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

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.* Other methods are more suitable for the analysis of large series of samples.* We conclude that this method is a useful tool for the laboratory diagnosis of NAITP.
Table 1. PCR-SSP conditions modified (*) from the method of Skögen et al.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Formamide (%)</th>
<th>MgCl2 (mM)</th>
<th>Specific primers (µM)</th>
<th>HGH primers (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-3a</td>
<td>0</td>
<td>1.5</td>
<td>0.75*</td>
<td>0.2*</td>
</tr>
<tr>
<td>HPA-3b</td>
<td>1</td>
<td>1.5</td>
<td>0.75*</td>
<td>0.2*</td>
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<tr>
<td>HPA-4b</td>
<td>0</td>
<td>3.5*</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>HPA-5a</td>
<td>0*</td>
<td>3.5</td>
<td>0.5</td>
<td>0.1*</td>
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<tr>
<td>HPA-5b</td>
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<td>3.5*</td>
<td>0.5</td>
<td>0.1*</td>
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<tr>
<td>HPA-6a</td>
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<td>3.5*</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>HPA-6b</td>
<td>1</td>
<td>3.5*</td>
<td>0.75</td>
<td>0.1</td>
</tr>
</tbody>
</table>

References

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).