PLATELET & GRANULOCYTE ALLOANTIGEN TYPING USING SEQUENCE-SPECIFIC PRIMER (SSP) PCR AMPLIFICATION

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INTRODUCTION

A polymerase chain reaction (PCR) amplification technique has been investigated for the phenotyping of two medically important polymorphic loci: the platelet alloantigen marker HPA1 and the granulocyte alloantigen marker NA. HPA1 phenotyping is important in predicting the development of alloimmune thrombocytopenia (Metcalfe & Waters 1993). NA phenotyping similarly facilitates the prediction of allo- & autoimmune neutropenia (Stein *et al* 1994). Both systems have been previously phenotyped using serological techniques which require isolation of specific cell types from blood samples - a method which is particularly difficult with fetal or neonatal samples and requires reagents of limited availability. In this study, the usefulness of each locus in paternity analysis was assessed by studying false family trios constructed by using an unrelated individual as the putative father. Twenty five meioses in the form of the mother : child pairs in the above trios were analysed for mutations and mode of inheritance. A population database of 60 Caucasians was analysed for any deviation from Hardy Weinberg equilibrium.

MATERIALS & METHODS

Both HPA1 and NA are diallelic systems which allow the use of sequence specific primers in paired PCR reactions for each sample - one reaction in each pair containing the primer complimentary to one of the two alleles found in the system. Samples are subsequently scored for presence or absence of the appropriate PCR product using agarose electrophoresis.

The technique of sequence specific primer PCR relies on the principle that a single base mismatch between the 3' terminal base of the primer and the target sequence will block progress of the Taq1 polymerase, which, unlike other polymerases, is unable to repair single base mismatches efficiently. Thus, alleles differing by only one base can be distinguished, since a mismatch will result in very low levels of amplification compared to those produced when primer and target site match precisely.

In the case of HPA1, the two alleles differ at a single base in position 196 of intron 2 of the GPIIIa gene (allele 1a = T, allele 1b = C) (Newman *et al* 1989).

The NA locus has 3 distinct base substitutions which distinguish the 2 alleles, occurring within intron EC-1 of the FcRIII-1 gene; so primers exploiting the base mismatches at 2 of these sites can initiate amplification at different positions to give a product of a distinct size for each of the two alleles (Stein *et al* 1994).

Samples: Blood samples of mother : child pairs from paternity investigations performed at the LHMC were used. Samples from medical students were used as fathers to construct false family trios and as additional samples for the database. All samples were identified as Caucasian from photographs.

DNA Extraction: DNA was extracted using 5% chelating resin (Lareu *et al* 1994).

Primers: HPA $^{1A}/_{1B}$ 5' TCA GGT CAC AGC GAG GTG AGG CC$^{A}/_{G}$ 3'
HPA common 5' CTG CAG GAG GTA GAG AGT CGC CAT AG 3'
NA 1 5' CAG TGG TTT CAC AAT GTG AA 3'
NA 2 5' CAA TGG TAC AGC GTG CTT 3'
NA common 5' ATG GAC TTC TAG CTG CAC 3'

Amplification: HPA1 : 98°C x 1 min.
68°C x 1 min. 30 cycles
72°C x 1 min.
(final extension - 5 mins)

NA: 95°C x 1 min.
68°C x 2 mins. 26 cycles
72°C x 1 min.
(final extension - 5 mins)

Both systems : 25µl PCR reaction volumes with 10µl DNA extract.
All primers at 0.5µM and nucleotides at 200mM. 1.0U of Taq per amp.

Electrophoresis: 12µl of PCR sample was electrophoresed in 1.6% FMC Seakem LE® agarose stained with ethidium bromide. 8 volts / cm. x 30 mins.

RESULTS

Typical results for HPA1 and NA are shown in figures 1 and 2. Since low levels of amplification *can* occur when primer and target site mismatch, faint non-specific bands can be found with some samples. However there is relatively low levels of activity in comparison to bands produced when primer and target site match, so that the relative intensity of bands can be important in the interpretation of results. Both bands should be of equal intensity and when one band is relatively weak this cannot be considered to provide evidence for presence of the corresponding allele. With NA typing, non specific amplification products can result in additional bands but these would not be confused with the true NA products.

In order to provide an indication that the PCR reaction was working correctly, Human growth hormone (HGH) primers have been used by some workers as an internal DNA control for each PCR reaction : producing a monomorphic band of similar size (439 bp) to both HPA & NA bands. Our experience suggested difficulties in obtaining suitable concentrations of control primers in the reaction mix to avoid competition between HPA or NA primers and the HGH primers. In many respects, the primer bands, clearly discernable in figure 1, act as a control.

The results of the phenotyping of 60 unrelated Caucasians (medical students & mothers from the false family trios) and the analysis of 25 false family trios for each system are outlined in tables 1 & 2.

Both systems produced phenotypes in expected ratios, with no deviation from Hardy Weinberg equilibrium.

Each system gave clear indications of normal Mendelian inheritance between the mother and child in all the families studied.

The exclusion rates are similar to those predicted by the allele frequencies determined from the population survey in each system.

FIGURE 1. HPA typing (left to right : +/-, +/+, +/+, -/+, -/+ = 1a, weak 1a1b, 1a1b, 1b, 1b)

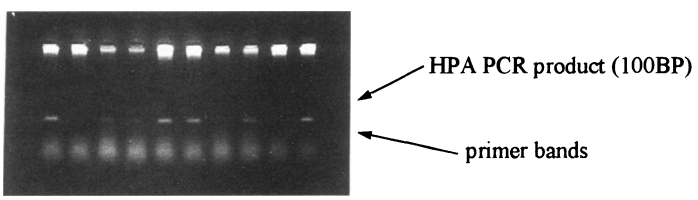

FIGURE 2. NA typing (left to right : +/+, +/+, +/+, +/-, -/+ = 2.1, 2.1, 2.1, 1, 2)

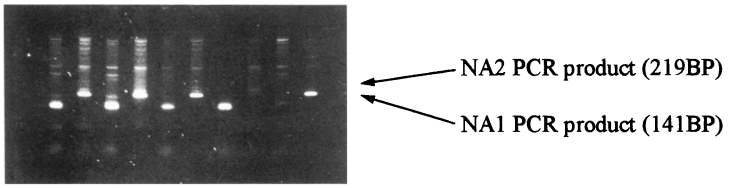

TABLE 1. HPA1 Analysis.

Phenotype	Obs. (%)	Expected	
1a	**40** (66.7)	**40.05**	HPA 1a = **0.817**
1a1b	**18** (30.0)	**17.94**	HPA 1b = **0.183**
1b	**2** (3.3)	**2.01**	χ^2 = < **0.001 (not Significant)**

Observed No.of Exclusions : **3/25 (12%)** Expected No. of Exclusions : **12.7%**

TABLE 2. NA Analysis

Phenotype	Obs (%)	Expected	
1	**9** (15.0)	**9.6**	NA 1 = **0.4**
2.1	**30** (50.0)	**28.8**	NA 2 = **0.6**
2	**21** (35.0)	**21.6**	χ^2 = **0.104 (not significant)**

Observed No.of Exclusions : **4/25 (16%)** Expected No. of Exclusions : **18.2%**

SUMMARY

It appears that SSP-PCR testing for the identification of the alleles in these two polymorphic systems provides a quick and simple method, where previous availability of reagents and adequacy of blood samples have made such typing difficult by other techniques.

The detection of the two polymorphisms described here and the further platelet and white cell polymorphic markers for which primers are becoming available, will provide a group of systems which could be used in problems of identification.

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