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CYP2C19*17 gene polymorphism–A pilot study

Parameshwara S[1]*, Manjula B[2], Gurupadappa K[3], Geetha Bhaktha[2], Ranjith Kumar GK[4]

[1] Department of Cardiology, Super-Speciality Hospital, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, India.
[2] Multidisciplinary Research Unit, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, India.
[3] Department of Biochemistry, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, India.
[4]Department of General Medicine, McGann teaching district hospital, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, India.

*Corresponding author:
Dr. Parameshwara S ,Assistant Professor,Department of Cardiology, Super-Speciality Hospital, Shimoga Institute of Medical Sciences ,Shimoga, Karnataka, India.,Mobile No.+91-9448730608
Email id: mrushivamoga@gmail.com

Abstract

Background: Cytochrome P450 2C19 (CYP2C19) play a versatile role in the metabolism of drugs. CYP2C19*17 variant allele increases the metabolic activity of the CYP2C19 resulting in ultra-rapid metabolizers phenotype in the individuals and decreases the therapeutic levels of substrate. Studies show that there are inter-ethnic differences in allele distribution.

Methods: We recruited 48 IHD patients, demographic and biochemical parameters collected and genotype for CYP2C19*17 were studied using nested PCR-restriction fragment length polymorphism (PCR-RFLP) method. Statistical analyses were carried out using SPSS software.

Results: The total allelic frequency of CYP2C19*17 was found to be 20% in our study population. Among the study subjects, 4.2% were homozygous mutant, 33.3% heterozygous and 62.5% homozygous wild genotype.

Conclusion: The frequency of CYP2C19*17 is less compared to other studies. This may be due to the small sample size and inter-ethnic differences. So further elaborate study is essential to evaluate the genotype-phenotype association and clinical utility of this variant.

Keywords: CYP2C19*17, genotype ultra-rapid metabolizers, IHD (Ischemic heart disease)

Introduction

Cytochrome P450C19 (CYP2C19) is a member of the Cytochrome P450 superfamily, which includes enzymes involved in the hepatic metabolism of xenobiotics, proton pump inhibitors and antiepileptic drugs. The CYP2C19 gene located on chromosome 10q24 and nearly 35 single nucleotide polymorphisms are known in the gene CYP2C19. The major gene polymorphism based on the substrate of the CYP2C19 gene were CYP2C19*2 (681G>A), CYP2C19*3 (636G>A) and CYP2C19*17 (−806C>T & −340C>T). CYP2C19*2 and *3 are associated with diminished enzyme activity, whereas CYP2C19*17 results in increased activity.[1]

Based on the CYP2C19 substrate metabolism the individuals classified as poor metabolizers/loss of function alleles (CYP2C19*2 and CYP2C19*3) and ultra-rapid metabolizers/gain of function allele (CYP2C19*17).[2-5]

The prevalence/incidence of CYP2C19 gene polymorphism varies worldwide due to ethnic differences and plays a major role in inter-individually in drug metabolism and response.[6] In a meta-analysis, it was reported that the highest percentage of poor metabolizers were found in India and ultra-metabolizers in Ecuadorian Mestizos and the probability of having a high frequency of non-normal predicted phenotype (CYP2C19*2, CYP2C19*3 and CYP2C19*17) in India is 80.1%.[6] Studies regarding ultra metabolizers in drug metabolism was least compared to poor metabolizer. Previous studies reported that CYP2C19*17 was associated with some adverse cardiovascular events (stent thrombosis, bleeding and high platelet reactivity) whereas other studies reported that CYP2C19*17 (−806C>T, rs12248560) is irrelevant with the clinical outcomes in cardiovascular diseases.[7-11] In the south Indian population the prevalence of CYP2C19*17 was found to be 19.2%[12] and 19.7%.[13]

Only a few studies were found in the literature regarding the ultra metabolizers in the Indian population. So, the present study was undertaken to evaluate the CYP2C19*17 gene polymorphism in ischemic heart disease (IHD) patients.

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Materials and methods

Study population: This is a cross-sectional study conducted in the tertiary health care hospital. Patients with IHD (Ischemic Heart Disease) were enrolled prospectively for genotyping of the CYP2C19*17 (−806C>T, rs12248560) polymorphisms. The patients were enrolled according to the inclusion criteria which include patients admitted to the Intensive Coronary Care Unit (ICCU) on the clopidogrel therapy with the age of >18 years and exclusion criteria which includes active neoplasm or history of neoplasm, Severe renal and hepatic insufficiency, Hemorrhagic diathesis, Hematocrit <35% or >50%, Pregnant women, Cancer patients with treatment and Concomitant GPIIb/IIIa inhibitor administration such as Abciximab, Eptifibatide, etc. Informed written consent was obtained from all the subjects before enrolment. Patient information was documented in a study proforma which included demographic data of individuals such as age, gender, body mass index (BMI) smoking, alcohol, and tobacco consumption, hypertension, type of occupation, Diabetes mellitus, family history of CVD and clinical profile.

Blood sampling: Venous blood samples (5ml) were collected in ethylene-diamine tetra- acetic acid (EDTA) vacutainer from each patient for genetic analysis.

Genetic studies: DNA was extracted from peripheral blood using the spin column kit method (HimediaHiPurA™ Blood Genomic DNA Mini Purification) and DNA yield was estimated by measuring absorbance at 260 nm using Nanodrop (Eppendorf BioSpectrometer® basic). Primers were designed using Primer3 and BLAST from National Center for Biotechnology Information (NCBI) and purchased from bioengineering (India). The forward and reverse primers for first and second PCR were as follows in 5' to 3' direction CGTTTCCCCTCTGCAGTGAT, GTTTGGAAGTTGTTTTGTTTTGCTA, TGGTGCCACACAGCTCATAG and TGGCGCATTATCTCTTACATCA. Amplification of the gene was carried out using nested polymerase chain reaction (PCR) using 2X PCR Taq Mixture (Himedia) in a thermal cycler (Biorad). For the first PCR amplification, the initial denaturation at 95^0C/10min, denaturation at 95^0C/1min, annealing at 55.3^0C/1 min and extension at $72\ ^0$C/45 sec was followed by 36 cycles with a final extension at $72\ ^0$C/5 min. For second PCR amplification, the initial denaturation at $95\ ^0$C/10min, denaturation at $95\ ^0$C/1min, annealing at $57.4\ ^0$C/1min, and extension at $72\ ^0$C/45 sec were followed by 35 cycles with a final extension at $72\ ^0$C/5 min and the PCR condition for first PCR. Restriction Fragment Length Polymorphism (RFLP) analysis for the second PCR-product was done using SfaNI restriction enzymes (New England Biolabs) to identify the genotypes of CYP2C19*17and bands were analyzed by 2% Agarose gel electrophoresis which was stained with ethidium bromide and the banding pattern was documented using Bio-Rad gel doc instrument.

Statistical analysis: The data obtained were coded and entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) (software version 22). Descriptive statistics such as frequencies, mean and standard deviation were calculated. Inferential statistics like analysis of variance (ANOVA) and Chi-square test were applied. The statistical significance was evaluated at a 5% level of significance. Allelic frequencies were calculated from genotype frequencies. Genotypes were tested for deviations from Hardy-Weinberg equilibrium.

Results

In this study, out of 48 people, 28 males and 20 females with a mean age of 56.8 ± 11.5 years. The demographic data and clinical profile of the study population as in **Table 1.** In our study population percentage of non-vegetarians, non-tobacco users, non-alcoholic and non-smokers, non-diabetic were more when compared to vegetarians, tobacco users, alcoholics, smokers, and diabetic. Risk factors such as diabetes, food habit, alcohol smoking, and tobacco consumption were not significant among the study population at a 5% level of significance (**data not shown**). Another risk factor of IHD is the lifestyle/type of work of the individuals. In this study, sedentary type lifestyle was significantly higher compared to a moderate and heavy type of occupation type. Except LDL, LDL/HDL ratio, hemoglobin, WBC and platelet count, other biochemical parameters were out of the normal range (**Table 2**).

Genetic analysis: Among the study population, the genotype frequency of wild type (CC) genotype had the highest frequency compared to heterozygous (CC) and mutant homozygous (TT) type genotype). The allelic frequencies were calculated using genotype frequencies which indicated that the wild allele (C) had a higher frequency (80%) than the mutant allele (T) (20%) in IHD patients (**Table 3**). The population was further stratified by gender and it was observed that males had a higher frequency of wild homozygous (CC), heterozygous (CT) and mutant homozygous (TT) type (33.3%, 20.8% and 4.2%) than females (29.2%, 12.5% and 0%) (**Table 4**).

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Discussion

The CYP2C19 variant CYP2C19*17 (rs12248560) is present in a promoter region of the CYP2C19 gene at the 50-flanking region of the exon 5 where cytosine (C) is replaced with thymine (T).[14-16] This replacement will modify the interaction of the transcriptional factor of the gene leads to variability.[17] This variability of the gene is associated with increase catalytic activity of the post-translation product.[14,16,18] Thus bioactivation of the drugs and their pro- drugs levels increases in the individuals and develop higher bleeding risk.[11,14,19,20] In a study with hepatic cells, the transcribe mRNA levels with the individuals heterozygous and homozygous mutant for CYP2C19*17 were found to be 1.8 and 2.9 fold higher than the homozygous wild type allele.[19] In another study, persons with CYP2C19*17/*17 exhibit 35– 40% lower omeprazole area under the plasma concentration-time curve values than the individuals having CYP2C19*1/*1, suggesting that this allele leads to an increased metabolizer phenotype and also reported that due to the increased transcriptional activity of CYP2C19*17 and electrophoretic mobility shift assays showed specific binding of human hepatic nuclear proteins to an element carrying −806T but not −806C.[15] Therefore many studies were conducted worldwide, but studies show that the incidence/prevalence of mutant was found to be less than 5% in the Asian population and is four times higher in Europeans and African populations.[21-22]

In our study population, the prevalence of the mutant homozygous was found to be 4.2% and carriers (heterozygous) were found to be 33.3% however in the South Indian Tamilian population the CYP2C19*17 allele frequency was found to be 19·2%.[12] Finally, it is still unclear that how patients with the CYP2C19 *17 allele would respond to drugs. More research should be carried out to find the protective and adverse bleeding effects of CYP2C19*17 in a large population with platelet activation and inhibition study between mutant, carriers and non-carriers.

Limitations

- It is a pilot study with a small sample size.
- It was a single-center study and hence the results cannot be generalized to the total population.

Conclusion

The frequency of CYP2C19*17 is less compared to other studies. This may be due to the small sample size and inter-ethnic differences. More research is needed to determine the CYP2C19*17 would be useful in daily clinical practice. So further elaborate study is necessary to evaluate the genotype-phenotype association and clinical utility of this variant to enhance the health of the patients.

Declarations

Conflicts of interest: The authors declare no conflict of interest.

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Table 1 Demographic features of the IHD patients

Demographic data	
Male (N, %)	28(58.3)
Female (N, %)	20(41.7)
Age (Mean ± SD)	56.8± 11.5
BMI (Mean ± SD)	25± 4.2
Vegetarians (N, %)	39(81.2)
Non-vegetarians (N, %)	9(18.8)
Tobacco user (N, %)	11(23)
Alcoholics (N, %)	13(27)
Smoking (N, %)	17(35.4)
Work type	
Moderate (N, %)	4(8.3)
Sedentary (N, %)	28(58.4)
Heavy (N, %)	16(33.3)
Diabetes (N, %)	15 (31.2)
Type of IHD	
ACS (N, %)	32(66.7)
NACS (N, %)	16(33.3)

SD: standard deviation, IHD: Ischemic heart disease BMI: Body mass index, ACS: Acute coronary syndrome, NACS:

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Table 2 Blood parameters of the IHD patients

Blood parameters (Mean ± SD)	
RBS	147.9± 96.7
Total cholesterol	212± 22
Triglycerides	231.9± 52
HDL	49.2± 8.6
LDL	114.5± 19.9
VLDL	37.1± 11.6
Cholesterol/HDL ratio	4.3± 1
LDL/HDL ratio	2.4± 0.6
CPKMB	34.1±18.3
Hemoglobin	12.4± 2.2
Total WBC Count	9675± 3078
Platelet count	2.6± 1.2

SD: standard deviation, RBS: Random blood glucose, HDL: high-density lipoproteins LDL: low-density lipoproteins, VLDL: very low density lipoprotein, CPKMB: Creatine phosphokinase-MB, WBC: white blood cells

Table 3 CYP2C19*17 genotype and allele data analysis

Genotype	**(N,%)**
Wild (CC)	30(62.5%)
Heterozygous (CT)	16(33.3%)
Mutant (TT)	2 (4.2%)
Allele	**Hardy-Weinberg equilibrium**
C (p)	0.8
T (q)	0.2

Table 4 Gender v/s CYP2C19*17 genotype analysis

Gender/genotype	**Wild**	**Heterozygous (carrier)**	**Mutant**
Female (N, %)	14 (29.2)	6 (12.5)	0 (0)
Male (N, %)	16(33.3)	10 (20.8)	2(4.2)
Total (N, %)	30 (62.5)	16 (33.3)	2 (4.2)