ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 01 | January - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ALLELIC VARIANT OF CYBA GENE (C242) IN ACUTE MYOCARDIAL INFARCTION

Cardiovascular

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ABSTRACT

Background: The polymorphism of CYBA gene may alter the risk of coronary heart disease. The aim of the present study was aimed to investigate the possible association between C242T (rs4673), a variant of the CYBA gene and its risk of developing CAD. **Methods:** As a case control study, blood samples from CAD and healthy volunteers were analyzed to determine lipids and lipoproteins level and PCR was done using Helini rs 4673 (C>T) human SNP genotyping PCR kit. **Results:** The level of cholesterol, triglyceride, LDL and VLDL were increased whereas the level of HDL was decreased in cases when compared to control group. The genotype frequency of cases was CC-0%, CT-42%, TT-58%. Controls: CC-33%, CT-22%, TT-10%. Allele frequency in cases are 20 and in controls 54. T allele frequency in cases are 76 and in controls 42. The mean TC value across genotypes, CT is 184.47mg/dl, TT-197 and CC-187.43. The mean TGL value across genotypes, CT is 193.35 mg/dl, TT-210.07 and CC-222.93. The mean HDL value across genotypes, CT is 39.09mg/dl, TT-39.10 and CC-46.75. The mean VLDL value across genotypes, CT is 38.67mg/dl, TT-42.01 and CC-44.58. The mean LDL value across genotypes, CT is 106.70 mg/dl, TT-115.87 and CC-96.10. **Conclusion:** From the above results confirmed that C242T polymorphism of the CYBA gene is a novel genetic marker for CAD subjects.

KEYWORDS

Coronary heart disease, CYBA gene, cholesterol, triglyceride, genotype

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of disability and premature death throughout the world. Globally, high proportions of deaths are due to myocardial infarction and it is estimated that more than 800,000 people suffer annually. It has been reported that 18.8% of total deaths are related to heart diseases and myocardial infarction is responsible for 59% of heart disease related deaths three quarters of it occurs in developing countries.[1]. It is a complex multi factorial and polygenic disorder, resulting from an interaction between a person's genetic makeup and various environmental factors.[2,3] There is a strong evidence, to suggest association of elevated levels of reactive oxygen species (ROSs) called oxidative stress is an important factor for CAD.[4]

Out of different types of ROS, the major sources is NADPH oxidase enzyme, which consists of several membrane bound and cytosolic proteins. One of the component proteins of the NADPH oxidase is p22phox. It is encoded by the CYBA gene that is located on chromosome 16q24 and spans 8.5 kb (6 exons and 5 introns).[5] The association between CAD risk and several polymorphic sites of the CYBA gene including C242T, C549T, A640G and promoter polymorphisms was investigated in previous studies. C242T (rs4673) is located in position 273853 of the CYBA gene's exon.[6] Single nucleotide polymorphism (SNP) involving replacement of histidine by a tyrosine at position 72, a substitution that produces a missense mutation, which leads C to T allele variant. Although there is supporting evidence which suggests that C242T can attenuate the oxidative function of NADPH oxidase, its actual role in CAD pathology remains to be elucidated.[7] The present study was aimed to investigate the possible association between C242T (rs4673), a variant of the CYBA gene and its risk of developing myocardial infarction in people attending our tertiary care hospital.

MATERIALS AND METHODS

This study was carried out during the period of six months from March 2019-August 2019. Out of 96 human subjects, 48 patients with acute coronary syndrome and 48 controls will be observed from Kilpauk Medical College Hospital, Chennai. All procedures concerning human subjects or patients were permitted by the Institutional Ethical Committee. Patients older than 40 years and younger than 70 years, subjects were complaints of chest pain within 24 hours of onset, ECG changes of ST-segment elevation, ST-segment depression, T-wave inversion, increased cardiac markers (CK-MB) 2 times the upper limit of normal are the inclusion criteria. Patients with liver, kidney disorder, chronic infection and Inflammation are the exclusion criteria. All procedures were carried out in the Department of Biochemistry and Department of Cardiology, Government Kilpauk Medical College, Chennai.

Data Collection

Informed consents were obtained from the subjects after explaining about study. The data was collected using a pre-structured performa. Baseline data including age, gender, occupation, detailed medical history, clinical examination and relevant investigations.

Blood Sample Collection

Blood samples were collected in EDTA vacutainer by venipuncture under strict aseptic precaution and used for the analysis of genetic and lipid profile. Serum was separated after centrifugation at 3000 rpm for 10 minutes and stored at -20°C and used for the analysis of total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides and very low density lipoprotein was measured on an automated platform using kit method COBAS C-311.

Genomic DNA Extraction

A total of 2 mL of a blood sample was used for DNA extraction. The DNA was extracted using HipurA Blood Genomic DNA miniprep (Himedia, India). The kit was used according to the manufacturer's instructions. DNA concentration and purity were measured using a UV-Vis spectrophotometer (Thermo Fisher Scientific, USA) and the extracted genomic DNA was used as a template and was kept at -20 °C until further use.

Polymerase Chain Reaction (PCR)

PCR was done using Helini rs 4673(C>T) human SNP genotyping PCR kit. The kit was used according to the manufacturer's instructions. The PCR products were electrophoresed with 1.5% Agarose gel in 1× TAE buffer. The gel was stained with ethidium bromide and visualised under a transilluminator.

Statistical Analysis

Results are expressed as the Mean±SD. SPSS version 21 package was used for statistical analysis.

RESULTS

Age Distribution

The age distribution of control and cases were shown in table 1. Out of cases (n=48) and controls (n=48), mean total age value of cases is 55.45 and controls is 53.52.

Table 1: Age Distribution Of Control And Cases

Group	Age in years

	Mean	SD
Cases	55.45	7.44
Controls	53.52	7.08

Gender Distribution

The gender distribution of control and cases were shown in table 2. Out of cases (n=48) and controls (n=48), 37 males and 11 females are in cases and 34 males and 14 females are in controls.

Table 2: Gender Distribution Of Control And Cases

Sex	Cases	Controls
Male	37	34
Female	11	14

Baseline Characteristics Of The Two Groups

The baseline characteristics of control and cases were shown in table 3. Out of 48 cases and controls, 9 persons in cases presented with family history (H/O) of coronary heart disease (CAD) and 16 in controls, which is statistically insignificant (p= 0.104). In cases 16 with history of diabetes mellitus (DM) and 20 in controls, which is statistically insignificant (p= 0.3). In cases 15 with history of hypertension (HT) and 16 in controls which is statistically insignificant (p= 0.827). 23 are smokers in cases and 27 in controls which is statistically insignificant (p= 0.414). 22 are alcoholics in cases and 28 in controls, which is statistically insignificant p= 0.220 (NS).

Table 3: Baseline Characteristics Of Control And Cases

Variable		Group	No of persons	P Value
Family H/O CAD	Cases (48)	YES	9	0.104 (NS)
		NO	39	
	Controls (48)	YES	16	
		NO	32	
H/O DM	Cases (48)	YES	16	0.3 (NS)
		NO	32	
	Controls (48)	YES	20	
		NO	22	
H/O HT	Cases (48)	YES	15	0.827(NS)
		NO	33	
	Controls (48)	YES	16	
		NO	32	
H/O Smoking	Cases (48)	YES	23	0.414(NS)
		NO	25	
	Controls (48)	YES	27	
		NO	21	
H/O Alcohol	Cases (48)	YES	22	0.220(NS)
		NO	26	
	Controls (48)	YES	28	
		NO	20	

Estimation Of Lipids And Lipoproteins Level

The mean cholesterol, triglyceride, HDL, VLDL and LDL of control and cases were depicted in table 4. The mean total cholesterol in cases was 201.44 mg/dl whereas in control mean total cholesterol was 172.08 mg/dl, which is statistically significant (p=0. 0028). The mean triglyceride level in cases was 222.47 mg/dl whereas, in control mean triglyceride level was 181.73 mg/dl, which is statistically significant (p=0. 046). The mean HDL in cases was 37 mg/dl whereas in control mean HDL was 43.75 mg/dl, which is statistically significant (p=0. 001). The mean VLDL in cases was 44.49 mg/dl whereas in control mean VLDL was 37.46 mg/dl, which is statistically significant (p=0. 045). The mean LDL in cases was 119.90 mg/dl whereas in control mean LDL was 90.87 mg/dl, which is statistically significant (p=0.004).

Table 4: Estimation of lipids and lipoproteins level of control and cases

Parameters	Cases	Controls	P value	Significant/non-significant
Mean±SD				
Total cholesterol	201.44±52.24	172.08±40.85	0.0028	Significant
Triglyceride	222.47±87.38	181.73±97.34	0.046	Significant
HDL	37±7.72	43.75±6.46	0.001	Significant
VLDL	44.49±17.47	37.46±19.46	0.045	Significant
LDL	119.90±53.04	90.87±44.05	0.004	Significant

Genotype Distribution Of CYBA Gene (c242t) (exon 4)

Table 5 shows the genotype distribution of CYBA gene of control and cases. The genotype frequency was cases: CC-0%, CT-42%, TT-58%. Controls: CC-33%, CT-22%, TT-10%. This was found to be in Hardy Weinberg equilibrium.

Table 5: Genotype distribution of CYBA gene of control and cases

Genotype	Cases	Controls
CC	0	16(33%)
CT	20 (42%)	22(46%)
TT	28(58%)	10(21%)
P value - 0.001		
Kruskal Wallis Test		
Significant		

Allele Frequency

Allele frequency of control and cases were illustrated in table 6 and Figure 1. C allele frequency in cases are 20 and in controls 54. T allele frequency in cases are 76 and in controls 42.

Table 6: Allele Frequency Of Control And Cases

Allele	Cases	Controls
C	20	54
T	76	42
	Among cases	Among controls
T allele frequency	79.00%	44%
Chi square test		
P value < 0.001		

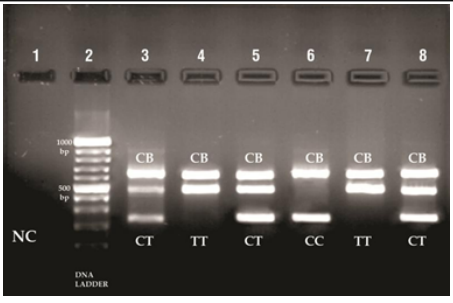

Figure 1: PCR products of cases and controls under UV illumination
C allele: PCR product size: 229bp
T allele: PCR product size: 454bp
Control PCR product size: 683bp

Comparison of serum TC, TGL, HDL, VLDL and LDL levels with different genotypes

Table 7 shows that comparison of lipids and lipoproteins level with different genotypes. The mean TC value across genotypes, CT is 184.47mg/dl, TT-197 and CC-187.43, which is statistically insignificant (p=0.482). The mean TGL value across genotypes, CT is 193.35 mg/dl, TT-210.07 and CC-222.93 which is statistically insignificant (p= 0.515). The mean HDL value across genotypes, CT is 39.09mg/dl, TT-39.10 and CC-46.75, which is statistically significant (p= 0.002). The mean VLDL value across genotypes, CT is 38.67mg/dl, TT-42.01 and CC-44.58, which is statistically insignificant (p= 0.515). The mean LDL value across genotypes, CT is 106.70 mg/dl, TT-115.87 and CC-96.10 which is statistically insignificant (p= 0.351).

Table 7: Comparison Of Lipids And Lipoproteins Level With Different Genotypes

Variables	Group	Mean	SD	P Value
Serum total cholesterol	CT	184.47	40.68	0.482(NS)
	TT	197	56.4	
	CC	187.43	36.76	
Triglycerides	CT	193.35	72.52	0.515(NS)
	TT	210.07	56.4	
	CC	222.93	140.20	
HDL	CT	39.09	7.3	0.002(S)
	TT	39.10	8.5	
	CC	46.75	5.6	
VLDL	CT	38.67	14.50	0.515(NS)
	TT	42.01	18.38	

	CC	44.58	28.04	
LDL	CT	106.70	41	0.351(NS)
	TT	115.87	55.44	
	CC	96.10	38.34	

DISCUSSION

The hypothesis in the current study is to establish the relationship between polymorphisms C242T (Exon 4) gene with lipid profile and occurrence of MI in our study population NADPH oxidase is the only enzyme whose sole function is to generate ROS, and it has been considered as the most important source of ROS in vascular wall and inflammatory cells. Most established risk factors for cardiovascular disease have been association with overproduction of ROS.[8,9] Moreover, ROS and NADPH oxidase have been implicated in numerous cell processes and pathogeneses associated with atherosclerosis. The association of the CYBA (C242T) rs4673 gene polymorphism with CAD has been widely studied with conflicting results.[10,11] Differences in the genetic background of the study populations, gene environment interaction, genetic heterogeneity, the study design and the statistic methods used might contribute to the disparate results. p22 phox, a heme-binding protein, contains two histidine residues at amino acids 72 and 94, respectively, and these are the potential heme-binding sites. The single nucleotide change at -72bp, where tyrosine substitutes the histidine residues, might modulate the activity and regulation of NADH/NADPH oxidase, which in turn regulates ROS production, influencing the progression of cardiovascular disease.[12,13]

This study was aimed to look for individuals with the single nucleotide change at the above mentioned site who are likely to have an increased risk of developing MI. The study group included 48 patients, who had documented MI, and the control group included 48 age, gender and risk factor matched groups. Fasting serum levels of TC, TGL, LDL, VLDL and HDL were estimated. TC, TGL, LDL, VLDL level was significantly higher in cases compared with controls. Serum HDL level was significantly lower in the cases than in the control group are consistent with the findings of various other studies, case-control studies in India have reported strong association of high total cholesterol, low HDL cholesterol and raised triglycerides with CHD or acute myocardial infarction.[14] Another case control study of premature CHD in Rajasthan (cases 165, controls 199) used multivariate logistic regression (odds ratio, 95% confidence intervals) to identify risk factors of importance. It was reported that smoking (19.41, 6.82–55.25), hypertension (8.95, 5.42–14.79), high LDL cholesterol (2.49, 1.62–3.84), high triglycerides (3.62, 2.35–5.59), high total:HDL cholesterol (3.87, 2.35–5.59), high fibrinogen (2.87, 1.81–4.55) and high homocysteine (10.54, 3.11–35.78) low HDL cholesterol (10.32, 6.30–16.91) were significant factors.[15,16]

The comparison of genotypes across the cases and controls reveals that TT homozygous genotype was higher among cases and CC genotype was seen more in controls, CT more or less equal among cases and controls. Genotype difference between cases and controls were statistically significant (p - 0.001). As far as frequency, T allele was higher among cases (0.76) as compared to controls (0.42) and frequency of C allele was higher among controls (0.54) as compared to cases (0.20). Such findings were in concordance with studies conducted in Italians 128 populations where they studied 494 individuals (276 patients/218 controls) and observed a significantly increased risk of developing CAD in subjects carrying a T allele of rs4673 ($p<0.01$). Cai and Harrison[17] found that the 242T allele was a modest risk factor for the presence of angiographically defined CAD in young (45 years of age) Australian Caucasian patients but not in the overall population. Another large prospective study shows that the 242T allele of the CYBA gene is associated with the progression of CAD, as determined by serial quantitative coronary angiography, in the LCAS population. [18,19] This study is in discordance to some previously reported data particularly studies carried out in Japan 122, china 129. Li et al.,[20] found that the frequency of the C242T mutation was similar in 149 subjects with CAD and 103 subjects without significant CAD but chest pain. The presence of the C242T allelic variants in the 98 study subjects had no effect on the mean serum levels of LDL cholesterol, total cholesterol, triglycerides. But had significant effect on mean serum HDL cholesterol ($p = 0.002$).[21]

CONCLUSION

The present study was done to find an association between CYBA gene polymorphism, lipid profile with myocardial infarction. The prevalence of the TC, TT genotype of the C242T polymorphism of the CYBA gene in CAD patients was significantly more frequent than that in control patients. To confirm that this polymorphism is a novel genetic marker for CAD subjects, investigations in a larger population and other ethnic populations are necessary.

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