A novel approach to Human Platelet Antigen (HPA)-1 typing in the UK: Real-time PCR genotyping

INTRODUCTION

Fetomatern alloimmune thrombocytopenia (FMAIT) is caused by maternal antibodies raised against alloantigens carried on fetal platelets. FMAIT is a significant cause of morbidity and mortality in newborns and is the most common cause of intracranial haemorrhage in full-term infants.

Incompatibility of human platelet antigen (HPA)-1 accounts for 80% of cases in the Caucasian population and arises when pregnant women with the HPA-1b platelet phenotype produce antibodies to fetal HPA-1a platelets. Rapid and accurate HPA genotyping is essential, so a diagnosis can be confirmed, HPA-matched components can be transfused to the thrombocytopenic infant and parents can be counselled for the risks associated with future pregnancies.

Laboratory diagnosis of FMAIT relies on the demonstration of maternal alloantibodies which react against paternal and fetal platelet antigens but not the mother’s own platelets. Unlike Haemolytic Disease of the Newborn, FMAIT can affect the first pregnancy.

HPAs are found on platelet glycoproteins involved with platelet activation and expressed as early as 16 weeks gestational age. The HPA-1 allele polymorphism is distinguished by a single nucleotide polymorphism (SNP) and has 3 possible genotypes: 1a1a, 1a1b and 1b1b.

STUDY DESIGN AND METHODS

Following withdrawal of an enzyme-linked immunosorbent assay (ELISA) used for HPA-1a typing at the Northern Ireland Blood Transfusion Service (NIBTS), an alternative method was required. Instead of using conventional polymerase chain reaction with sequence specific primers (PCR-SSP) used by other UK laboratories, an ‘in-house’ real-time PCR method for platelet genotyping was evaluated.

For the qualification, thirty six samples of DNA were isolated from donors of known HPA-1a types using the MagNA Pure Compact System and Nucleic Acid Isolation Kit I (Roche Diagnostics). The limit of detection was established using serial dilutions of DNA target sequences, whose concentration was determined using a Nanodrop™ Lite (Thermo Scientific).

Taqman mastermix, primers and fluorogenic probes complementary to HPA alleles 1a and 1b were used for real-time PCR using a Roche LightCycler® 430 and endpoint genotyping analysis. Controls from synthesised HPA-1 oligonucleotides and donors with known HPA-1 types (1a1a, 1a1b and 1b1b) were included.

RESULTS

The HPA-1 genotype results of thirty six samples analysed were 100% concordant with the results using conventional PCR-SSP. This method has shown to be extremely sensitive, with a limit of detection estimated at 16.1 copies of DNA.

Table 1. HPA-1 Genotypes tested and their frequency in the Caucasian population

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Glycoprotein</th>
<th>Genotype</th>
<th>Caucasian Population frequency (%)</th>
<th>No. of samples tested at NIBTS (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1</td>
<td>GP1a</td>
<td>a/a</td>
<td>72</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a/b</td>
<td>26</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b/b</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Real-time PCR HPA-1 genotyping has been successfully established at NIBTS and will be used to facilitate the diagnosis of FMAIT. It further enables NIBTS to screen donors in an effort to maintain and expand the bank of known HPA-1a negative donors.

The test demonstrated to be sensitive, specific and reproducible, and does not require a gel electrophoresis step. These benefits alongside the low cost, speed and accuracy demonstrate that it would be feasible to introduce this assay as a routine screening test, to identify pregnancies at risk of FMAIT in the future. NIBTS plan to expand HPA genotyping using real-time PCR to include HPA-2, HPA-3, HPA-5 and HPA-15.

ACKNOWLEDGMENTS

The authors would like to thank the Irish Blood Transfusion Service for providing samples of known HPA-1 types and supplying the HPA-1a negative platelet for the above case.

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REFERENCES


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