short report
Haematologica 1997; 82:600-601

USE OF A RAPID METHOD FOR GENOTYPING HUMAN PLATELET ANTIGEN SYSTEMS IN NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

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ABSTRACT

We applied a polymerase chain reaction-sequence specific primer (PCR-SSP) method developed by other researchers to study 4 families of newborns with neonatal alloimmune thrombocytopenia (NAITP) in which serology had provided inconclusive human platelet antigen (HPA) typing data. This method allowed for the identification of the newborn HPAs which were incompatible with their respective mothers. They were HPA-2b, -1b, -3a, and -5b. This PCR-SSP is a useful tool for improving the ability to identify the incompatible HPA in NAITP.

Key words: platelet antigens, genotyping, neonatal alloimmune thrombocytopenia

Platelet antibody detection in the mother' serum and human platelet antigen (HPA) typing of both the parents and the newborn may facilitate the diagnosis and management of neonatal alloimmune thrombocytopenia (NAITP). Often the low patient platelet count and the scarcity of high quality antisera cause difficulties in serological typing.

Recently, a number of DNA typing methods based on polymerase chain reaction (PCR) have been developed. In this study, we applied a PCR-SSP method developed by Skögen *et al.*[1] with slight modifications on the evaluation of NAITP cases in which we were unable to identify the antigen involved with serological methods.

Materials and Methods

We examined 4 NAITP newborns and their parents. Genomic DNA was extracted according to Gustincich *et al*.[2] For HPA typing we used the sequence-specific primers described by Skögen *et al.*[1] Two primers which amplify a region of the human growth hormone (HGH) gene were used as an internal control in each amplification mixture. The PCR profiles were as follows: 9°C for 3.5 min, then 30 cycles at 94°C for 30 seconds, 65°C for 1.5 min (57°C for HPA-5 and -6), and 72°C for 30 seconds. At the end of the procedure, a final extension step was carried out at 72°C for 6.5 min. PCR products were analyzed according to Skögen *et al*.[1] Table 1 summarizes the PCR conditions modified from those described in the original method.[1]

Results and Comments

We could recognize the alleles involved in all four NAITP cases (HPA-2b, HPA-1b, HPA-3a and HPA-5b) (Figure 1). During implementation in our laboratory, we introduced some modifications to further simplify the method developed by Skögen *et al*. DNA was extracted with a rapid method, only 2 of the 4 HGH control primers were employed for all allele determinations, the time of PCRs was reduced and only 2 different annealing temperatures instead of 3 were used. As a result, we obtained a complete genotyping of all 6 HPAs using only 2 PCR runs in about 2 and a half hours. We could not evaluate the efficiency of our amplification protocol with the HPA-4b and HPA-6b specific primers because we did not find any individuals with these alleles.

Other DNA typing methods have been described, but some of them are time consuming and require very particular expertise, and so their use has not spread outside specialized centers.[3-5] Other methods are more suitable for the analysis of large series of samples.[6] We conclude that this method is a useful tool for the laboratory diagnosis of NAITP.

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Received April 24, 1997; accepted August 5, 1997.

Table 1. PCR-SSP conditions modified (*) from the method of Skögen *et al.*[1]

Allele	*Formamide (%)*	*MgCl2 (mM)*	*Specific primers (µM)*	*HGH primers (µM)*
HPA-3a	0	1.5	0.75*	0.2*
HPA-3b	1	1.5	0.75*	0.2*
HPA-4b	0	3.5*	0.5	0.1
HPA-5a	0*	3.5	0.5	0.1*
HPA-5b	0*	3.5*	0.5	0.1*
HPA-6a	1	3.5*	0.5	0.1
HPA-6b	1	3.5*	0.75	0.1

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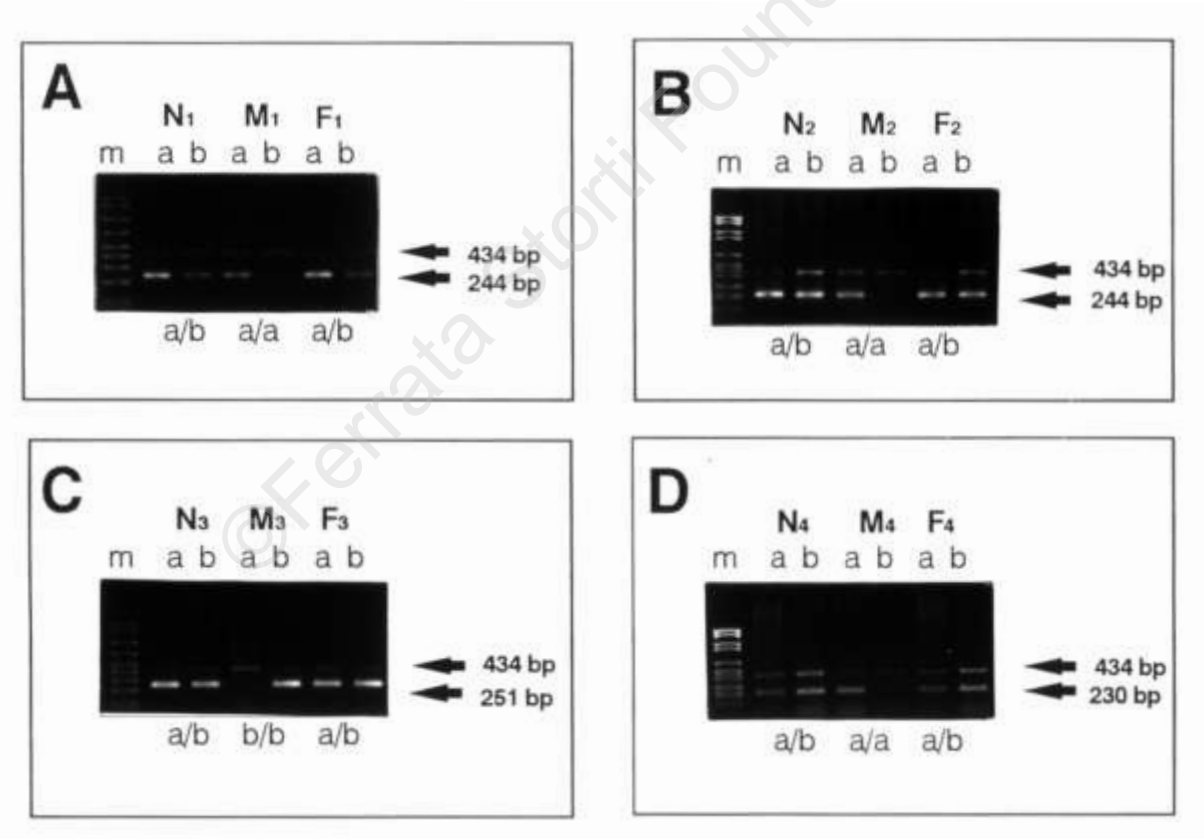

Figure 1. PCR-SSP genotyping of the HPA system involved in 4 NAITP cases. (N=newborn; M=mother; F=father). The specificity of the PCR-SSP (a or b) and DNA size standard, used as a marker (m), (AmpliSize standard, BioRad) are indicated at the top of each lane. HPA genotypes are indicated at the bottom of each lane. The HGH (control) PCR product (434 bp) and the allele-specific product (230-251 bp) are indicated by arrows.
Panel A: N1M1F1 = family of NAITP case 1 (typing of HPA-2). B: N2M2F2 = family of NAITP case 2 (typing of HPA-1). C: N3M3F3 = family of NAITP case 3 (typing of HPA-3). D: N4M4F4 = family of NAITP case 4 (typing of HPA-5).