An alloantibody to a high-prevalence MNS antigen in a person with a GP.JL/M^k phenotype

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The low-prevalence MNS blood group antigen TSEN is located at the junction of glycophorin A (GPA) to glycophorin B (GPB) in several hybrid glycophorin molecules. Extremely rare people have RBCs with a double dose of the TSEN antigen and have made an antibody to a high-prevalence MNS antigen. We report the first patient who is heterozygous for *GYP.JL* and *M^k*. During prenatal tests, an alloantibody to a high-prevalence antigen was detected in the serum of a 21-year-old Hispanic woman. The antibody detected an antigen resistant to treatment by papain, trypsin, α-chymotrypsin, or DTT. The antibody was strongly reactive by the IAT with all RBCs tested except those having the M^{k}M^k, GP.Hil/GP.Hil, or GP.JL/GP.JL phenotypes. The patient's RBCs typed M+N–S+/–s–U+, En(a+/–), Hut–, Mi(a–), Mur–, Vw–, Wr(a–b–), and were TSEN+, MINY+. Reactivity with *Glycine soja* suggested that her RBCs had a decreased level of sialic acid. Immunoblotting showed the presence of monomer and dimer forms of a GP(A-B) hybrid and an absence of GPA and GPB. Sequencing of DNA and PCR-RFLP using the restriction enzyme *Rsa*I confirmed the presence of a hybrid *GYP(A-B)*. The patient's antibody was determined to be anti-En^{a}FR. She is the first person reported with the GP.JL phenotype associated with a deletion of *GYPA* and *GYPB* in *trans* to *GYP.JL*.
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The antigens of the MNS blood group system are carried on glycophorin A (GPA), glycophorin B (GPB), or various hybrid molecules thereof. The antigens arise from single amino acid substitutions in GPA or GPB, the novel amino acid sequences formed at the junction of GPA to GPB or the junction of GPB to GPA, or expression of amino acids encoded by the pseudo exon of the *GYPB* gene.[1]

The low-prevalence MNS RBC antigens, TSEN and MINY, are located at the junction of GPA (3′ end of *GYPA* exon 3) to GPB (5′ end of *GYPB* exon 4) in the GP(A-B) hybrid associated with GP.JL (Mi.XI), and in the GP(B-A-B) hybrid associated with GP.Hop (Mi.IV).[2,3] These rearranged genes can be detected by altered *Rsa*I restriction enzyme sites.[4] However, these hybrid genes are not distinguished by this restriction enzyme from the hybrid genes that encode hybrid glycophorin molecules that carry Hil, namely GP.Hil (Mi.V), GP.Mur (Mi.III), GP.Bun (Mi.VI), and GP.HF (MI.X).[1,4]

TSEN+ RBCs are usually found because of a discrepant S typing or by detection of an antibody to a low-prevalence antigen.[2,5,6] Extremely rare people have RBCs with a double dose of TSEN and have made an antibody to a high-prevalence MNS antigen.[7,8] We report a person who is heterozygous for *GYP.JL* and *M^k* (the null allele in the MNS system).

Case Report

We report a case of a 21-year-old group O Hispanic woman in her second pregnancy. During prenatal tests, an alloantibody to a high-prevalence antigen lacking from her RBCs was detected in her serum. The proband had no history of transfusion. Her first pregnancy ended in miscarriage for reasons unrelated to the antibody; her second pregnancy resulted in the birth of an apparently healthy baby girl whose RBCs typed as group A, D+. RBCs from the baby's cord blood sample were positive in the DAT (3+) with anti-IgG. An eluate prepared from these RBCs was reactive with all RBCs except those of the mother. The baby's serum also contained the antibody, albeit weakly reactive. The mother and baby were discharged the day after the baby was born, and there is no indication in the records that the baby required special care.

Material and Methods

Serologic testing of patient RBCs and serum was performed by standard tube hemagglutination. Treatment of intact RBCs with papain, trypsin, α-chymotrypsin, or 200 mM DTT; DNA extraction from WBCs; DNA sequencing; and PCR-RFLP were performed

by standard methods.[9,10] Immunoblotting of membranes prepared from RBCs was also performed by standard method under nonreducing conditions using a commercial monoclonal anti-GPA/B (E3, Sigma Biologicals, St. Louis, MO) as the primary antibody and a peroxidase-conjugated rabbit anti-mouse IgG as the secondary antibody (MP Biomedicals, Aurora, OH).

Results

Hemagglutination

The alloantibody in the proband's serum reacted strongly (4+) by the IAT with all RBCs tested, including Wr(a+b–) RBCs, but did not react with RBCs having the M^{k}M^k (MNS null phenotype), GP.Hil/GP.Hil (Mi.V/Mi.V), or GP.JL/GP.JL (Mi.XI/Mi.XI) phenotypes, all of which are Wr(a–b–). The antigen detected by the antibody was resistant to treatment of RBCs with papain, trypsin, α-chymotrypsin, or DTT. These results are consistent with the presence of anti-En^{a}FR in the patient's serum. The antibody had a titer of 128 by saline-IAT. RBCs from the proband's mother, brother, and sister typed TSEN– and were strongly agglutinated (4+) by the patient's serum. Other family members were not available for testing.

The proband's RBCs typed M+N–S+/– (positive with four polyclonal and one monoclonal [MS95] anti-S, and negative with one polyclonal and one monoclonal [MS94] anti-S), s–U+, En(a+/–) (weakly positive with five and negative with four polyclonal anti-En^a), He–, Hut–, Mi(a–), Mt(a–), Mur–, Vw–, Wr(a–b–) (with two polyclonal and five monoclonal anti-Wr^b), and were TSEN+, and MINY+, suggesting a GP.JL phenotype. The proband's RBCs were pedestrian with regard to other blood group antigens.

The proband's RBCs were strongly agglutinated by *Glycine soja* lectin, suggesting they had a decreased level of sialic acid. The results of testing the proband's RBCs with monoclonal anti-GPA directed at different epitopes on GPA (anti-GPAMSer1, anti-GPAMGly5, anti-GPA1-26, anti-GPA38-44, anti-GPA34-48, and anti-GPA49-55) suggested that the proband's RBCs express the *N*-terminal portion of GPA.

Immunoblotting

Immunoblotting of RBC membranes made from the proband's sample with monoclonal anti-GPA+GPB (E3, Sigma) showed the presence of monomer and dimer forms of a hybrid GP (A-B) and the absence of GPA and GPB (Fig. 1).

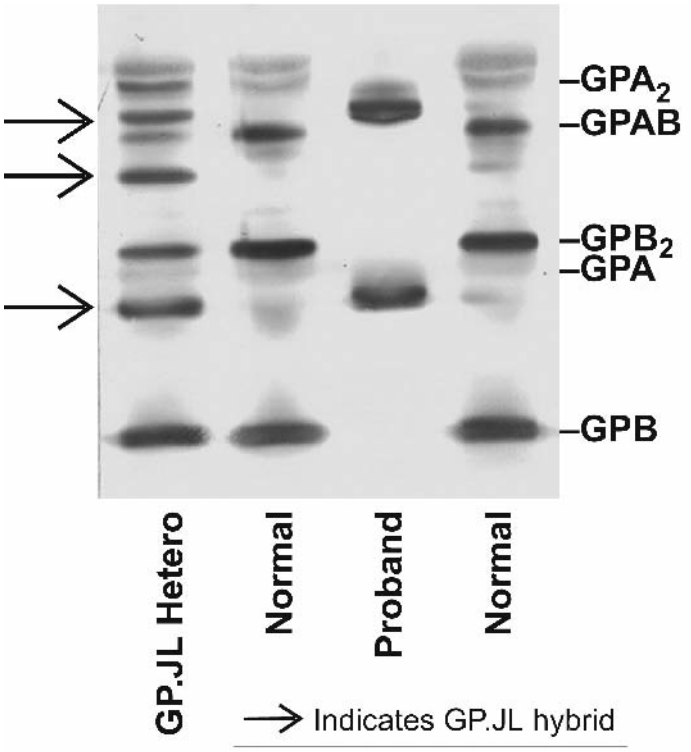

Fig. 1. Immunoblot of RBC membranes using Mab anti-GPA+GPB. As is typical with Mab anti-GPA+GPB (E3, Sigma), stronger staining was obtained with GPB than with GPA. "GP.JL Hetero" is from a control, known heterozygous *GYP.JL,* unrelated person. The RBCs from the "Normal" in lane 2 typed M+N–S+s+ and in lane 4 typed M+N+S+s+. The arrows indicate the GP.JL glycoprotein: monomer (lowest band) and homodimer (upper band) in both "Proband" and "GP.JL Hetero" samples, and the heterodimer, GPB/GP.JL, in the "GP.JL Hetero" sample.

DNA analyses

Sequencing of genomic DNA isolated from the proband's WBCs confirmed the presence of a *GYP(A-B)* hybrid in which exon 3 is from *GYPA* and exon 4 is from *GYPB* (Fig. 2). This change ablates an *Rsa*I restriction enzyme site at nucleotide 242 (T>G) and introduces an *Rsa*I site at nucleotide 266 (A>T). PCR-RFLP analysis after *Rsa*I digestion gave bands of 206 bp and 143 bp for the proband's hybrid glycophorin gene, compared with bands of 182 bp and 167 bp for *GYPA* (Fig. 3).

Analysis of DNA isolated from WBCs from the proband's mother, brother, and sister showed that the hybrid *GYPA-GYPB* gene was not present.

Discussion

We describe the first person with a probable *GP.JL/M^k* genotype who has made an alloanti-En^{a}FR. Anti-En^a is a broad term representative of a group of antibodies that react with different epitopes on GPA, the glycoprotein carrying MN. Anti-En^a may be an

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GYPA Ex4          ttttctttgcacatgccttacttatttggacttacattgaaattttgctttataggAGAA
GYP.JL (proband)  ---------------c---a-----------------------------------------
GYPBS Ex4         ---------------t---t-----------------------------------------

GYPA Ex4          AGGGTACAACTTGCCCATCATTTCTCTGAACCAggtatgttaatatttgacaaagaataa
GYP.JL (proband)  -T--G--------T-----G----A---T-------------------------------
GYPBS Ex4         -T--G--------T-----G----A---T-------------------------------
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Fig. 2. Alignment of gDNA nucleotide sequences using *GYPA*-specific primers for the flanking regions of exon 4 of the proband, compared with normal *GYPA* and *GYPB*. Uppercase letters indicate nucleotides in exon 4, and lowercase letters indicate nucleotides in intron 3 (upper line) and intron 4 (lower line). Dashes (–) represent consensus nucleotides using *GYPA* as the reference sequence. In the Proband, exon 4 is *GYPB*-specific.

autoantibody or, as in this case, an alloantibody recognizing a portion of GPA that is altered on or lacking from the RBCs of the individual who made the antibody. The antibody present in the proband's serum is directed at a ficin- (and papain-) resistant epitope on normal GPA close to the RBC membrane, which is lacking on her RBCs. This is in contrast to anti-En^{a}FS and anti-En^{a}TS, which are antibodies directed at, respectively, ficin-sensitive and trypsin-sensitive antigenic determinants at the *N*-terminal of this region on GPA.

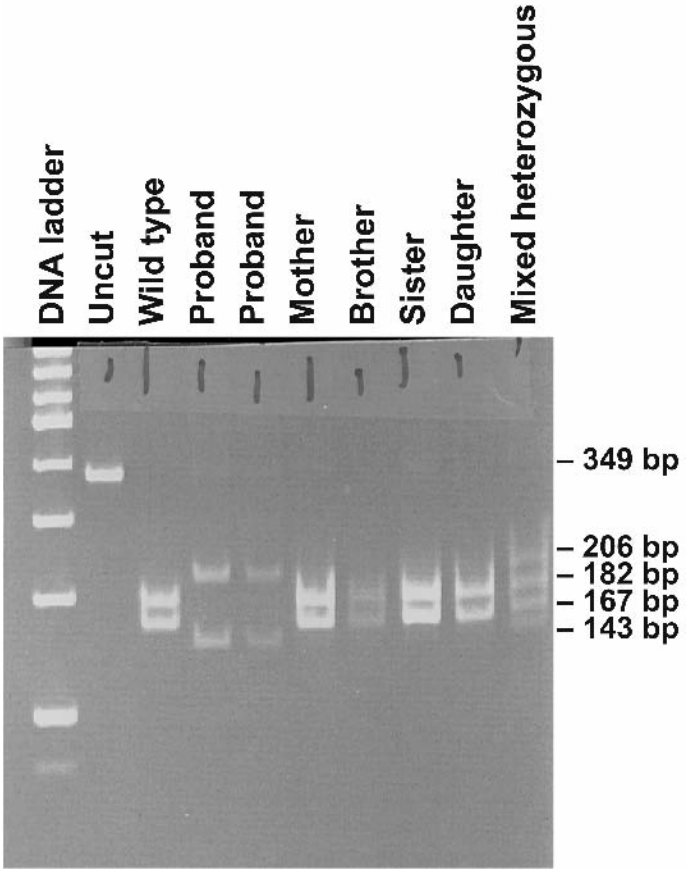

Fig. 3. PCR-RFLP of *GYPA* exon 4 using *Rsa*I. An 8% polyacrylamide gel demonstrating a band pattern of 182 bp and 167 bp for the "normal" *GYPA* control sample and for four relatives of the proband. The "Proband" sample gave bands of 206 bp and 143 bp. A sample containing equal parts of the proband's DNA and "normal" DNA gave bands of 206 bp, 182 bp, 167 bp, and 143 bp.

The proband's RBCs are TSEN+, MINY+ and appear to have a double dose of these antigens as a result of the presumed (based on the *Glycine soja* and immunoblotting results) presence of M^k in *trans* to *GYP.JL.* The S antigen on the proband's RBCs is altered, as would be expected, because her RBCs are TSEN+. The Wr(b–) status of her RBCs is also expected because, although Wr^b is associated with a point mutation on band 3, Wr^b expression requires the presence of amino acid residues 59 to 76 of GPA that are lacking from RBCs with a double dose of GP.JL and GP.Hil.[11,12] This explains why previously reported cases of individuals homozygous for genes encoding GP.JL (Mi.XI) were described as having anti-Wr^b, anti-Wr^b/En^{a}FR, or mixtures thereof in their serum.[7,8,13] Indeed, anti-Wr^b is an anti-En^{a}FR. However, not all anti-En^{a}FR are anti-Wr^b, and there is heterogeneity in these antibodies.

The altered *Rsa*I sites provide a means to perform PCR-RFLP analysis to detect the genes encoding TSEN and Hil.

The baby appeared to be unaffected by the mother's antibody, and it is tempting to speculate that the difference in ABO type (mother group O, baby group A) may have had a protective effect.

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