบว่าผู้บริจาคโลหิตรายนี้มี รูปแบบยีนของ GYPB*sD allele แบบ homologous ซึ่งเป็นสาเหตุที่ทำให้เกิดผลไม่สอดคล้องกันในการตรวจแอนติเจน s นี้ รายงานนี้จึงเป็นรายงานแรกที่มีการกล่าวถึงรูปแบบยีน GYPB*sD allele ในระดับ homozygous ในคนไทย ที่ทำให้เกิดผลลบปลอมในการตรวจแอนติเจน s ด้วยน้ำยา mono-clonal anti- s clone P3YAN3

คำสำคัญ : ● หมู่โลหิต MNS ● แอนติเจน s^D ● แอนติเจน s

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Introduction

The MNS blood group system was identified after the ABO system and is recognized as highly complex, like that seen with the Rh system¹. This complexity arises from a high mutation rate in the closely related genes (> 95% homology), known as *GYP A* and *GYP B*¹, resulting in hybrid gene variants that are not typically observed in other blood groups. The MNS antigens are located on glycophorin A (GPA) and glycophorin B (GPB) on the red blood cell (RBCs) membrane.

Within the MNS system, antigen expression is governed by three genes-*GYP A*, *GYP B*, and *GYP E*-located on chromosome 4 (4q31.21).¹ The *GYP A* gene contains seven exons encoding the clinically significant M and N antigens, while the *GYP B* gene is composed of six exon-like regions, one of which is a pseudo-exon (exon 3), encoding the S and s antigens. Similarly, *GYP E* consists of six exon-like regions, two of which are pseudo-exons (exon 3 and 4), which are normally not expressed on RBCs except when new gene rearrangements yield *GYP E* variants. Notably, these three genes share over 95% sequence similarity to each others.⁴

The s^D antigen, or MNS23 is encoded by *GYP B* c.173C>G. It was first described in the Dreyer family, a Caucasian South African¹, and identified in the East Asian population from 1,000 Genomes Project.⁵ The s^D antigen has been reported with weak s expression.⁶ The case of severe hemolytic disease of the fetus and newborn (HDFN) in a pregnant Thai woman revealed the presence of anti- s^D in her plasma⁷, which reacted to 2% of group O blood donors.⁷ This observation represented the first investigation of the s^D antigen frequency in the Thai population, revealing a higher prevalence than in other populations and underscoring its clinical significance in causing severe HDFN or potential transfusion reactions.

A subsequent Chinese study identified four blood donors initially typed as s-positive using monoclonal anti-s clone P3BER but as s-negative with monoclonal anti-s clone P3YAN3, rendering the results inconclusive with this discrepancy. Further analyses revealed that

one donor possessed a novel variant affecting exon 4 of *GYP B*. While the other three had a heterozygous c.173C>G, which is the s^D allele in conjunction with an S allele. These genetic findings demonstrated that the s^D allele can alter s antigen expression, potentially causing false-negative results with monoclonal anti-s clone P3YAN3⁸.

Following US-FDA guidance for Labeling of Red Blood Cell Units with Historical Antigen Typing Results, antigen testing in blood donors requires the use of more than one monoclonal reagents derived from different clones that yield concordant results.^{9,10} National Blood Centre, Thai Red Cross Society performed red cell phenotyping in blood donors to provide safe blood transfusion to hospitals following US-FDA guidance. In the event of discrepant findings between clones, additional testing must be performed to ensure accurate outcomes. Molecular testing can predict an antigen's expression at the genetic level (called predicted phenotype). However, commercial reagents designed for specific targets may occasionally generate inaccurate results. Consequently, if molecular testing with commercial reagents fails to align with the observed phenotype, DNA sequencing is necessary to clarify the cause of the inconsistency. The aims of this case report are to investigate discrepancy phenotyping results between different monoclonal anti-s using serological and molecular techniques and the first to describe homozygous s^D antigen expression causing a false-negative reaction in s antigen testing using clone P3YAN3.

Case presentation

A Thai female blood donor at the National Blood Centre, Thai Red Cross Society, had phenotyping performed and showed discordant s antigen results with the following reagents: s positive with monoclonal anti-s clone P3BER (Merck Millipore, USA) but s negative with monoclonal anti-s clone P3YAN3 (Ortho Clinical Diagnostic, USA). The donor's MNS blood group antigens-tested using two different standard monoclonal antisera were S-Mi(a-), as shown in Table 1.

Table 1 Phenotyping results of donor's RBC

Antigen	Antiserum/Test	Antigen typing results		
		CAT	CTT	PCR-SSO
S	Anti-S (P3S13JS123, Ortho)	Negative	Negative	Negative
	Anti-S (MS-94, Merck)			
	ID core XT (Grifols)			
s	Anti-s clone P3YAN3 (Ortho)	Negative	Positive	Positive (Possible U variant)
	Anti-s clone P3BER (Merck)			
	ID core XT (Grifols)			
Mi ^a	Anti-Mi ^a (In house human monoclonal, National Blood Centre, Thai Red Cross Society)	Negative	Negative	Negative
	ID core XT (Grifols)			

CAT, column agglutination test (serological technique); CTT, conventional tube test (serological technique);

PCR-SSO, polymerase chain reaction sequence-specific oligonucleotide (molecular technique)

Table 2 Titration results of RBCs with different dilutions of anti-s (P3BER)

Cell type	Serial dilution of anti-s clone P3BER with normal saline										
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Total
Donor's RBCs	12	12	12	12	10	8	5	2	0	0	73
S-s+ cells 1	12	12	12	10	8	8	8	5	5	0	80
S-s+ cells 2	12	12	12	10	8	8	5	5	5	0	77
S+s+ cells 1	12	12	12	10	8	5	5	2	0	0	66
S+s+ cells 2	12	12	12	10	8	5	5	2	0	0	66

Cell 1 and cells 2 are from panel cells (In house human monoclonal, National Blood Centre, Thai Red Cross Society)

To further characterize these results, a titration of the monoclonal anti-s clone P3BER reagent was performed against the donor's red blood cells (RBCs). Results were recorded using the scoring system⁹ following the Association for the Advancement of Blood and Biotherapies (AABB)'s guideline. The results were compared to four additional donors; two exhibiting the same phenotype which is homozygous s (S-s+ cells); and two with heterozygous s phenotype (S+s+ cells). These findings shown in Table 2 suggest that while s antigen expression was somewhat slightly diminished, it was still stronger than that observed in heterozygous S+s+ RBCs. This implies that the donor is most likely homozygous for a variant of the *GYPB*s* allele, which is *GYPB*s^D/GYPB*s^D* confirmed by Sanger sequencing. The fact that the monoclonal anti-s

clone P3YAN3 yield negative results supports that this donor has homozygosity s^D and not heterozygosity s/s^D. Results of the comparison between donors' RBCs with other RBCs with S-s+s^D + phenotypes, tested with both monoclonal anti-s clones P3YAN3 are shown in Table 3. The results supported that only *GYPB*s^D/GYPB*s^D* will affect s phenotyping using monoclonal anti-s clone P3YAN3 but not if only a single *GYPB*s^D* present.

DNA subsequently extracted from the donor's sample to investigate predicted s antigen expression using a molecular technique via PCR-SSO by the BeadChip ID coreXT kit (Grifols, Spain) gave results predicting the donor's RBCs to be S-s+ while also indicating a possible U variant shown in Table 1. Further Sanger sequencing of the *GYPB* gene-encompassing exons 1-2,

Table 3 The phenotyping results compared with S-s+s^D+ cells

Phenotypes of donors	Anti-S clone MS-94	Anti-S clone P3S13JS123	Anti-s clone P3YAN3	Anti-s clone P3BER
S-s+s ^D + 1	0	0	+	+
S-s+s ^D + 2	0	0	+	+
Donor's RBCs	0	0	0	+

pseudo-exon 3, and exons 4-6-revealed a homozygous or hemizygous nucleotide substitution at position 173 (c.173C>G), consistent with the s^D + phenotype.

Discussion

Phenotype testing in blood donors using two distinct monoclonal antibody clones may yield discordant results, precluding definitive determination of the correct phenotype. Molecular testing was therefore employed to establish the accurate phenotype and facilitate the appropriate blood allocation to patients. In this case, due to inconsistent serological results, a commercial molecular assay was utilized but failed to provide conclusive resolution. Subsequent sequencing analysis was required to investigate the cause of the false-negative reaction with the monoclonal anti-s clone P3YAN3.

The analysis revealed the donor harbored the c.173C>G variant, which corresponds to the s^D + antigen. This variant has been documented to cause weakened s antigen expression. Previous reports from China indicate that S+s+s^D+ heterozygous donors may yield false-negative results for s antigen when tested with clone P3YAN3.⁶ However, this donor exhibited s^D + with either homozygous or hemizygous zygosity. This finding suggests an abnormality in glycophorin B (GPB) expression resembling the JENU-negative phenotype.¹² The JENU- phenotype caused by homozygous *GYP*^{Mur}* with pseudo-exon 3 activation and affecting amino acid change on GPB where s antigen is expressed. The JENU- phenotype can give false negative result with monoclonal anti-s clone P3BER while positive results with monoclonal anti-s clone P3YAN3, in contrast to s^D + phenotype that affect s expression and gave negative

result with monoclonal anti-s clone P3YAN3 with homozygous *GYPB*s^D* allele. Should this donor require transfusion, identifying compatible blood may prove challenging due to potential alloimmunization against GPB antigens. Definitive zygosity characterization required familial segregation studies, warranting further investigation to confirm zygosity of *GYPB*s^D* allele.

The MNS blood group system exhibits complexity comparable to the Rh system and holding greater clinical significance in Thais than in Caucasians. For instance, the Mi(a+) antigen occurs in 10-22% of Thais¹³ but in less than 1% of Caucasians.¹ The prevalence of numerous other MNS antigens remains uncharacterized in Thais, complicating assessments of their clinical relevance. Nevertheless, the s^D antigen is a clinically significant antigen implicated in severe HDFN in Thais⁶, with an approximate population frequency of 2%.⁶ Establishing antigen frequencies will inform the design of population-appropriate screening cells to detect clinically significant antibodies and optimize the provision of safe blood products.

This case report showed the importance of confirmation of phenotyping using serological techniques following US-FDA guidance and how to investigate when discrepancy results appear. Understanding limitations of serological methods persist, as commercial antisera are unavailable for certain antigens. Testing with human plasma may exhibit low specificity and sample volume constraints. Molecular techniques for population frequency studies thus offer significant utility for enhancing blood product safety and establishing population-specific blood banking standards. It is important to study prevalence of antigens in each individual population that may be

helpful to understand background of immigration and how to improve transfusion standard specific with certain populations.

Conclusion

This case report documents the first instance of homozygous s^D antigen expression causing a false-negative reaction in s antigen testing using clone P3YAN3. The s antigen expression levels were assessed by serological titration and confirmed via Sanger sequencing. These results indicated the clinical significance of the s^D antigen in the Thai population. Previous serological studies on s^D antigen frequency revealed a prevalence of approximately 2% among Thais. However, when the s^D antigen-categorized as a low-incidence antigen-is expressed in a homozygous state, it can lead to aberrant expression of glycophorin B (GPB), which may lead to formation of alloantibodies specific to GPB.

The methods employed in this report indicate the importance of confirming phenotyping results using multiple monoclonal antibodies for each antigen. Furthermore, they demonstrate how molecular techniques can be utilized to conclusively determine phenotypes, thereby enhancing the safety of blood transfusion for patients.

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