



Original Article

Role of cytochrome epoxygenase (CYP2J2) in the pathophysiology of coronary artery disease in South Indian population

Javeed Ahmad Tantray^{a, b}, K. Pratap Reddy^a, Kaiser Jamil^{b, *}, Shiva Kumar Yerra^c^a Department of Zoology, Osmania University Hyderabad, Telangana, India^b Dept. of Genetics, Bhagwan Mahavir Medical Research Centre, 10-1-1, Mahavir Marg, Hyderabad-500004, Telangana, India^c Department of Cardiology, Mahavir Hospital and Research Centre Hyderabad, Telangana, India

ARTICLE INFO

Article history:

Received 5 September 2018

Accepted 5 November 2018

Available online 1 December 2018

Keywords:

Coronary artery disease

Cytochrome

Expression

Cholesterol

ABSTRACT

Background: The cytochrome P-450 2J2 (CYP2J2) is known to be one of the major enzymes of epoxygenase pathway of arachidonic acid in extrahepatic tissues, which produces series of regioisomeric *cis*-epoxyeicosatrienoic acids (EETs) such as 5,6-, 8,9-, 11,12-, and 14,15-EETs. In the present study, we analyzed the impact of a genetic variant in CYP2J2 on coronary artery disease (CAD) in the Telangana region of Indian population.

Material and methods: The case–control study consisted of 100 CAD cases and 110 healthy controls. The deoxyribonucleic acid was extracted using the salting out method. Genotyping and gene expression was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism and real-time-PCR methods.

Results: In the present study, the percentage of smokers, alcoholics, hypertensive patients, and diabetics was high. Increase in fasting glucose, urea, creatinine, fasting triglycerides, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), total cholesterol/high-density lipoprotein (TC/HDL), LDL/HDL, homocysteine, and C-reactive protein levels were significantly higher in patients with CAD than in controls ($p < 0.001$). CYP2J2 G-50T was associated with CAD ($p = 0.04$). The mRNA expression of CYP2J2 showed altered gene expression in this study among CAD patients in comparison with control ($p = 0.01$).

Conclusions: A functionally relevant polymorphism of the CYP2J2 gene was independently associated with an increased risk of CAD.

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1. Introduction

Notable progresses have been observed in the treatment of coronary artery disease (CAD). Hence, CAD mortality faced a progressive decline in the past two decades. Strong case exists for the efficacy and safety of primary prevention of CAD through lifestyle changes. Further awareness and primary prevention efforts need to be extended to both public health and clinical arenas. Our previous study showed that essential changes in life leading to preventive measures include smoking avoidance or cessation, lowering alcohol consumption, modifying intakes of foods and nutrients, weight control, and physical activity.¹ Despite these progresses, incidence

of new and recurrent CAD remains elevated.² The functional role of cytochrome P-450 2J2 (CYP2J2) gene have been identified as targets for investigating the role of CYP2J2 variants in the risk of cardiovascular diseases, such as hypertension and CAD, paying particular attention to the functional polymorphisms.^{3–5} In the present study, we aim to investigate the relation between CYP2J2 gene and CAD in South Indian population.

Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid which is released from the sn2 position of membrane phospholipids by the activity of phospholipases (PLs), and among them, the role of cytosolic(c) PLA2 is noteworthy. Free AA can be metabolized to eicosanoids through three major pathways: (i) the cyclooxygenase pathway, which generates prostanoids; (ii) the lipoxygenase pathway, which generates leukotriene and hydroxyeicosatetraenoic acids; (iii) the cytochrome P450 (CYP) pathway, which includes CYP epoxygenase and CYP ω -hydroxylase enzymes. CYP epoxygenases, such as members of the CYP2C and CYP2J families, metabolize AA to four biologically active

* Corresponding author. Dept. of Genetics, Bhagwan Mahavir Medical Research Centre, Hyderabad-500004, Telangana, India. Fax: +914066631500.

E-mail addresses: javeedh3@gmail.com (J.A. Tantray), pratapkreddy@gmail.com (K.P. Reddy), kj.bmmrc@gmail.com (K. Jamil), shivakumar.yerra@gmail.com (S.K. Yerra).

epoxyeicosatrienoic acids (EETs; 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET). The CYP epoxygenase inserts an oxygen atom on a carbon atom attached to one of the double bonds of AA, and the double bond is reduced as the epoxide forms.⁶ Epoxygenase enzymes are localized in endothelial and vascular smooth muscle cells, and also in astrocytes and cardiomyocytes.^{7–9} Many evidences suggest that alteration in EET pathway contribute to the pathophysiology of hypertension, including blood pressure elevation, endothelial dysfunction, and end-organ damage. The infusion of angiotensin II, a potent vessel constrictor, elevates blood pressure in various animal models.¹⁰ Recently, a protective role of CYP2J2-derived EETs in heart failure was found.¹¹ Thus, CYP2J2-derived EETs may be a target for the development of drugs to prevent cardiac hypertrophy and cardiomyocyte apoptosis in heart failure.

2. Materials and methods

2.1. Study population

A total of 100 CAD patients as defined by angiographically documented coronary artery stenosis of >50% severity and 110 healthy controls were recruited from Mahavir Hospital and Research Centre, Hyderabad. All patients gave an informed consent that explicitly provided permission for DNA analysis and collection of relevant clinical data. Three milliliters of blood was drawn from an arm vein into a sterile tube containing ethylenediamine tetraacetic acid. At the same time, a complete clinical history, including cardiovascular risk factors, was obtained from all study patients. The study protocols were approved by the ethics committee of Bhagwan Mahavir Hospital and Research Centre, Hyderabad, India. Estimation of total cholesterol (TC),¹² triglycerides (Fossati and Lorenzo, 1982),¹³ and high-density lipoprotein (HDL)¹⁴ was also determined. Low-density lipoprotein cholesterol (LDL-C) was calculated by using the Friedewald's formula. $LDL-C \text{ mg\%} = TC - (HDL-C + VLDL-C)$. VLDL cholesterol was calculated by using following formula $mg\% = \text{Triglycerides} \times 1/5$. For blood analysis, the following cutoff values were defined as abnormal: $TC \geq 200 \text{ mg/dl}$, triglycerides $\geq 180 \text{ mg/dl}$, $HDL-C \leq 40 \text{ mg/dl}$, and $LDL-C \geq 150 \text{ mg/dl}$.

2.2. Genotype analysis

Genomic DNA was extracted using a standard salting out extraction method.¹⁵ The proximal promoter of CYP2J2 G-50T was analyzed for genetic variants by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). A 373-bp fragment of CYP2J2 G-50T was amplified by primers shown in Table 1. The CYP2J2-50T allele has an *Alu* I cleavage site, whereas the wild type CYP2J2-50G allele does not. As such, the homozygous GG genotype was detected by the presence of a single 373-bp

fragment, whereas the heterozygous GT genotype yielded three digested fragments that were 99, 274, and 373 bp in length (Fig. 1). Homozygous TT genotypes were also observed in the study cohort.

Lane 1 shows the molecular weight marker (ladder); lane 2, 3, 4 shows GG genotype; lane 5 and 7 shows GT genotype; and lane 6 shows TT genotype.

2.3. Real-time PCR

Gene expression for CYP2J2 gene was performed in 20 CAD cases and 20 controls using real-time polymerase chain reaction (RT-PCR). Total RNA was extracted using an RNeasy Mini kit (Qiagen, Germany). The concentration and purity of extracted RNA were determined by measuring the absorbance at 260 and 280 nm using a spectrophotometer. All samples with a minimum concentration of 100 ng/μL at 260/280 between 1.8 and 2 were included in the study. Reverse transcription was performed in a personal Master Cycler (Bio-Rad CFX 96), using 1 μg of total RNA in the presence of random hexamer (50 ng/μL) and reverse transcriptase (50 U/μL) in a total volume of 20 μL, including also 10 X TaqMan RTBuffer, MgCl₂ solution (25 mM), deoxyribonucleotide triphosphate (dNTPs) mixture (10 mM), an RNase inhibitor (20 U/μL), and nuclease-free water. The reaction mixture was incubated for 10 min at 25 °C, 60 min at 42 °C, heated for 5 min at 95 °C, and then at 4 °C for a minimum of 2 min. The resulting cDNA was stored at –20 °C until further use. RT-PCR was performed, with 1 μL of cDNA, 12.5 μL EvaGreen, and with specific primers (Table 1) synthesized from Bioserve Biotechnologies Ltd (Hyderabad, India): A three-step PCR was standardized using a Bio-Rad thermo cycler and carried out with initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30s, and extension at 72 °C for 45 s. A final extension at 72 °C for 5 min was carried out. Gene expression levels were normalized to the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Amplification products were visualized by ethidium bromide staining after separation by run of 2% agarose gel and the respective bands are analyzed using GelDoc.

3. Results

Clinical characteristics of the two groups are described in Table 2. The CAD patients were younger in age (mean age 48.3 ± 7.4 years vs 55.4 ± 10.5 years, p value = 0.001) and had a higher proportion of men (69% vs 56.4%, p value = 0.06) as compared with controls. Most of the CAD patients were in the age group 45–50 years or 50–55 years (34% and 25%, respectively). The onset of the disease was most commonly in the age group 41–45 years, suggesting that most of the patients develop CAD after 40 years of age.

Table 1
Primers used in the present study.

Primer sequences of CYP2J2 G-50T used for PCR-RFLP	
Forward primer	5'- TTTCTGAGACCGTGCGTG -3'
Reverse primer	5'- CAGGTGCGACTGCTCGAAG -3'
Primer sequences of the gene CYP2J2 gene used for real-time PCR	
CYP2J2: F	5TCCATCCTCGGACTCTCTAC-3
CYP2J2: R	5-GGCCCGTCTTTTGAGAAAGT-3
GAPDH: F	5-AAGGTGAAGTCGGAGTCAA-3
GAPDH: R	5-AATGAAGGGTCATTGATGG-3

PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction- restriction fragment length polymorphism.

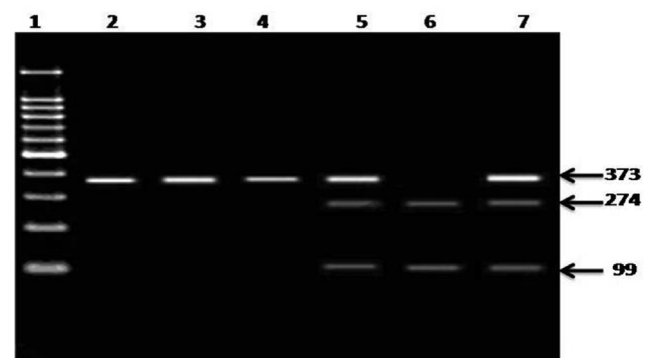


Fig. 1. Number of GG, GT, and TT in CYP2J2 G-50T gene among coronary artery disease cases and controls.

Table 2
Demographic details of CAD patients and controls of this study.

Variables	Patients with CAD (n = 100)	Patients without CAD (n = 110)	p-Value
Age (mean ± SD)	48.2 ± 7.4	55.4 ± 10.5	0.001
Male	69 (69%)	62 (56.36%)	0.06
Age male (mean ± SD)	47.3 ± 9.5	46.9 ± 9.3	0.75
Female	31 (31%)	48 (43.63%)	0.06
Age female (mean ± SD)	52.4 ± 8.7	57.3 ± 10.3	0.003
Smokers	65 (65%)	40 (36.4%)	0.001
Alcoholic	20 (20%)	5 (4.5%)	0.001
Hypertension	35 (35%)	15 (13.6%)	0.004
DM	30 (30%)	9 (8.2%)	0.001
Family history of DM	24 (24%)	13 (11.8%)	0.02

CAD, coronary artery disease; DM, diabetes mellitus; SD, standard deviation.

Compared with the controls, the CAD patients had a higher prevalence of smoking (65% vs 36.4%, p value = 0.001), alcohol use (20% vs 4.54%, p value = 0.001), hypertension (35% vs 13.6%, p value = 0.004), diabetes (30% vs 8.2%, p values = 0.001), and family history of premature CAD (24% vs 11.8%, p value = 0.02). Among CAD patients, 25% had single-vessel disease (SVD), 35% had double-vessel disease (DVD), and 40% had triple-vessel disease (TVD) (Table 3).

3.1. Studies on biochemical risk factors

Lipid levels such as TC, triglyceride, HDL-C, LDL-C, and VLDL of the study groups are shown in Table 4. The difference was significant for TC, LDL-C, and triglyceride (TG) when cases of CAD were compared with controls. TC (236 ± 15.5 vs 202 ± 10.3 mg/dL; $p < 0.0001$), LDL-C (140.8 ± 5.7 vs 102 ± 5.5 mg/dL; $p < 0.001$), triglycerides (193 ± 2.4 vs 121 ± 2.6 mg/dL; $p < 0.0001$), CHO/HDL-C ratio (3.2 ± 1.4 Vs 3.8 ± 2.2 ; $p < 0.001$), and LDL/HDL-C ratio (5.3 ± 2.3 Vs 4.2 ± 1.1 ; $p < 0.001$) were higher in patients with CAD, whereas levels of HDL-c (40 ± 2.9 vs 40.5 ± 2.8 mg/dL; $p < 0.20$) were lower. Fasting glucose levels were also higher in cases (201 ± 10.5) than in controls (122.3 ± 5.4 , $p < 0.001$). Blood urea (59.0 ± 5.6 vs 43.3 ± 2.2) and serum creatinine levels (2.0 ± 0.2 vs 1.01 ± 0.3) showed significant deviation between the two groups ($p < 0.001$).

C-reactive protein (CRP) levels were elevated in patients (Table 4). In some of the patients, the CRP levels appeared to reflect disease activity. CRP is used to gauge disease activity in several chronic inflammatory and infectious diseases. There was a significant difference in the levels of CRP between the two groups cases (7.5 ± 2.4 vs controls 6.3 ± 0.9 , $p = 0.001$).

3.2. Genomic results

The frequency of the G/G, G/T, and TT genotypes were present in 72 (72%), 21 (21%), and 7 (7%) of the 100 patients with CAD and 97 (88.18%), 12 (10.90%), 1 (0.90%) of the 110 control patients, respectively (Table 5).

Table 3
Clinical features of CAD cases on angiography.

Angiographic findings	No.	Percentage
SVD	25	25%
DVD	35	35%
TVD	40	40%
Total	100	

values are represented in numbers and percentages (%) in each age group.
CAD, coronary artery disease; SVD, single-vessel disease; DVD, double-vessel disease; TVD, triple-vessel disease.

Table 4
Biochemical Parameters in CAD patients and controls.

Parameter	CAD Cases (n = 100)	Controls (n = 110)	p value
Homocysteine (μmol/l)	19.7 ± 1.8	10.7 ± 1.50	<0.01
Serum creatinine (mg/dl)	2.0 ± 0.2	1.01 ± 0.3	<0.001
Urea (mg/dl)	59.0 ± 5.6	43.3 ± 2.2	<0.001
Fasting blood sugar (mg/dl)	201 ± 10.5	122.5 ± 5.4	<0.001
Serum cholesterol (mg/dl)	236 ± 15.5	202 ± 10.2	<0.01
LDL cholesterol (mg/dl)	140.8 ± 5.7	102 ± 5.5	<0.001
Serum triglycerides (mg/dl)	193 ± 2.4	121 ± 2.6	<0.01
HDL cholesterol (mg/dl)	40 ± 2.9	40.5 ± 2.8	0.20
Total/HDL ratio	3.2 ± 1.4	3.8 ± 2.2	<0.001
LDL/HDL ratio	5.3 ± 2.3	4.2 ± 1.2	<0.01
CRP	7.5 ± 2.4	6.3 ± 0.9	<0.001

Data are represented as mean, standard deviation, and p -value.
CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein;

Table 5 shows results for the G-50-T polymorphism, both heterozygous GT and homozygous mutant TT genotypes were more in patients than in controls; and the difference between patients and controls was statistically significant [odds ratio (OR) 2.17, 95% confidence interval (CI) 1.0–4.68, p -value 0.04 and OR 8.20, 95% CI 0.99–67.90, p -value 0.05]. Homozygous wild type GG genotypes were more in controls among CAD patients, and GG genotype may be protective for CAD (OR 0.34, 95% CI 0.16–0.71, p -value 0.004). Allele G and T in CAD patients compared with controls were statistically highly significant (OR 0.32, 95% CI 0.16–0.61, p -value 0.006 and OR 3.12, 95% CI 1.62–5.9, p -value 0.006) (Table 5).

3.3. CYP2J2 gene expression in CAD patients

The relative gene expression of CYP2J2 was quantified in 20 CAD samples by performing RT-PCR. The gene expression levels were determined as a ratio between CYP2J2 and the reference gene GAPDH to correct for variation in the amount of RNA. The relative ratio is presented as the fold change in gene expression normalized to an endogenous reference gene and relative to the control.

Among the 20 CAD samples, 10 of 20 (50%) showed CYP2J2 gene—altered expression in CAD patients, whereas all the 20 control samples showed normal expression (Table 6). In 10 samples which showed altered expression, six were upregulated and four were downregulated. The upregulation was six times higher in two samples, five times higher in one sample, three times higher in two samples, and two times higher in one sample, whereas as down-regulation showed a sixfold increase in one sample, threefold increase in one sample, and two fold increase in two samples (Fig. 2). In the present study, increased mRNA expression was defined as ≥ 2.0 folds, 'normal' expression ranging from 0.5001 to 1.9999 folds, and decreased mRNA expression was ≤ 2.0 folds. The mRNA expression of CYP2J2 was upregulated in this study among CAD patients in comparison with control. The CAD patients group is significantly different from the target gene-control group ($p = 0.01$).

3.4. Correlation between CYP2J2 G-50T and exogenous factors

CYP2J2 G-50T genotypes (GG, GT, and TT) were correlated with demographic factors such as gender, smoking, and angiographic findings to investigate the effect of genetic polymorphism in modulating the risk of developing CAD. When CYP2J2 genotypes were correlated with gender, it was noted that there was insignificant difference between men (GG, GT, and TT genotypes were in 75.36% (52 patients), 17.39% (12 patients), and 7.24% (5 patients), respectively) and women [GG, GT, and TT genotypes were in 64.51%

Table 5

Genotypic distribution of CYP2J2 G-50T polymorphism in CAD patients and controls.

Polymorphism	Genotype	CAD n (%)	Control n (%)	Odds ratio	95% CI	p-value
CYP2J2 G-50T	GG	72 (72%)	97 (88.18%)	0.34	0.16–0.71	0.004
	GT	21 (21%)	12 (10.91)	2.17	1.0–4.68	0.04
	TT	7 (7%)	1 (0.91)	8.20	0.99–67.90	0.05
	G	165	206	0.32	0.16–0.61	0.006
	T	35	14	3.12	1.62–5.9	0.006

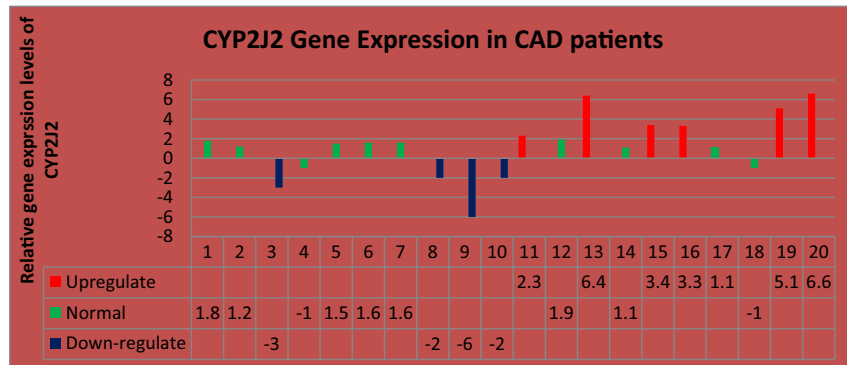
Data are represented as odds ratio, 95% CI, and p-value ($p < 0.05$ significant).
CI, confidence interval.

Table 6

Relative expression of CYP2J2 in CAD patients' and healthy patients' blood samples.

Gene expression	Cases (n = 20)	Controls (n = 20)	Odds ratio	95% CI	p Value
CYP2J2 Altered Expression	10 (50%)	0 (0%)	41.0	2.18–770.1	0.01
CYP2J2 Normal Expression	10 (50%)	20 (100%)	0.02	0.00–0.45	0.01

CYP2J2 expression was expressed as the $2^{-\Delta\Delta CT}$.
CI, confidence interval.

**Fig. 2.** Relative expression of CYP2J2 in coronary artery disease patients and healthy patients.

(20 patients), 29.03% (9 patients), and 6.45% (2 patients), respectively]. We also investigated the association between CYP2J2 genotypes versus smoking. In patients, we observed that smokers with CAD were 76.92% (50 patients), 16.92% (11 patients), and 6.15% (4 patients) with GG, GT, and TT genotypes than nonsmokers 22 patients (62.85%), 10 patients (28.57%), and 3 patients (8.57%), respectively. The association between CYP2J2 and angiographic findings showed SVD was found in 18 patients (72%), six patients (24%), and one patient (4%), DVD in 25 patients (71.42%), eight patients (22.85%), and two patients (5.71%), TVD in 29 patients (72.5%), seven patients (17.5%), and four patients (10%) with GG, GT and TT genotypes, respectively (Table 7).

4. Discussion

In the present study, we found a higher percentage of smokers, alcoholics, diabetics, and hypertensive patients. The diagnostic parameters such as fasting glucose, urea, creatinine, fasting triglycerides, TC, LDL-C, TC/HDL, LDL/HDL, homocysteine, and CRP levels were significantly higher in patients of CAD than in controls ($p < 0.001$). Hence, the present study depicts that modifiable risk factors account for much greater share of CAD, than the non-modifiable factors.

Recent data demonstrate that human CYP2J2 gene is highly polymorphic,^{16–18} and it has been proposed that genetic

Table 7

Relationship of CYP2J2 G-50T genotypes with gender, smoking, and angiographic findings.

CYP2J2 genotype	Male (n = 69)	Female (n = 31)	Statistics
GG (72)	52	20	OR 1.6, 95% CI 0.6–4.2, P 0.26
GT (21)	12	9	OR 0.5, 95% CI 0.1–1.3, P 0.19
TT (7)	5	2	OR 1.13, 95% CI 0.2–6.1, P 0.88
CYP2J2 genotype	Smokers (n = 65)	Nonsmokers (n = 35)	Statistics
GG (72)	50	22	OR 1.9, 95% CI 0.80–4.82, P 0.13
GT (21)	11	10	OR 0.5, 95% CI 0.19–1.35, P 0.17
TT (7)	4	3	OR 0.6, 95% CI 0.14–3.31, P 0.65
Angiographic findings	SVD (n = 25)	DVD (n = 35)	TVD (n = 40)
GG (72)	18	25	29
GT (21)	6	8	7
TT (7)	1	2	4

CI, confidence interval; SVD, single-vessel disease; DVD, double-vessel disease; TVD, triple-vessel disease.

polymorphisms within the gene might contribute to expression and/or activity of the enzyme and, in turn, affect the biosynthesis of EETs. Several single nucleotide polymorphisms (SNPs) have been identified within human *CYP2J2* gene; however, only one common SNP (G-50T substitution within the proximal promoter of the gene) was found to be functionally important. The G-50T substitution interrupts a critical binding site for Sp1 transcription factor and, thereby, results in decreased *CYP2J2* promoter activity *in vitro* and reduced levels of *CYP2J2* epoxygenase metabolites *in vivo*.^{17,18}

Few studies about the association between *CYP2J2* polymorphisms and the cardiovascular risk have provided inconsistent results. The study by Spiecker et al (2004) showed a functionally relevant polymorphism of the *CYP2J2* gene (rs890293) independently associated with an increased risk of CAD. This result was supported by the study of Ping Yin Liu showing the polymorphism of *CYP2J2* (rs890293) was an important risk factor for the development of MI in younger groups in Taiwanese.¹⁹ In addition, a low risk of CAD was reported by Lee in African-Americans carrying *CYP2J2* variant alleles (rs890293), but no significant association was observed in Caucasians.²⁰ Our previous study suggested that genetic testing of *CYP2C19* may help in prescribing a dose in accordance with genetic makeup and represent the initial steps toward the development of diagnostic tests and therapeutic strategies that will substantially improve human health. Minor abnormalities of chromosomal changes were also found in CAD patients taking clopidogrel drug but were not found to be the cause for CAD.²¹

The frequency of genotypes of *CYP2J2* (G-50T) such as G/G genotypes were 72% in CAD patients compared with 88% of GG in controls (Table 5), whereas the results for the G-50-T polymorphism showed both heterozygous GT and homozygous mutant TT genotypes more in patients than in controls; and the difference between patients and controls was statistically significant (OR 2.17, 95% CI 1.0–4.68, *p*-value 0.04 and OR 8.20, 95% CI 0.99–67.90, *p*-value 0.05). Homozygous wild type GG genotypes were more in controls among CAD patients; hence, it is suggested that GG genotype may be protective for CAD (OR 0.34, 95% CI 0.16–0.71, *p*-value 0.004). Allele G and T in CAD patients compared with controls were statistically highly significant (OR 0.32, 95% CI 0.16–0.61, *p*-value 0.006 and OR 3.12, 95% CI 1.62–5.9, *p*-value = 0.006) (Table 5).

Expression studies among the 20 CAD samples showed altered expression of *CYP2J2* gene (6 upregulated and 4 downregulated) in 50% of the patients, whereas all the 20 control samples (100%) showed normal expression (Table 6). This study group of CAD patients showed significantly higher expression of *CYP2J2* gene than the control group (*p* = 0.01). G-50T has been earlier shown^{17,18} downregulating the expression of *CYP2J2* gene activity. The incidence of G-T polymorphism in our cases was 21%; hence, we decided to further investigate its functional significance by qRT-PCR by taking 10 cases with this polymorphism (G-50T). The other 10 cases were other polymorphisms (GG and TT) of our cases. Our results did not show any elevation of *CYP2J2* gene with G-T polymorphism which contradicts with earlier studies.^{17,18} This difference could be due to ethnic population of India which has complex genetic diversity. However, the two polymorphisms (GG and TT) were associated with altered expression of *CYP2J2* gene; therefore, the functional investigation of these two polymorphisms needs to be explored. Overall, our results showed that these polymorphisms determine the expression of *CYP2J2* gene and plays an important role in the CAD by elevating the expression of *CYP2J2* gene.

4.1. Study limitations

This study covered a relatively small number of patients in a single center study, so future studies of larger patient populations are necessary to assess this finding.

5. Conclusion

In conclusion, we found that G-50T may be a novel polymorphism of the *CYP2J2* gene associated with CAD in Telangana's (southern region) population of India. Furthermore, *CYP2J2* could serve as a useful biomarker for early detection of CAD—which may be confirmed by using more number of samples.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ihj.2018.11.011>.

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