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A New Low Frequency Alloantigen (Kha^b) Located on Platelet Glycoprotein IIIa as a Cause of Maternal Sensitization Leading to Neonatal Alloimmune Thrombocytopenia

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BACKGROUND AND CASE REPORT

A total of 34 human platelet alloantigens (HPA) have been identified to date,¹ and all have been implicated in Neonatal Alloimmune Thrombocytopenia (NAIT), in which maternal antibodies destroy fetal and neonatal platelets that carry an incompatible HPA inherited from the father. All of the recently discovered HPA occur with low frequency (typically 0.1%) in Caucasian populations. We report a case of NAIT, in which maternal serum contained strong IgG antibodies reactive with a new low frequency HPA localized on paternal platelet glycoprotein (GP)IIIa.

A gravida 7 para 4 Latino/Hispanic female gave birth at 29 weeks gestation due to decreased fetal movement to an infant with prominent petechiae and 43,000 platelets/ μ L. The infant was treated with IVIgG and 3 platelet transfusions over a 2 week period. The third transfusion increased the platelets from 11K/ μ L to 240K/ μ L, and the counts remained normal thereafter. One of the previous three live-born infants delivered was also reported to have had thrombocytopenia, indicating that the mother was probably immunized from a prior pregnancy.

METHODS AND RESULTS

Samples from the parents were submitted to BloodCenter of Wisconsin (BCW) for diagnostic testing and all studies were approved by the BCW Internal Review Board. NAIT workup consisted of modified antigen capture ELISA (MACE) serology for detection of antibodies against HPA-1 to -5, MACE cross-match of maternal serum with paternal platelets, and parental DNA genotyping, all as previously described.² Site directed mutagenesis (Quick Change II XL, Agilent, Santa Clara, CA), full length GPIIIa and GPIIb cDNAs in the mammalian expression vectors pcDNA3.1/Zeo and Neo, respectively

CONFLICT OF INTEREST

All authors declare no conflicts of interest relevant to the content of this manuscript.

WEB RESOURCES www.ncbi.nlm.nih.gov/projects/SNP accessed July 30, 2014.

(Invitrogen, Carlsbad, CA), and transformation in One Shot Top 10 *E. coli* (Life Technologies, Carlsbad, CA) were all performed as previously described.³

NAIT workup showed the parents were incompatible for HPA-2b and -15a. Maternal serum showed no HPA-2b or -15a antibodies, but did have HLA antibodies and strong IgG antibodies reactive only with paternal GPIIb/IIIa by MACE (Figure 1a). Bidirectional Sanger sequencing of paternal *ITGB3* from genomic DNA, which encodes GPIIIa, showed a novel single nucleotide polymorphism (SNP) c.98C>T, p.Thr33Met (p.Thr7Met mature protein), we termed Kha^b. The new SNP (ss#1221285311) has been deposited in the NCBI dbSNP database.

To confirm that the maternal antibody specifically recognized Kha^b, site directed mutagenesis of full length wild type (WT) *ITGB3* was performed changing nucleotide C to T at position 98 followed by transformation into *E. coli* and selection of antibiotic resistant colonies. Mutated *ITGB3* and WT *ITGA2B*, were co-transfected into HEK293 cells and grown in zeomycin-neomycin selection media. Cells expressing high levels of GPIIb/IIIa were sorted by FACSaria (Becton Dickinson, San Jose, CA). Maternal serum reacted only with HEK293 cells expressing GPIIb/IIIa with Met33 in GPIIIa (Figure 1b) confirming that her antibody was specific for this form of the protein. It was not possible to obtain DNA from the infants affected with thrombocytopenia to confirm that they had c.98C/T in *ITGB3* as expected.

DNA genotyping of 100 unrelated Caucasian U.S. blood donors for c.98C>T in *ITGB3* found none with T at nt. 98, indicating that the SNP probably occurs at a very low frequency in this population. The father was Cambodian, but we were not able to obtain DNA from this population to screen for the frequency of Kha^b.

SUMMARY

We have documented a case of NAIT caused by maternal antibodies recognizing a novel mutation (Kha^b, c.98C>T) in *ITGB3* and expressed on GPIIIa. Kha^b brings the total number of low frequency HPA expressed on GPIIIa to 13, and further documents the importance of including a crossmatch of maternal serum with father's platelets in laboratory investigations of suspected NAIT.

References

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Figure 1. Characterization of novel SNP c.98C>T in *ITGB3* and confirmation of specificity of cognate maternal antibody

A) Glycoprotein (GP)IIb/IIIa was captured with a monoclonal antibody after incubation with maternal serum in the modified antigen capture ELISA (MACE). Maternal serum antibody reacts in MACE only with GPIIb/IIIa from paternal platelets (black bar), but not with GPIIb/IIIa from two different random platelets (light and dark gray bars). B) GPIIb/IIIa MACE shows maternal serum reactivity only with HEK293 cells expressing GPIIIa with methionine at amino acid 33 (MUT/Met33/Kha^b), but not with cells expressing wild type GPIIIa with threonine at amino acid 33 (WT/Thr33) or untransfected cells (UT).