Genotyping for Human Platelet Antigen-1 Directly From Dried Blood Spots on Cards

To the Editor:

Neonatal alloimmune thrombocytopenia (NAITP) is the commonest cause of severe thrombocytopenia in neonates, with intracerebral haemorrhage leading to neurologic damage in utero being a recognized complication. The condition is caused by maternal alloimmunization to antigenic epitopes of paternal origin in one of the biallelic human platelet antigen (HPA) systems, usually HPA-1, resulting in antibody-mediated destruction of fetal platelets. For HPA-1, the difference between the 2 alleles a and b is a single amino acid substitution of leucine (a allele)/proline (b allele) at position 33 in glycoprotein (GP) IIa. Severe NAITP usually arises in an HPA IbIb mother carrying an HPA Ia/Ib fetus. Because there are no routine screening programs for NAITP, the condition may go unrecognized in the neonatal period, but affected children may present with neurologic problems later. Investigation of such children should ideally include HPA-1 genotyping, particularly when the father is heterozygous for the HPA-1a antigen. Methods for HPA genotyping by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis or PCR using sequence-specific primers (SSP) are now well established using genomic DNA extracted from whole blood samples. However, the means to type prospectively on fresh capillary blood and retrospectively on dry capillary blood from neonates and babies would be advantageous. In the United Kingdom, all neonates have a heelprick blood sample taken at 9 days for the detection of phenylketonuria and hypothyroidism. These samples are stored on cards (Guthrie cards) that for medicolegal reasons, are retained for 15 years. We have developed a method for genotyping for HPA-1 using Guthrie cards, based on an established method for whole blood genotyping consisting of PCR followed by RFLP analysis.

A piece of Guthrie card (kindly provided by Dr A. Heeley, Peterborough Hospital NHS Trust, Peterborough, UK) approximately 1 mm by 2 mm was cut out and placed directly into a PCR reaction mixture consisting of 4 pmol/L of each of the sense (5'C TT CTG ACT CAA GTC CTA ACG3') and antisense (5'ATA AGC TTA GCT ATT GGG AGG AAG YGG TAG GCC CTG3') amplification primers, 2 U Tag polymerase (Promega, Madison, WI), 0.2 mmol/L dNTPs, and enzyme reaction buffer (Promega M188A supplied with enzyme) containing magnesium at a final concentration of 1.5 mmol/L. No initial DNA extraction or quantitation was required. Primary amplification of a 482-bp segment from the GPIIIa gene containing the leucine/proline 33 polymorphism consisted of a hot start of 95°C for 10 minutes and 72°C for 5 minutes, followed by 30 cycles of 1 minute at 95°C, 1 minute at 50°C, and 1 minute at 72°C, with a final product extension step of 3 minutes at 72°C (Biometra TRIO-Block; Biometra, Tampa, FL). To enhance the specificity of primer annealing, 5 μL of this product was then subjected to a second 20 cycle round of amplification as above, except that a 5-minute 95°C hot start and a 61°C annealing temperature were used. Digestion with Msp-I (New England Biolabs, Inc, Beverly, MA) yielded two fragments of 197 and 279 bp for the genotype HPA-1 (a+b−); three fragments of 106, 173, and 197 bp for HPA-1 (a−b+); and four fragments of 106, 173, 197, and 279 bp for HPA-1 (a+b+). Separation and detection of fragments was performed by 8% polyacrylamide (acyrulamide:bisacrylamide, 19:1) gel electrophoresis followed by ethidium bromide staining and UV visualization.

Six platelet donors previously genotyped from whole blood DNA (2 each of HPA Ia/Ia, Ia/Ib, and Ib/Ib) were retyped by the above method from four drops of capillary blood collected directly onto blank Guthrie cards and stored for 3 months at room temperature before use. RFLP analysis results were 100% concordant with those originally produced by genotyping DNA extracted from whole blood (Fig 1A).

The method was then used to type 19 infants of HPA-1a−negative mothers as part of a regional survey of NAITP from cards stored for up to 17 months. Typical results of 4 infant samples are shown in Fig 1B. Clear results were obtained in all cases (18 HPA-1aIb and 1 HPA-1bIb).

This method can be applied to any small volume samples, including cordocentesis and fingerprick samples from children. In addition, the HPA-1b allele has recently been reported as a risk factor for coronary artery disease. Further population studies will be facilitated by a genotyping method that does not require DNA extraction and that allows easy transportation, storage, and testing of large numbers of samples.

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Fig 1. RFLP analysis of Guthrie card samples from (A) control donors (previous whole blood genotype is shown) and (B) 4 unknown infant samples.
REFERENCES


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