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SHORT COMMUNICATION

CYP11B2 gene polymorphism among coronary heart disease patients and blood donors in Malaysia

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Abstract

Various previous studies have reported the implication of CYP11B2 gene polymorphism in the pathophysiology of cardiovascular diseases. In particular, the -344T/C polymorphism, which is located at a putative binding site for the steroidogenic transcription factor (SF-1) has been associated with essential hypertension, left ventricular dilation and coronary heart disease. In the present study, we aim to determine the allele and genotype frequencies of the CYP11B2 gene in patients with clinical manifestation of coronary heart disease and confirmed by angiography and blood donors and to calculate the association of the gene polymorphism with CHD. A total of 79 DNA from patients with coronary heart disease admitted to the National Heart Institute and 84 healthy blood donors have been genotyped using polymerase chain reaction technique followed by restriction enzyme digestion (RFLP). Results of the study demonstrated that out of 79 for the patients, 40 were homozygous T, 10 were homozygous C and 29 were heterozygous TC. The frequencies of genotype TT, CC and TC for patients were 0.5, 0.13 and 0.36 respectively. The frequencies of allele T and C in patients were 0.68 and 0.31 respectively. While for the blood donors, 40 subjects were of homozygous T, 7 were homozygous C and 37 were heterozygous TC. The genotype frequencies for the TT, CC and TC were 0.47, 0.08 and 0.44 respectively. The frequency of the allele T was 0.69 and allele C was 0.3. Chi-Square analysis showed that there was no significant difference in the genotype and C allele frequencies between the CHD patients and the blood donors. Our study suggests that there is lack of association between -344T/C polymorphism of CYP11B2 gene and coronary heart disease.

Key words: CYP11B2 gene, -344 T/C polymorphism, coronary heart disease

INTRODUCTION

Coronary heart disease is a major cause of morbidity and mortality, therefore, much effort has been focused on identifying risk factors for the disease. These risk factors include biochemical and physiological parameters as well as genetic predisposition. Aldosterone is the principal mineralocorticoid hormone which plays a major role in regulating sodium balance and volume homeostasis.[1] Aldosteron synthase is a mitochondrial cytochrome P450 enzyme, which catalyses the terminal steps of aldosteron synthesis. This enzyme is encoded by the CYP11B2 gene located on chromosome 8, band q24.3. Several common polymorphisms have been described in the promoter of the CYP11B2 gene.[2] Of these, the -344T/C polymorphism, which is located at a putative binding site for the steroidogenic transcription factor (SF-1), has been attracting interest with regards to its relation to cardiovascular diseases. Several studies have suggested that the C allele of this gene polymorphism is associated with genetic predisposition to cardiovascular diseases such as hypertension and myocardial infarction.[3,4] In the present study, we aim to investigate the -344T/C polymorphism of the CYP11B2 gene in patients with coronary heart disease admitted to the National Heart Institute and to assess whether the polymorphism is associated with genetic predisposition to coronary heart disease by comparing the allele frequencies in the patients with respective allele frequencies in healthy blood donors.

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MATERIALS AND METHODS

A total of 79 patients with confirmed coronary heart disease as documented by angiography were enrolled into the study. Eighty-four samples of healthy blood donors were obtained as controls. Informed consent were obtained from each patient.

Genomic DNA was isolated from peripheral leucocytes using the QIAamp blood extraction kit (Qiagen, Hilden, Germany). The -344T/C polymorphism of the CYP11B2 gene was determined by the analysis of restriction fragment length polymorphism (RFLP). The DNA fragment containing -344T/C of the CYP11B2 gene was amplified by polymerase chain reaction (PCR). The primers used in the PCR were 5'-CAG GAG GAG ACC CCA TGT GAC-3' (sense) and 5'-CCT CCA CCC TGT TCA GCC-3' (antisense). The PCR condition included a cycle at 95^0 C for 5 min, 35 cycles of denaturation at 94^0 C for 15s, annealing at 67^0 C for 15s, and polymerization at 72^0 C for 5 min. The amplified fragments were digested with Hae III restriction enzyme (Research Biolabs) and were subjected to electrophoresis on 2% ethidium bromide stained agarose. The uncut -344T allele had a size of 273 bp, and cut fragments (C allele) had a size of 202 bp. Results were calculated as genotype and allele frequencies. The results from the patients and healthy blood donors were analysed using the Chi-square Test for statistical significance. P value less than 0.05 was considered to be significant.

RESULTS

Of the 79 patients tested, 40 were homozygous T, 10 were homozygous C and 29 were heterozygous TC. The frequencies of genotype for TT, CC and TC for the patients were 0.51, 0.13 and 0.37 respectively. The allele frequencies for T and C in patients were 0.69 and 0.37 respectively.

For the blood donors, 40 out of 84 were homozygous T, 7 were homozygous C and 37 were heterozygous TC. The genotype frequencies for TT, CC and TC for the controls were 0.48, 0.08 and 0.44 respectively, and the allele frequencies for T and C were 0.689 and 0.31 respectively.

The observed genotype frequencies were in agreement with those expected under the assumption of Hardy-Weinberg equilibrium. Using the Chi-square Test, there was no significant difference ($P>0.05$) in the genotype and allele frequencies between the patients and the blood donors.

DISCUSSION

Kupari *et al* first reported that the -344C allele of CYP11B2 gene polymorphism was associated with left ventricular size in Finnish young adults without clinical heart disease.[5] They also reported that the -344C allele was associated with higher systolic blood pressure and increased risk of myocardial infarction especially when the C allele coexisted with habitual smoking.[5] However, conflicting results were observed in later studies.[6,7]

In the present study, no association was observed between CYP11B2 gene -344 T/C polymorphism and coronary heart disease. Moreover, the CC genotype frequencies noted in this study were lower in both patient (0.13) and control (0.08) groups compared to the respective frequencies reported for the Europeans[4,6] and the Japanese.[3] The discrepancy of results from the present study may be attributed to various factors such as the different background characteristics of the study populations, sample size and the influence of environmental factors. Furthermore, the Malaysian population is not highly homogenous ethnically, and this may also account for the difference. Further large scale study with matched patients and controls and taking consideration of potential confounding factors is needed to confirm the results.

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