Published in final edited form as:

Transfusion. 2010 August ; 50(8): 1855–1856. doi:10.1111/j.1537-2995.2010.02660.x.

A third allele of the HPA-5 (Br) platelet alloantigen system identified in investigating a case of neonatal thrombocytopenia

Julie A. Peterson, PhD, Maria L. Gitter, MS, Bradley Pietz, MS, MBA, Daniel Bellissimo, PhD, Brian Curtis, MS, MT(ASCD)SBB, and Richard Aster, MD

Blood Research Institute and Platelet & Neutrophil Immunology Laboratory, Blood Center of Wisconsin and the Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

Julie A. Peterson: julie.peterson@bcw.edu

HPA-5a/b (Brb/a) was identified in the late 1980s as an antigen system capable of causing maternal immunization leading to neonatal alloimmune thrombocytopenia (NAIT).¹ Subsequent studies showed that the HPA-5a/b antigens result from a single-nucleotide polymorphism (G>A1600; “A” of the ATG start codon is here designated Nucleotide 1) in the gene encoding platelet (PLT) glycoprotein (GP) Ia (α 2 integrin), leading to a Glu (HPA-5a)>Lys (HPA-5b) amino acid substitution at Residue 505 in GPIa.² Further studies showed that maternal-fetal incompatibility for HPA-5 antigens is second only to incompatibility for HPA-1a as a cause of NAIT.³ HPA-5a and -5b is thought to be a diallelic system with gene frequencies approximately 0.89 and 0.11, respectively. Here, we describe a third allele of HPA-5 identified in a Caucasian individual while studying paternal DNA in a suspected case of NAIT.

A full-term male infant born to a primiparous woman had a normal blood count except for a PLT level of $38 \times 10^9/L$. PLTs were $39 \times 10^9/L$ on Day 4, but increased to normal on Day 8. The infant had physical features of Down’s syndrome and Trisomy 21 was confirmed in genetic studies. No maternal antibody reactive with paternal PLTs was detected using flow cytometry and immunoprecipitation assays. Using polymerase chain reaction (PCR) with sequence-specific priming method, no incompatibility between father and mother was identified for HPA-1, -3, and -5 alloantigen systems. When this study was done, melting point analysis⁴ was being evaluated to type DNA for HPA antigens. In this technique, fluorescence is produced when PCR products containing the nucleotide sequence being typed anneal to sequence-specific “reporter probes.” As the reaction mixture is heated, the DNA complexes melt (with resulting loss of fluorescence) at temperatures that are allele-specific (Fig. 1). The mother’s DNA produced a single peak at 50°C, indicating that her genotype was HPA-5a/a. The father’s DNA produced two peaks, one corresponding to HPA-5a and the other intermediate between the HPA-5a peak and the one expected for HPA-5b (Fig. 1). Because of this atypical finding, the father’s DNA encoding the HPA-5 mutation was sequenced using standard methods. It was found that father’s DNA contained both the expected G at Position 1600 and an unexpected T substitution at Position 1601 (Table 1), providing an explanation for the atypical melting curves obtained when his DNA was typed for HPA-5 (Fig. 1). The substitution of A at Nucleotide 1601 to T leads to amino acid substitution at Position 505 from glutamic acid into valine creating a third allele of HPA-5. “T” at position 1601 was identified in one individual in the course of screening 20 DNA samples from NAIT cases when the melting point technique was being evaluated. However, no similar examples of T1601 were identified in typing 128 normal Caucasian

CONFLICT OF INTEREST

The authors have no disclaimers to make or conflicts to disclose.

subjects for HPA-5 by direct sequencing. Additional normal subjects are being typed to obtain a better estimate of the frequency of this allele.

It was not possible to obtain DNA from the infant to determine whether he inherited T1601. However, since no maternal antibody reactive with PLTs or GPIa/IIa from the father was identified and transient thrombocytopenia is common in newborns with Down's syndrome,⁵ it seems unlikely that the low PLT counts were the result of maternal immunization against GPIa containing Val at Position 505.

Knowledge of the GPIa mutation identified in this case will be helpful if other examples are identified in future evaluations of NAIT cases. It is possible, however, that T1601 will not be detected in assays that utilize sequence-specific primers to type for HPA-5a/b antigens.

References

1. Kiefel V, Santoso S, Katzmann B, Mueller-Eckhardt C. The Bra/Brb alloantigen system on human platelets. *Blood*. 1989; 73:2219–23. [PubMed: 2471562]
2. Santoso S, Kalb R, Walka M, Kiefel V, Mueller-Eckhardt C, Newman PJ. The human platelet alloantigens Br(a) and Brb are associated with a single amino acid polymorphism on glycoprotein Ia (integrin subunit alpha 2). *J Clin Invest*. 1993; 92:2427–32. [PubMed: 7901236]
3. Kaplan C, Morel-Kopp MC, Kroll H, Kiefel V, Schlegel N, Chesnel N, Mueller-Eckhardt C. HPA-5b (Br(a)) neonatal alloimmune thrombocytopenia: clinical and immunological analysis of 39 cases. *Br J Haematol*. 1991; 78:425–9. [PubMed: 1873226]
4. Curtis BR. Genotyping for human platelet alloantigen polymorphisms: applications in the diagnosis of alloimmune platelet disorders. *Semin Thromb Hemost*. 2008; 34:539–48. [PubMed: 19085653]
5. Thuring W, Tonz O. Neonatal thrombocyte values in children with Down's syndrome and other autosome trisomies. *Helv Paediatr Acta*. 1979; 34:545–55. [PubMed: 161560]

Fig. 1. Melting curve analysis of HPA-5 alleles with LightCycler (Roche Diagnostic, Indianapolis, IN). The first negative derivative of the fluorescence versus temperature ($-dF/dT$) graph shows the melting temperature (T_m) of HPA-5a probe/DNA complex (50°C) and the HPA-5b probe/DNA complex (40°C). There is a single curve at 50°C for an HPA-5a/a individual, a single curve at 40°C for an HPA-5b/b individual, and curves at both 40 and 50°C for an HPA-5a/b individual.

TABLE 1

Alleles of HPA-5

Allele	Nucleotides 1600/01/02	Amino Acid 505	Gene frequency
HPA-5a	GAG	Glutamic acid	0.89
HPA-5b	AAG	Lysine	0.11
Present case	GTG	Valine	?