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Association of *CYP2C8*, *CYP2C9* and *CYP2J2* gene polymorphisms with myocardial infarction in South Indian population

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ABSTRACT

Background: Cardiovascular diseases (CVDs) are the major cause of mortality and morbidity worldwide. Myocardial infarction (MI) is a complex multi-factorial, polygenic disorder arising from an interaction between genetic makeup of individuals and various environmental factors. *CYP2C8, CYP2C9* and *CYP2J2* gene involved in the metabolism of arachidonic acid, generates epoxyeicosatrienoic acids (EETs) that mediate dilation of coronary arteries improving post-ischemic cardiac contractile function, reduce vascular inflammation, and increase intravascular fibrinolysis. The study is aimed at analyzing the association of *CYP2C8, CYP2C9* and *CYP2J2* gene polymorphisms and MI risk in the South Indian population.
Methods: This retrospective study consisted of 287 MI patients, 279 risk control patients and 321 healthy individuals. Blood samples were collected from all the subjects and DNA was isolated using standard phenol–chloroform method. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and real time-polymerase chain reaction (RT-PCR) methods were used for genotyping. To test the potential independent association between polymorphisms and the risk of MI, Multiple-logistic regression analysis was performed.
Results: Our findings displayed a significant association between *CYP2J2*7* ($p = 0.04$; OR = 2.0) polymorphism and MI while comparing cases with to risk controls. We did not observe any association of *CYP2C8*2, CYP2C8*3, CYP2C9*2* and *CYP2C9*3* with MI.
Conclusion: Our results suggest that individuals with any conventional risk factor for MI along with *CYP2J2*7* variant allele may be predisposed to risk of MI in South Indian population.

Introduction

Cardiovascular diseases (CVDs) are the major cause of mortality and morbidity worldwide [1]. CVDs are the outcome of numerous risk factors like age, body mass index (BMI), smoking, alcohol intake, hypertension, diabetes mellitus, hypercholesterolemia, less physical activity, tobacco use, stress, low intake of fruits, vegetables and raised waist to hip ratio. Worldwide, heart attacks are the leading cause of death among men and women. World health organization reported 17.3 million cardiovascular deaths in 2008, out of which 7.3 million was due to heart attack. It is estimated that there may be 23.3 million deaths due to heart diseases by 2030 [2]. Nitric oxide (NO) also known as endothelium derived relaxing factor (EDRF) produced by the vascular endothelial cells, is a key molecule which regulates vascular tone. In addition, the arachidonic acid metabolites, epoxyeicosatrienoic acids (EETs) also known as endothelium derived hyperpolarizing factor (EDHF) formed by drug metabolizing enzymes *CYP2C8, CYP2C9, CYP2J2* isoforms in human endothelial cells, plays vital physiological roles in maintenance of vascular tone (vasodilatation), *NOS3* regulation, vascular smooth muscle migration and fibrinolysis [3–6]. Drug metabolizing enzymes (DMEs) are a

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diverse group of proteins which metabolizes vast array of xenobiotic compounds such as drugs, environmental pollutants, and endogenous compounds. Polymorphisms in *CYP2C9* are analyzed to determine the clearance of drugs such as ibuprofen, indomethacin, flurbiprofencelecoxib, valdecoxib, etc. *CYP2C8* also plays a vital role along with *CYP2C9* for the clearance of ibuprofen [7]. It is also shown that CYP2C9 and CYP2C8 are inhibited by danazol, nicardipine, ketoconazole and lansoprazole. There is also evidence that sulfamethoxazole inhibit the enzyme activities of CYP2C9 and trimethoprim inhibit the enzyme activities of CYP2C8 [8]. The activity of CYP2J2 is reduced by more than 90% by drugs such as ketoconazole, lansoprazole, loratadine, miconazole, danazole, nicardipine and verapamil [9]. As all these drugs are commonly used for ailments such as myalgia, gastritis, etc., they also have important effect on activities of these enzymes. In humans *CYP2C* and *CYP2J* gene families are expressed in the endothelium, myocardium, and kidney. Vasodilatation, anti-hypertension, pro-angiogenesis, anti-atherosclerosis are some of the cardiovascular effects of CYP epoxygenases and EETs [10]. From previous reports it is evident that *CYP2C8*, and *CYP2C9* genes are highly polymorphic in nature and their allelic variant frequencies differs based on ethnicity [11].

Human Cytochrome P450 [CYP] Allele Nomenclature Committee has so far described 14 variant alleles for *CYP2C8* gene among which *CYP2C8*2* (I269F) and *CYP2C8*3* (R139K, K399R) has shown to decrease paclitaxel 6′-hydroxylase activity [12]. *CYP2C8* metabolizes arachidonic acid to biologically active EETs (11,12-EET and 14,15-EET) and therefore influences the circulating levels of EETs [13]. Thus allelic variants of *CYP2C8* could produce an influencing pathological and physiological change in humans [14]. Human Cytochrome P450 [CYP] Allele Nomenclature Committee has so far described 58 variant alleles for *CYP2C9* gene among which *CYP2C9*2* and *CYP2C9*3* are the most common polymorphisms [15]. Both these variants are associated with decreased metabolism of *CYP2C9* substrates [16]. *CYP2J2* metabolizes arachidonic acid primarly to 11,12-EET. This eicosanoid by inhibiting endothelial nuclear factor-B, possesses a potential anti-inflammatory effect. EETs, such as 5,6-, 8,9-, and 14,15-EET, influence important vasodilatation functions effected via the mechanism of smooth muscle cell relaxation. Reports also suggest that EETs possess various other properties such as antioxidant, antiapoptotic, antimigratory and antithrombotic [17]. *CYP2C8*, *CYP2C9* and *CYP2J2* genes are involved in the pathogenesis of acute myocardial infarction (AMI) and therefore can be considered as candidate genes for MI [17,18].

The current study was designed out of our curiosity to find the association of these candidate *CYP* genes and the risk of MI among South Indian population. To the authors knowledge, the current study was the first study to evaluate the role of the allelic variants of *CYP* genes in patients with common cardiovascular diseases such as MI and hypertension. Literature surveys also showed that there are no studies done to evaluate the risk assessment of *CYP2C8*, *CYP2C9*, and *CYP2J2* on MI patients in an Indian population. Thus the aim of the present study is to find the influence of interethnic differences and the risk modifying effects of *CYP2C8*, *CYP2C9* and *CYP2J2* alleles on MI risk in Indian population.

Methods

Subjects

The retrospective study consisted of three groups. Group1 comprised of 287 MI patients (MI diagnosis was based on World Health Organization criteria), group2 comprised of 279 risk control patients who had one of the conventional risk factor for coronary heart disease (hypertension) and group3 comprised of 321 healthy individuals. All the study subjects were interviewed using standardized questionnaire to know about the status of their lifestyle, smoking, alcohol and drug intake. The study subjects were aged between 25 and 75 years of either gender and unrelated ethnic Tamilians recruited from inpatients and out patients ward of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) Hospital, Pondicherry, India. Subjects were selected from families who were residing in South India for at least three generations and spoke Tamil as their mother tongue. The Institute Human Ethics Committee approval was obtained for the study. The study procedure was explained in detail and written informed consent was obtained from all the volunteers.

Genotyping

Volunteers were requested for 5 mL of blood and were collected from antecubital vein in polypropylene tubes containing 100 μL of 10% ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The plasma was separated by centrifugation and was used for lipid profile analysis. Standard phenol–chloroform method was used to extract the genomic DNA from the peripheral leukocytes. After extraction, DNA was diluted to a concentration of 50 ng/μL and was stored at −20 °C. All the subjects were genotyped for *CYP2C8**2 and *CYP2C9*2* alleles by polymerase chain reaction–restriction fragment length polymorphism method (PCR-RFLP). Amplification was checked on 1% agarose gel. 8% or 12% polyacrylamide gels were used to identify electrophoresed enzyme-digested products. The details of the primers used for amplification, enzymes used for RFLP analysis and their band patterns are given in Table 1. Genotyping of *CYP2C8**3, *CYP2C9**3 and *CYP2J2*7* was performed by real-time PCR allelic discrimination method (Applied Biosystems, Foster City, CA, USA). The thermocycler, kits for amplification and allelic discrimination were obtained from Applied Biosystems, Foster City, CA, USA. The assay ids for *CYP2C8**3, *CYP2C9**3 and *CYP2J2*7* were C__25625782_20, C__27104892_10 and C__9581699_80, respectively.

Statistical analysis

Statistical Package for Social Sciences (SPSS windows version 16) was used for analyzing the genotype data. The demographic details with continuous variables were compared by Student '*t*' test

Table 1
Primers and restriction enzymes used for genotyping of *CYP2C8*2 and CYP2C9*2* alleles by PCR-RFLP method.

Gene	SNP	Primers	Product Size (bp)	Annealing Temperature (°C)	Restriction enzyme	Major allele (1)	Minor allele (2)
CYP2C8	805A > T	F – 5′AAAGTAAAAGAACACCAAGC3′ R – 5′AAAATCCTTAGTAAATTACA3′	167	56.9	Mbo I	69, 65, 33	98, 69
CYP2C9	430C > T	F – 5′TACAAATACAATGAAAATATCATG3′ R – 5′CTAACAACCAGACTCATAATG3′	690	53.0	Ava II	521, 169	690

F – Forward; R – Reverse; bp – base pairs.
(1) Band patterns observed under ultra violet light in the presence of wild type allele.
(2) Band pattern observed under ultra violet light in the presence of variant allele.