HPA-1b Gene Polymorphism in Coronary Artery Disease Patients with Myocardial Infarction in North-east Peninsula Malaysia

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Abstract **-**Platelet aggregation at the site of ruptured plaques in coronary arteries is the principal mechanism responsible for the crisis of myocardial infarction (MI) in patients of coronary artery disease (CAD). Polymorphism of the Human Platelet Antigen (HPA-1) gene may render platelets more sensitive for thrombus formation at ruptured plaques and therefore increase the risk of MI. Of the 113 CAD patients with MI, 106 (93.8%) were HPA-1a/1a; 7 (6.2%) were HPA-1a/1b; and none were HPA-1b/1b. In the control category, 107 (91.5%) were HPA-1a/1a; 10 (8.5%) were HPA-1a/1b; and none were HPA-1b/1b. HPA genotype was determined in Malay patients prediagnosed CAD with MI (median age= 52.2 years; p=0.05). The control group consisted of healthy Malay subjects from the same area, (median age=42.2 years; p=0.05). Genotyping was performed using allele-specific polymerase chain reaction (PCR) amplification of a 244-bp product. Sequence-specific primers were used to discriminate between the HPA-1a/1b alleles. We found no significant difference in frequencies of HPA-1b allele between patient and control of the Malay population in north east Peninsula Malaysia.

Keywords**—**Atherosclerosis, Coronary artery disease, Myocardial infarction, HPA-1b gene polymorphism

I. Introduction

CARDIOVASCULAR disease (CVD) is the leading cause of death globally. In 2008, there were 17.3 million deaths from CVD, of which 7.3 million died of ischemic heart disease, 6.2 million from stroke or other forms of cerebrovascular disease [30].

In Malaysia, CVD is the number one killer, accounting for nearly 30% of all medically certified deaths in the country [15].

Coronary artery disease (CAD) is the gradual build-up of plaque in the coronary arteries and may subsequently restrict the blood flow to the heart. The features include the loss of elasticity and reduction in the luminal diameter of the arteries. It could eventually occlude the blood flow in the affected arteries, effectively cutting off blood supply to the affected cardiac muscles, thus causing coronary heart disease (CHD) and myocardial infarction (MI). MI can result from occlusion of arteries from ruptured plaques and platelet-rich thrombi formed.

The occurrence of a sudden plaque rupture may result in an acute MI when the freed thrombus causes an occlusion in the artery supplying blood to the cardiac muscle.

The first stage of a myocardial infarction most often happen when the thin cap covering a plaque is ruptured. This is followed by platelet adhesion, aggregation and then fibrin clotting at exposed subendothelial tissue of the ruptured site. As a result, there will be obstruction of the artery. In severe cases, there will be total obstruction and the muscle beyond dies, and MI results [7].

The contributing risk factors for CAD, CHD and MI are multitude, some of which are yet unknown. They consist of environmental factors which are modifiable and the non-modifiable factors such as genetics, age, gender and others. Among the non-modifiable factors, genetic factors are well known risk factors. There are more than 250 genes that may play a role in CAD and MI. These involved mutations in some genes that increase or decrease risks of CAD and MI [28].

A polymorphism of the platelet glycoprotein (GPIIIa) which is a membrane receptor for fibrinogen and von Willebrand factor mediates platelet aggregation and fibrinogen binding is known to play a role in the pathogenesis of acute coronary syndromes [27].

The molecular basis of GPIIIa polymorphism in persons with HPA-1a have a leucine at position 33 of mature glycoprotein IIIa whereas persons with HPA-1b have a proline at this position, which result from C→T mutation at 1565 nt position in exon 2 of the glycoprotein IIIa gene [16]. The HPA-1b allele is suggested to be a risk factor for coronary thrombosis. This association was found to be stronger in patients who were younger than age 60 years at the time of their acute MI [8], [27].

GP IIb/IIIa is highly polymorphic and carries the HPA-1 and other diallelic antigen systems [13]. HPA-1b allele has been suggested as an inheritable, independent risk factor for MI [27].

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II. Methods And Materials

Blood samples were collected from 113 patients of Malay ethnicity with an established diagnosis of CAD and MI of both genders (aged 30 to 60). The patients were recruited at the Cardiology Unit, Hospital Universiti Sains Malaysia, Kubang Kerian, North-East Peninsula Malaysia. For the control group, 117 healthy blood donors, matched with the case patients for age, race, and gender with no documented history of CAD or MI were recruited from the Transfusion Medicine Unit of the same hospital. All the subjects with coagulopathies, platelet disorders, psychosis, pregnancy and those who had received blood or blood products in the past three months, were excluded from the study.

DNA was prepared according to GENE√ALL TM Blood SV mini Protocol. The primers used, HPA-1a and HPA-1b were originally described by Skogen [25] and were obtained commercially from Invitrogen Customs.

The allele-specific PCR was performed as described by Hurd [10] with some modifications. The final reaction mixture contained 0.1uL platinum Taq DNA polymerase (5.5U/µl), 2.5 µL 10x reaction buffer, 1.5 uL MgCl, 0.5 uL dNTP, 0.8 uL of each specific primer, and 1.6uL of each internal control primer. 20µl of the mastermix was aliquoted into the desired number of 0.2ml tubes. Subsequently, the required volume of the sample DNA and control DNA (1-5µl) was added. The PCR was run using an automated thermal cycler (Eppendorf Gradient) under the following conditions. The samples were denatured at 94ºC for 5 minutes and then 30 amplification cycles were performed. Each cycle consisted of a denaturation phase at 94ºC for 1 minute, annealing of primer at 65ºC for 2 minutes and primer extension at 72ºC for 1 minute. After the 30th cycle, the extension reaction was continued for another 10 minutes at 72ºC. The amplification products were analyzed by agarose gel electrophoresis and ethidium bromide staining.

The statistical analysis was performed with SPSS version 18. Genotype distribution and allele frequencies were compared by cross-tables using the Fisher's exact test. Significance level was established at a value of $P \leq 0.05$.

III. Results

Distribution of HPA-1 genotypes is shown in Table I. Allele frequencies in both groups are in accordance with those predicted by the Hardy-Weinberg equilibrium. Of the total 230 individuals, 17 were HPA-1b positive corresponding to only 7.4% of the total group. The prevalence of HPA-1b amongst patients and controls was not significantly different (p=0.335). Allele frequencies for HPA-1b were 3.7% in total. In the patient group, the HPA-1b allele frequency was 3.1% and among controls it was 4.3%, indicating no significant risk of CAD with MI attributable by the HPA-1b allele.

IV. Discussion

Cardiovascular disease, in particular coronary artery and other atherosclerotic disease, is the leading cause of death in many countries and carry enormous socioeconomic burden. In many developing countries as well as newly industrialized countries such as Malaysia, the incidence of cardiovascular diseases is rapidly rising making it one of the top five causes of morbidity and mortality, thereby creating an enormous financial burden.

Thus the value of early identification of persons at higher risk for development of CAD with MI based on detection of genetic risk factors, so as to pursue vigorous life style modification to minimize exposure to modifiable risk factors is an attractive proposition.

In the Malaysian context, over the past two decades, ethnic Malay CAD mortality rate has increased at a rate of 3.4 times compared to 2.2 and 1.9 times for ethnic Chinese and Indians respectively during the same period [11]. The selection of the CAD group which consisted of younger age subjects (30-60 years) in this study was due to the fact that inherited risk factors would have a greater influence on the development of CAD and MI than elderly patients, where the influence of metabolic and behavioral risk factors become increasingly more important. The study was also confined to only a single race (the Malay ethnicity) and conducted in north-eastern Peninsula Malaysia whose population consisted of a rather homogenous population of the Malay ethnicity that allows for an ideal setting in studies that could be influenced by genetic susceptibility.

The frequency of the HPA-1b polymorphism has been shown to differ significantly in various different geographical areas and ethnicities [12], [17], [24], [26] and, to our

Table I
Genotype and Allele Frequencies (%) in CAD with MI Patients and Control Subjects. P* Values were Calculated by Fisher's Exact Test for Comparison of Subjects who had the 1A/1A Genotype with those who had the 1A/1B Genotype. P* Values ≤0.050 is Considered Significant.

	Patients n (%)	Controls n (%)	P* Value
HPA-1a/1a	106(93.8)	107 (91.5)	0.335
HPA-1a/1b	7(6.2)	10 (8.5)	0.335
HPA-1b/1b	0	0	-
HPA-1b allele frequency	0.03	0.04	0.335
Total	113	117	

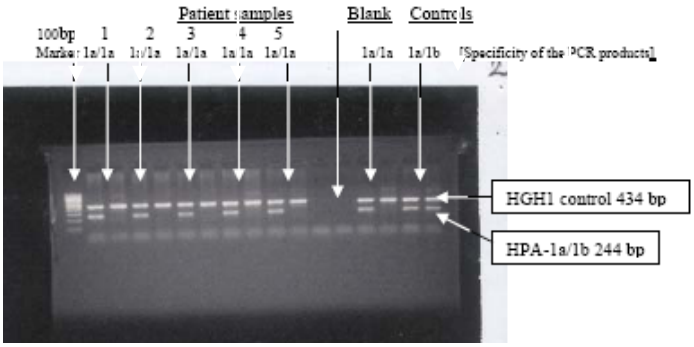

Fig. 1. Platelet alloantigen typing by PCR with allele-specific primers. Two separate PCR procedures are required to identify the 'a' and 'b' alleles of each HPA system. The specificity of the PCR (a or b) is indicated at the top of each line. The HGH1(control) PCR products (434 bp) and the allele-specific products (244 bp for HPA-1a/1b) are indicated.

knowledge, published data on a Malay population in this aspect is scarce.

The HPA-1b genotypic frequency on a Malay community in Kelantan is estimated at approximately 10% [19] amongst healthy individuals as against 8.5% in this study. As the study was restricted to a small area with limited sample size, the data for control subjects on the present study may not accurately reflect the population genetics of the Malay ethnicity in Malaysia as a whole. Nevertheless, this figure is much higher than previous reports on the other Asian ethnic groups such as the Taiwanese, Indonesians, Thais, Filipinos, Japanese and Koreans [23]. The Europeans and American Whites, on the other hand have remarkably higher gene frequencies of HPA-1b than Asians and Blacks.

We found no significant difference between the HPA-1b allele polymorphism in patients with CAD and healthy control group. Among the patients, the allele frequencies of HPA-1a and HPA-1b were 0.97 and 0.03 respectively. While among the control group, it was 0.96 and 0.04 respectively. The genotype frequencies of HPA-1a/1a, HPA-1a/1b and HPA-1b/1b were 93.8%, 6.2% and 0% respectively among the CAD group. This is somewhat similar to the non-CAD control group of 91.5%, 8.5% and 0% respectively. Thus, the allele frequencies and genotypic frequencies of the CAD group and the non-CAD group does not showed any significant difference (p=0.335).

Although the results of this study did not accord to the original findings by *Weiss et al*., (1996) and many other subsequent studies that ensued which showed an association [1]-[4], [14], [18], [31], it is interesting to note that there were an almost equal number of studies that failed to demonstrate such an association between this polymorphism and CAD as well [5], [6], [8], [9], [20]-[22].

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