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TRANSFUSION

CASE REPORT

Hemolytic disease of the fetus and newborn caused by anti-s^D antibody in a GP.Mur/Mur Thai mother and review of the prevalence of s^D in Thai blood donors

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Abstract

Background: Low-prevalence antigen s^D (MNS23) is encoded by *GYPB* c.173C > G. Hemolytic disease of the fetus and newborn (HDFN) due to anti-s^D is rare. A mother delivered a newborn whose red blood cells (RBCs) were DAT-positive and was later diagnosed with HDFN. Serum from the mother was incompatible with the father's RBCs and was used to screen 184 Thai blood donors. This study aimed to investigate the cause of HDFN in a Thai family and determine the prevalence of s^D in Thai blood donors.

Materials and Methods: Three family members and four blood donors were investigated in the study. Massively Parallel Sequencing (MPS) was used for genotyping. Standard hemagglutination techniques were used in titration studies, phenotyping, and enzyme/chemical studies. Anti-s, anti-Mi^a, anti-JENU, and anti-s^D reagents were used in serological investigations.

Results: The mother was *GYP*Mur/Mur*. The father and the four donors were *GYPB*s/s^D* predicting S − s + s^D+. The baby was *GYP*Mur/sD* and his RBCs were Mi^a+, s + ^{w} with anti-s (P3BER) and JENU+^{w}. RBCs from two *GYPB*s^D*-positive blood donors reacted with anti-s^D (Dreyer). Proteolytic enzyme α-chymotrypsin-treated s^D+ cells did not react with anti-s^D (Wat) produced by the GP.Mur/Mur mother but reacted with the original anti-s^D (Dreyer).

Discussion: This is the first report of HDFN due to anti-s^D in the Asian population. The genotype frequency for *GYPB*s^D* in a selected Thai blood donor population is 2.2% (4/184). Anti-s^D should be considered in mothers with Southeast Asian or East Asian background when antibody identification is unresolved in pregnancies affected by HDFN.

KEYWORDS

anti-s^{D}, haemolytic disease of the fetus and newborn, MNS blood group system, prevalence of s^{D} in Thai blood donors, s^{D} antigen

1 | INTRODUCTION

MNS blood group antigens are carried on glycophorin A (GPA), glycophorin B (GPB), or hybrids of GPA/GPB.[1] GPB, encoded by *GYPB* gene, express high-prevalence antigens 'N', U, JENU and polymorphic antigens S and s.[1,2] The genetic basis for the s antigen is *GYPB* c.143C (p.Thr48).[1,2]

Expression of the s antigen (MNS4) has been reported to be affected qualitatively as observed in hybrid glycophorins GP.Mur and GP.Bun,[3–5] and quantitatively — weak s expression was associated with the s^{D} (MNS23) antigen first observed in the Dreyer family.[6,7] The molecular basis for the s^{D} antigen is *GYPB* c.173C > G (rs374811215).[7,8] In Caucasian and mixed-race populations in South Africa, the prevalence of s^{D} was 0.1%.[6,7] Recently, data from the 1000 Genomes Project was analyzed for uncommon MNS alleles and *GYPB*s^{D}* allele was identified in the East Asian population.[9]

Anti-s^{D} was first produced by Mrs Dreyer, a Caucasian South African, and the second case was with Mrs B-C (ethnicity not reported).[6,10] The antibody in Mrs B-C's case was initially reported recognizing an antigen on the Rh-associated glycoprotein (RhAG) molecule. However, subsequent investigations determined that the antibody was anti-s^{D}.[10,11] Anti-s^{D} reacts strongly in the indirect antiglobulin test (IAT). To our knowledge, these two cases are the only clinical reports that involved anti-s^{D} in pregnancy. In both cases, anti-s^{D} antibody caused severe hemolytic disease of the fetus and newborn (HDFN) in their second-born child but not first-born.[6,10]

2 | CASE PRESENTATION

A mother of Thai ethnicity, 37 weeks into her third pregnancy, delivered a baby who 7 h post-delivery developed symptoms of jaundice with a hematocrit of 26% (Reference Range, RR 39–51%). The baby's RBCs were direct antiglobulin test (DAT)-positive. On day 2, hematocrit decreased to 19%, hemoglobin level was 4.8 g/dL (RR: 13–17 g/dL), total bilirubin was 12.9 mg/dL (RR: 0.2–1.2 mg/dL) and direct bilirubin was 1.1 mg/dL (RR: <0.05 mg/dL). Day 7 peripheral blood smear showed RBCs that were moderately polychromatophilic and slightly microspherocytic. The baby was diagnosed with severe HDFN and glucose-6-phosphate dehydrogenase deficiency. For the next 9 days, the baby received phototherapy three times, a single dose of intravenous immunoglobulin (IVIg), and three blood transfusions (each at 30 ml leukocyte-poor packed RBCs). The baby's father is also of Thai heritage. The mother had no history of blood transfusion. Her first pregnancy was terminated and her second pregnancy delivered a newborn without complication. RBC phenotypes for the family are presented in Table 1.

Serum from mother and baby were antibody screen negative. The mother's serum and eluate prepared from the baby's DAT-positive cord blood cells were strongly positive (3+) with father's RBCs by IAT. Mother's serum was negative with a panel of cells expressing low-prevalence antigens Hop+, St(a+), Mt(a+), Vw+, Hut+, C^{w}+, Kp(a+), Mi(a+) and positive with seven out of 184 Group O blood donors at the Thai National Blood Centre. An antibody to a low-prevalence antigen was suspected.

3 | METHODS

3.1 | Study samples

Samples from the father, mother, and baby were investigated (phenotyping, antibody screening, antibody titrations, and enzyme/chemical studies) at the National Blood Centre, Bangkok. The mother's serum was used to screen selected 184 Group O Thai Red Cross blood donors (TRCBD). Of the seven blood donors (7/184) that were positive, only four returned for next donation.

Blood samples from the mother, father, baby, and the four blood donors (TRCBD-1, TRCBD-2, TRCBD-3, and TRCBD-4; all of Thai ethnicity) were sent to Australian Red Cross Lifeblood (Lifeblood), Brisbane for DNA sequencing, anti-s^{D} phenotyping, and enzyme studies.

TABLE 1 RBC phenotype of the family

Father	Group B, D + C + E − c + e+, Jk(a + b−), S−, Mi(a−), Fy(b−), Di(a−) K−, s+
Mother	Group B, D + C + E + c + e+, Jk(a + b−), S−, Mi(a+), Fy(b−), Di(a−) K−, s−/+
Baby	Group B, D + C + E + c + e+, Jk(a + b−), S−, Mi(a+), s+

3.2 | Hemagglutination tests

At the National Blood Centre (Bangkok), hemagglutination tests were performed using the test tube method or using column agglutination technology (ID Card LISS/Coombs, BioRad or DG Gel Cards, Grifols) according to manufacturer's recommendation. At Lifeblood (Brisbane), standard hemagglutination tests were performed using the test tube method.

3.3 | Massively parallel sequencing (MPS)

Genomic DNA (gDNA) was isolated from EDTA-whole blood samples and quantitated as previously described.[12] DNA from seven individuals were genotyped by MPS (MiSeq, Illumina) and the sequencing data were analyzed as previously described.[13]

3.4 | Gel PCR assay for *GYPB*s^D*

Allele-specific primers were designed to genotype for *GYPB*s^D*. Primer sequences and the PCR assay protocol are provided in Table S1.

3.5 | Antibody titration study

Anti-s (P3BER) reacts with s expressed on GPB but not with s expressed on GP.Mur.[3,4,14] Twofold serial dilutions of anti-s P3BER (Merck, Millipore) and anti-s polyclonal (CSL) reagents, diluted out to 1/1024 dilution, were prepared. Each dilution was tested against RBCs from the father, baby, control S − s +, and S + s + by IAT. Hemagglutination reactions were assessed and given an agglutination score (from 0 to 12 scoring scale). Reaction scores were added together to give a titration score. A score difference of 10 or more between control and test sample is considered significant.

3.6 | Phenotyping for s, Mi^a, JENU, and s^D

Anti-s reagents (P3YAN3, Ortho; P3BER, Merck Millipore; polyclonal, CSL), anti-Mi^a (in-house polyclonal), anti-JENU and anti-s^D (Dreyer) antisera were used for phenotyping.

3.7 | Enzyme and chemical studies

At the National Blood Centre (Bangkok), the mother's serum (Wat) was tested against a panel of cells: RBCs from the father, TRCBD-1 and TRCBD-2. These cells were treated with trypsin, papain, aminoethylisothiouronium bromide, and α-chymotrypsin (Sigma-Aldrich, C4129) to investigate antibody specificity.

At Lifeblood (Brisbane), s^D+/s^D− panel RBCs were tested against serum from the mother (Wat) and anti-s^D (Dreyer). In the IAT, these panel cells were used untreated and treated with α-chymotrypsin (Sigma-Aldrich, C3142, Batch SLBZ9942, Purity: 96%, Conc: 5 mg/mL).

4 | RESULTS

4.1 | MNS genotype by MPS

Sequencing showed that the mother was *GYP*Mur/Mur* (data not shown) and the father was homozygous *GYPB* c.143C and heterozygous *GYPB* c.173C/G interpreted as *GYPB*s/s^D*, (Figure 1), predicting S − s + s^D+. The *GYPB*s^D* sequence, obtained from the father was submitted to NCBI (GenBank OK345035 and ClinVar SCV001950176). The baby was *GYP*Mur/s^D* and the four blood donors were *GYPB*s/sD*. The genotype frequency for *GYPB*s^D* in Thai blood donors was 2.2% (4/184). No hybrid glycophorin gene variants were detected in the father and the four donors (TRCBD-1–4).

GYPA analysis showed that the father, mother, baby, TRCBD-2, and TRCBD-3 were *GYPA*M/M*. TRCBD-1 and TRCBD-4 were *GYPA*M/N*. No other unexpected MNS blood group gene variants were identified.

4.2 | Gel PCR *GYPB*s^D* genotyping

DNA from the father (positive control) produced a 434 bp HGH band and a 93 bp band specific for *GYPB*s^D* allele, Figure S1. The mother was *GYPB*s^D*-negative and all four donors were *GYPB*s^D*-positive consistent with MPS genotyping.

4.3 | Titration studies with anti-s reagents

Using anti-s P3BER, the titration score difference between the father and control RBCs S + s + was 10, and control S − s + was 11, Table S2. This demonstrates that the father's RBCs, although it carried a single dose of GPB.s, reacted weaker than S + s + control RBCs. The baby's titration score gave a difference of at least 15 compared to control RBCs suggesting that the baby express weak s (s + ^w). Using anti-s polyclonal, the

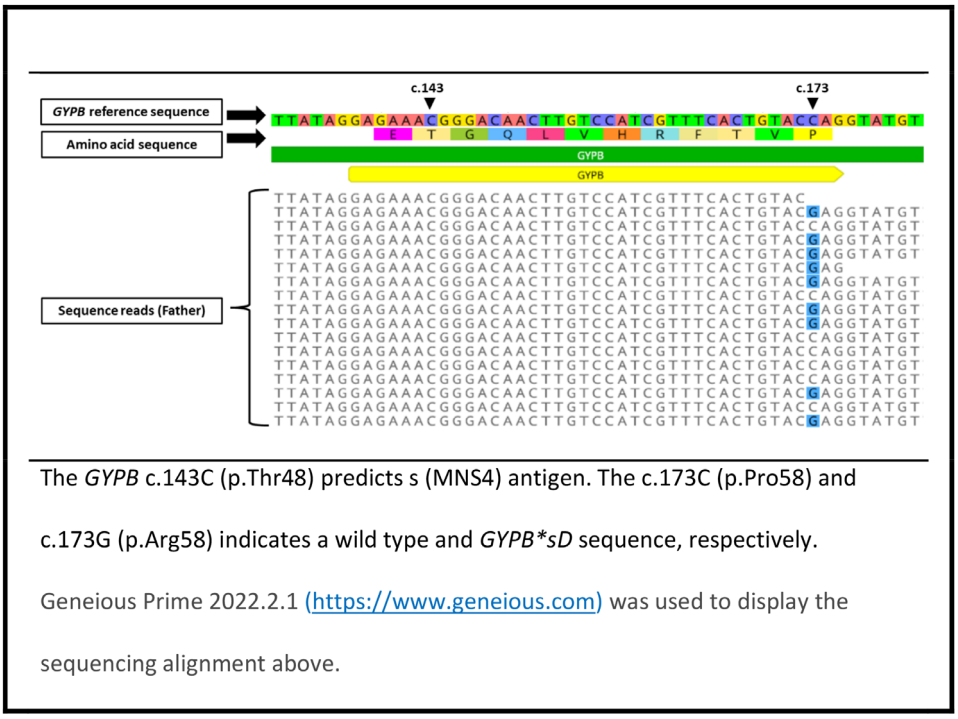

FIGURE 1 *GYPB* exon 4 DNA sequencing alignment. The *GYPB* c.143C (p.Thr48) predicts s (MNS4) antigen. The c.173C (p.Pro58) and c.173G (p.Arg58) indicate a wild-type and *GYPB*s*^{D} sequence respectively. Geneious Prime 2022.2.1 (https://www.geneious.com) was used to display the sequencing alignment above. [Color figure can be viewed at wileyonlinelibrary.com]

reactivity profile between test cells (father and baby) and control cells was not significantly different, Table S2.

4.4 | Anti-s, anti-Mi^{a} and anti-JENU phenotyping

The mother's RBCs were positive with anti-s P3YAN3 and negative with anti-s P3BER. This reactivity pattern is consistent with the profile reported for GP.Mur/Mur.[3,4] The baby's cells were Mi(a+).

Anti-JENU was positive with control S + s + (2+) and father's RBCs (2+) while the baby's cells were weakly positive (1+). JENU is not carried on GP.Mur. The baby's GP.Mur/GPB.s^{D} cells reactivity with anti-JENU suggests GPB.s^{D} express JENU, however, it is weakly expressed (JENU+^{w}).

4.5 | Anti-s^{D} (Dreyer) phenotyping

At Lifeblood, TRCBD-3 and TRCBD-4 RBCs were tested with anti-s^{D} and both were positive (3+). RBCs from the father, mother, baby, TRCBD-1 and TRCBD-2 were received hemolysed and were therefore not suitable for phenotyping.

4.6 | Anti-s^{D} (Wat) against enzyme/chemical-treated *GYPB*s*^{D}-positive RBCs

The above results showed that the antibody from the mother has anti-s^{D} specificity. From this point onwards, the mother's sample will now be referred as anti-s^{D} (Wat).

Performed earlier in the investigations in Bangkok, RBCs from Father, TRCBD-1 and TRCBD-2 were treated with enzymes and chemical before the IAT step. Hemagglutination reaction showed that the epitope recognized by anti-s^{D} (Wat) was resistant to trypsin and aminoethylisothiouronium bromide, partially sensitive to papain, and sensitive to α-chymotrypsin, Table 2A.

4.7 | Anti-s^{D} (Wat) and anti-s^{D} (Dreyer) against α-chymotrypsin-treated s^{D}+ RBCs

At Lifeblood, anti-s^{D} (Wat) reacted with untreated s^{D}+ RBCs (2+) but did not react with α-chymotrypsin-treated s^{D}+ RBCs, Table 2B. This is consistent with the reactivity pattern observed in Table 2A.

Anti-s^{D} (Dreyer) reacted to both untreated (3+) and α-chymotrypsin-treated s^{D}+ RBCs (2+), Table 2B. Slightly weaker reaction was observed in α-chymotrypsin-treated s^{D}+ than untreated RBCs. The reactivity profile for anti-s^{D} (Dreyer) on α-chymotrypsin-treated s^{D}+ RBCs observed in this study is consistent with the previous report.[6,15]

5 | DISCUSSION

Amongst the antibodies to MNS antigens, many are considered clinically significant causing HDFN and hemolytic

TABLE 2 Serological profile for anti-s^D (wat)

A. Anti-s^D (wat) versus panel of RBCs			
	Father	**TRCBD-1**	**TRCBD-2**
Untreated	2+	2+	2+
Trypsin	2+	2+	2+
AET bromide	2+	2+	2+
Papain	w	1+	1+
α-chymotrypsin	0	0	0
B. Anti-s^D (Wat) and anti-s^D (Dreyer) versus s^D+/s^D− panel cells			
		anti-s^D (Wat)	**anti-s^D (Dreyer)**
Untreated:	RC #138, s^D+	2+	3+
Untreated:	RC #894, s^D+	2+	3+
Untreated:	Abtectcell III, s^D−	0	0
α-chymotrypsin-treated:	RC #138, s^D+	0	2+
α-chymotrypsin-treated:	RC #894, s^D+	0	2+
α-chymotrypsin-treated:	Abtectcell III, s^D−	0	0

Note: RC #138, RC #894, and Abtectcell III (02421301) are all Group O. 0 (negative); + (positive).
Abbreviations: AET, aminoethylisothiouronium; TRCBD, Thai red cross blood donor; w, weak.

transfusion reactions.[2] Only two cases of HDFN due to anti-s^D were ever reported.[6,10] In both cases, the second-born child was affected with severe HDFN but not the first child. The clinical pattern in these HDFN cases is consistent with the HDFN case report in this study. All three cases suggest that the mothers were alloimmunized to s^D during pregnancy and that succeeding pregnancies, where the baby is s^D+, is at risk of HDFN.

The Thai population has a unique MNS blood group profile.[16] In Thais, the prevalence for Hop (0.68%),[15,17] Mt^a (0.94%),[18] and Mi^a (9.7%)[17] is higher than most population groups. Of the 9.7% (243/2500) Mi^a + Thai blood donors, 99.2% (241/243) are GP.Mur.[17] Individuals who are GP.Mur/Mur are also JENU−.[3] In this study, the baby is s^D+ and JENU+^{w}. However, the s^D− JENU− mother did not develop anti-JENU but only anti-s^D that reacted with her baby's s^D+ RBCs causing HDFN. To our knowledge, this is the third case of HDFN due to anti-s^D and the first in the Asian population.

The reactivity profile for anti-s^D (Wat) is different from anti-s^D (Dreyer) in α-chymotrypsin-treated s^D+ RBCs suggesting that these two anti-s^D antisera recognize distinct epitopes on GPB.s^D. An explanation for this inconsistent pattern is probably due to the glycophorin profile of the two anti-s^D producers. Anti-s^D (Wat) was produced by a GP.Mur/Mur mother while the original anti-s^D (Dreyer) was produced by a GPB.s/s mother.

The s^D antigen is rare. Its prevalence was only ever determined in South Africans.[6] In this study, s^D was identified in a Thai family and in Thai blood donors. Of the seven blood donors who reacted with anti-s^D (Wat), only four were confirmed to express s^D and/or carry the *GYPB*s^D* allele. We report that the prevalence of s^D in a selected Group O Thai blood donor population is 2.2% (4/184), however, it could be as high as 3.8% (7/184).

The use of s^D+ RBCs in antibody screening cell panel can help detect and determine the incidence of anti-s^D in the Thai patient population. Anti-s^D should be considered in mothers with Southeast Asian or East Asian background when antibody identification is unresolved in pregnancies affected by HDFN.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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