

## GENE FREQUENCIES OF HUMAN PLATELET ALLOANTIGENS IN KELANTAN, MALAYSIA

Dear Sir,

The gene for the  $\beta_3$  (GPIIIa) subunit of the integrin  $\alpha_{IIb}\beta_3$  (GPIIb-IIIa) present on the platelet membrane contains the platelet antigen alleles (PIA) in a homozygous form ( $PI^{A1/A1}$ ) or the heterozygous or polymorphic form ( $PI^{A1/A2}$ ) in humans. This GPIIIa subunit serves as a receptor for adhesive proteins (viz. fibrinogen and von Willebrand factor) and is critical for platelet aggregation.<sup>(1)</sup> The structural basis of  $PI^{A1/A2}$  polymorphism arises from a single base difference, a thymidine or a cytosine, which leads to an amino acid difference at position 33 in the mature  $\beta_3$  subunit: a leucine in  $PI^{A1}$  or a proline in  $PI^{A2}$ .

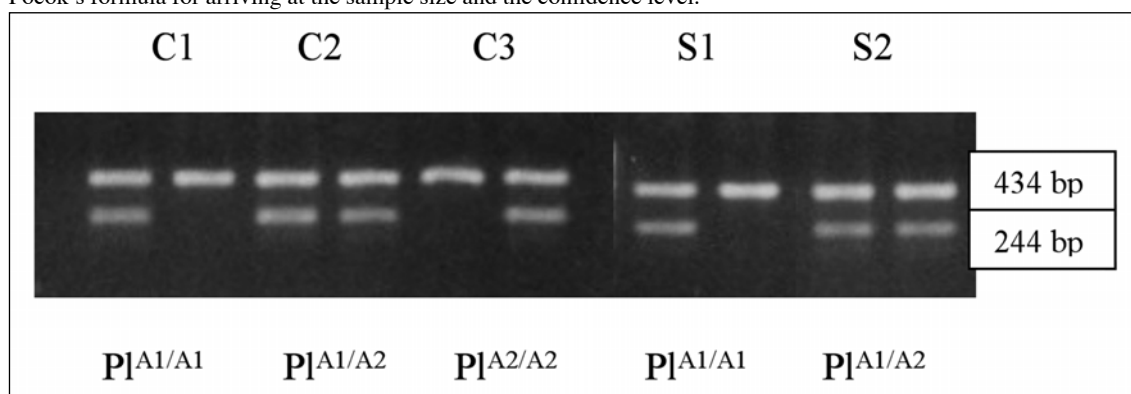
Till 1996, significant work on the single nucleotide polymorphisms (SNPs) present in the PIA system, especially the alleles in the gene for GPIIIa, had not been undertaken in detail and correlated with platelet behaviour. It was Weiss et al who identified the heterozygous (polymorphic)  $PI^{A1/A2}$  allelic state as a significant risk factor leading to platelet hyperadhesion and hence to thrombotic events like myocardial infarction and unstable angina, particularly in the younger Caucasian males.<sup>(2)</sup>

Since the initial report that the  $PI^{A2}$  polymorphism is a cardiovascular risk factor, many population-based studies had been done at many centres to determine the natural occurrence of  $PI^{A1/A2}$  polymorphism amongst the various ethnic groups. Searching the literature indicated that no previous studies had been done on the  $PI^{A1}$  and  $PI^{A2}$  allele frequencies in the Malaysian population.

We conducted a study on a sample, which included 160 Kelantan residents who were apparently healthy and unrelated to one another. Their ages were between 15 and 50 years, with a mean age of 33 years. There were 150 Malays, of which 20 were males and 130 females. The study also included ten Chinese patients as the cohort of the Chinese population in Kelantan is small. Using 2 ml of venous blood, isolation of DNA was performed with a DNA isolation kit. (QIamp Blood Kit, Qiagen, Research Biolabs, Kuala Lumpur, Malaysia). The genotypes were determined using allele-specific polymerase chain reaction (PCR) amplification. Briefly, exon 2 of the human platelet glycoprotein IIIa (ITGB3) gene was amplified using primers that amplify a 244 base pair fragment of genomic DNA that contains the polymorphic site responsible for the amino acid substitution causing a given platelet alloantigen.

Two PCR procedures were undertaken to achieve alloantigen genotyping of an individual using the Allele Specific Oligonucleotide (ASO) technique.<sup>(3)</sup> One, using one allele-specific primer with the consensus primer, is used to identify that corresponding allele, and the second allele is identified in a reaction using the complementary allele-specific primers. Thus, in homozygous individuals, amplification occurred only when the corresponding specific primer is present in the reaction solution. In heterozygous individuals, amplification occurred in both reactions.

The results of PCR amplification viewed by gel electrophoresis are shown in Fig. 1. We used the single proportion Pocok's formula for arriving at the sample size and the confidence level.



**Fig. 1** Platelet alloantigen products of sample 1 (S1) represent  $PI^{A1/A1}$  homozygous state and sample 2 (S2) represent  $PI^{A1/A2}$  heterozygous state. In addition to the Human Growth Hormone (HGH) PCR product at the 434 bp, a distinct band at the 244 bp is present. This band has the predicted size for the amplification product corresponding to the platelet alloantigen.

Our results of the gene frequency pattern of  $PI^{A1}$  and  $PI^{A2}$  alleles among the 150 Malays are tabulated in Table I along with those reported from other countries. The results of the genotypes followed the Hardy-Weinberg Equilibrium. This study proved that the allele frequency of the homozygous  $PI^{A1}$  allele is much more predominant (95.4%) than the heterozygous  $PI^{A2}$  allele (4.6%) in the residents of Kelantan. It was noted that among the ten Chinese subjects,

none had the PI<sup>A2</sup> polymorphism. Though the number of subjects studied is small, the results compared well with the frequencies reported among the Asian populations, especially those in the Far East, particularly Thailand and the Philippines. In the western populations, and strangely in the Indian reports as well, the gene frequency of the PI<sup>A2</sup> allele remains higher (nearly 16%–28%) with the maximum prevalence in the African-American populations.

The observed results of the PI<sup>A1</sup> and PI<sup>A2</sup> allele frequencies in the Malay population in Kelantan is similar to that seen in the Thai and Filipino population (PI<sup>A1</sup> = 0.98 and PI<sup>A2</sup> = 0.02).<sup>(4)</sup> Thus, the inheritance of PI<sup>A2</sup> allele could contribute to similar thrombotic risks as seen in other Southeast Asian population. Our small sample study was not designed to look into the association between the thrombotic events and the incidence of PI<sup>A2</sup> allele in the 160 subjects in this study. Larger cohorts need to be studied to ascertain the association between this PI<sup>A2</sup> allele and thrombotic events.

Yours sincerely,

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**Table I. Comparison of allele frequency of PI<sup>A1/A1</sup> and PI<sup>A1/A2</sup> between our study\* and those from other world populations.**

Population	Frequency of PI <sup>A1/A1</sup>	Frequency of PI <sup>A1/A2</sup>
Taiwanese	0.998	0.002
Japanese	0.998	0.002
Chinese	0.995	0.005
Indonesian	0.991	0.009
Thai	0.985	0.015
Filipino	0.98	0.02
Kelantanese* (Malay)	0.954	0.046
Arab	0.84	0.16
UK	0.84	0.16
German	0.83	0.17
Indian	0.794	0.196
Spanish	0.78	0.22
Caucasian-American	0.74	0.26
African-American	0.72	0.28

## REFERENCES

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