Gstp1 Gene Polymorphism and Oxidative Stress in Coronary Artery Disease

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Abstract

Objectives: The primary purpose of this study is to analyze single nucleotide polymorphism (SNP) rs1695 of GSTP1 and oxidative stress in Coronary artery disease (CAD) and its association with CAD. **Experimental Design:** The study involved 50 CAD subjects as a case and 30 healthy subjects as control. Venous blood was drawn to analyze oxidative stress markers and antioxidants. For the genetic analysis of SNPs of GSTP1, genomic DNA was extracted, amplified by a polymerized chain reaction and detected by sequencing reaction. **Result**: Conventional risk factors, elevated MDA levels, and low levels of antioxidants were significantly ($p < 0.000$) associated with CAD. **Conclusion:** Present study could not observe a significant association of *GSTP1* gene SNP with CAD and traditional risk components of CAD. However, there was no significant difference in allele (A and G) frequencies, and genotype ('AA',' AG' and 'GG') frequencies between cases and control groups studied. Present data of the South-Indian Tamil population agrees with the allele and genotype frequencies of the other South Asian people.

Keywords: Coronary artery disease, *GSTP1* gene, SNP polymorphism, oxidative stress, Conventional risk factors

Introduction

"Coronary artery disease (CAD) is the most frequent kind of cardiovascular disease" [1]. It has complicated pathogenesis that includes a variety of lifestyle and environmental factors. Some risk factors for CAD are modifiable, such as hypertension (HTN), hyperlipidemia, smoking, diabetes, obesity, lack of physical exercise, bad diet, and stress. In contrast, others, including age, sex, and family history, are non-modifiable. "In addition to these risk factors, oxidative stress-mediated by reactive oxygen species (ROS) has been implicated in the aetiology of CAD in several experimental and clinical studies" [2]. Cardiovascular disease is thought to develop due to an imbalance between the generation of reactive oxygen species (ROS) and the intrinsic antioxidant defence system, resulting in oxidative stress. "Natural antioxidants such as vitamin A, ascorbic acid (vitamin C), and alpha-tocopherol (vitamin E), as well as antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, help to maintain this balance" [3]. Endothelial dysfunction and atherosclerosis can be caused by increased lipid peroxidation or depletion in the antioxidant defence caused by metabolic disorders or a poor lifestyle.

CAD is a complicated polygenic disease, and multiple gene variations are thought to influence genetic risk. "Individual genetic variants in genes may play a minor role in illness pathogenesis, but the cumulative effect of multiple polymorphisms is likely to be more relevant in disease pathogenesis" [4]. "Glutathione S-transferases (GST) are phase-II detoxification enzymes found in the mitochondria and cytoplasm that play a key role in conjugating electrophilic substances (xenobiotics and endogenously produced oxidative stress products) with glutathione to reduce oxidative stress and protect cells" [5]. Reduced GST activity can increase susceptibility to oxidative stress, increasing exposure to inflammatory disorders such as CAD. GSTs are thus regarded as one of the essential defence mechanisms against the negative consequences of oxidative stress.

The glutathione S-transferase P1 (GSTP1) gene is 2.8 kilobytes in length and has seven exons. It is located on chromosome 11's long arm (11q13.3). "Exon 5 (rs1695) of GSTP1*B and exon 6 (rs1138272) of GSTP1*C have genetic polymorphisms, but GSTP1*A is the wild type. The rs1695 SNP is found in exon 5 of the GSTP1 gene and is a functional SNP. An A–G substitution causes GSTP1*B at position 313 in exon 5, which results in the amino acid isoleucine being replaced by valine at position 105. (Ile105Val)" [6]. Genetic variations influence individual vulnerability to CAD in the GST genes, which result in a virtual lack of enzyme function.

Because genetic background influences susceptibility to oxidative stress, there has been much interest in finding new genetic variants that can be used as oxidative stress markers and can help predict the risk of oxidative stress-related disorders. As a result, the current study examines the significance of GSTP1 gene polymorphism in CAD in the South Indian population. The association of genetic polymorphism in *GSTP1* in patients with CAD is not known in the ethnic community of the South Indian population. Hence the primary purpose of this study is to analyze single nucleotide polymorphism rs1695 of GSTP1 and its association with CAD in the South Indian Population. We also attempted to compare the present data of the South-Indian Tamil population with the allele and genotype frequencies of the other world populations. This study also aimed to detect the linkage of oxidative stress and antioxidants with CAD and also with GSTP1gene polymorphism rs1695 in CAD.

Materials and Methods

The Research Ethics Committee accepted the research procedure of the Saveetha Medical College, Tandalam, Chennai. All subjects and control individuals provided written informed consent. The study involved 50 subjects diagnosed with coronary artery disease who are recruited based on the patient directory of the Institute. Clinically well-characterized cases were chosen from the inpatients and outpatients registered at the hospital. The control group consisted of 30 healthy adults who were age and sex-matched. Detailed clinical and other relevant data were recorded using proforma. Five millilitres of venous blood was drawn from each subject, and the subsequent investigations oxidative stress marker MDA and antioxidants SOD, GST and vitamin E were performed using commercially available kits.

Following procedures were done for genetic analysis of SNPs of GSTP1.

1. "Isolation of Genomic DNA- The genomic DNA was isolated using the QIAamp DNA Blood Midi Kit from 2ml of peripheral blood samples" (QIAGEN).

2. Quantification of the isolated DNA -All the isolated DNA samples were quantified in Fluorometer. The concentration of the samples varied from 23ng/ul to 66ng/ ul.

3. Amplification of the SNP region- The region is harbouring the variant rs1695 was successfully amplified through a polymerase chain reaction in a thermal cycler (Eppendorf) using the custom-designed primers. The primers 5'ACCCCAGGGCTCTATGGGAA3' and 3'TGAGGGCACAAGAAGCCCCT5" has amplified a 176bp product. All 80 samples were successfully amplified. A representative gel image is given in Fig.1.

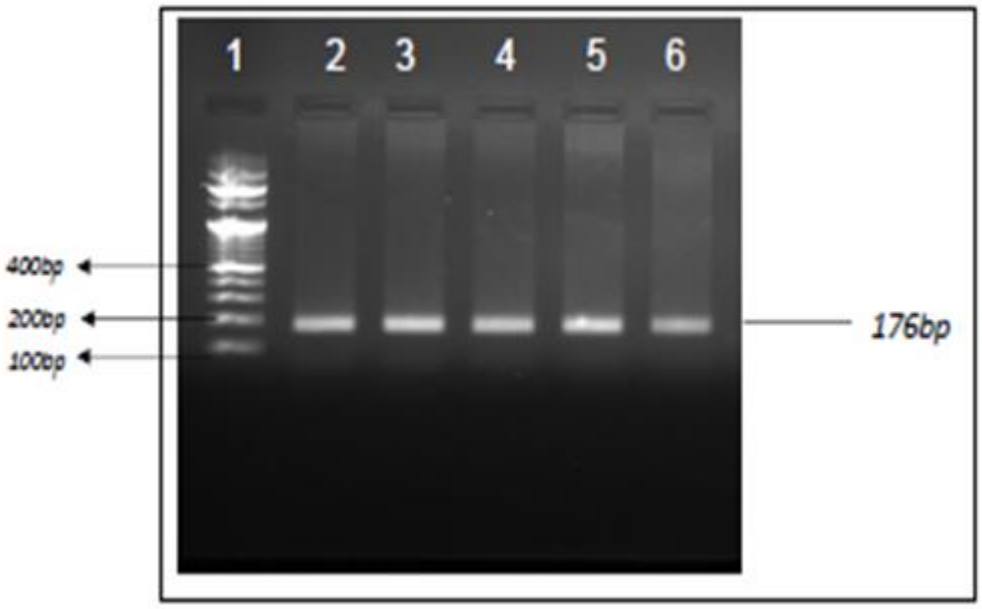


Figure 1. PCR amplification of the 176bp fragment of the *GSTP1* harbouring the rs1695 polymorphism

4. The rs1695 SNP Polymorphism- The rs1695 is a functional SNP located within the exon 5 of the *GSTP1* gene. It is an A to G non-synonymous polymorphism that leads to a change in the amino acid sequence in the final protein product, Isoleucine to Valine, at position 105 of the protein.

5. Sequencing -After the PCR, the product was purified, as mentioned in the materials and methods section. Sequencing PCR was carried out using this purified product, and finally, a sequencing reaction was performed in a genetic analyzer. Representative sequence images showing the polymorphic region is given in figures 2,3 and 4.

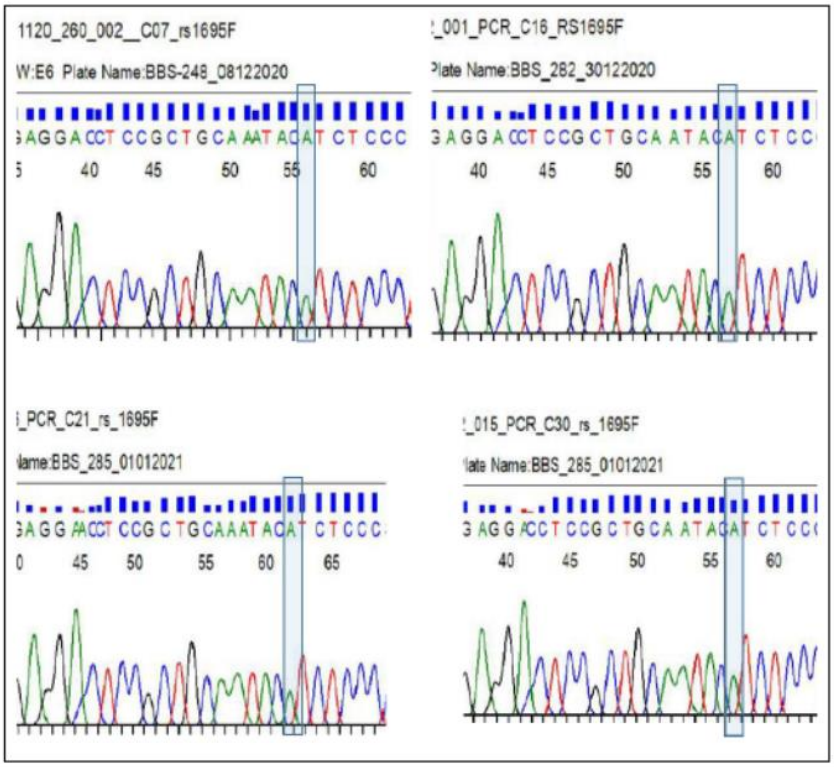


Figure 2. The genotype 'AA' as shown by sequencing in four representative samples

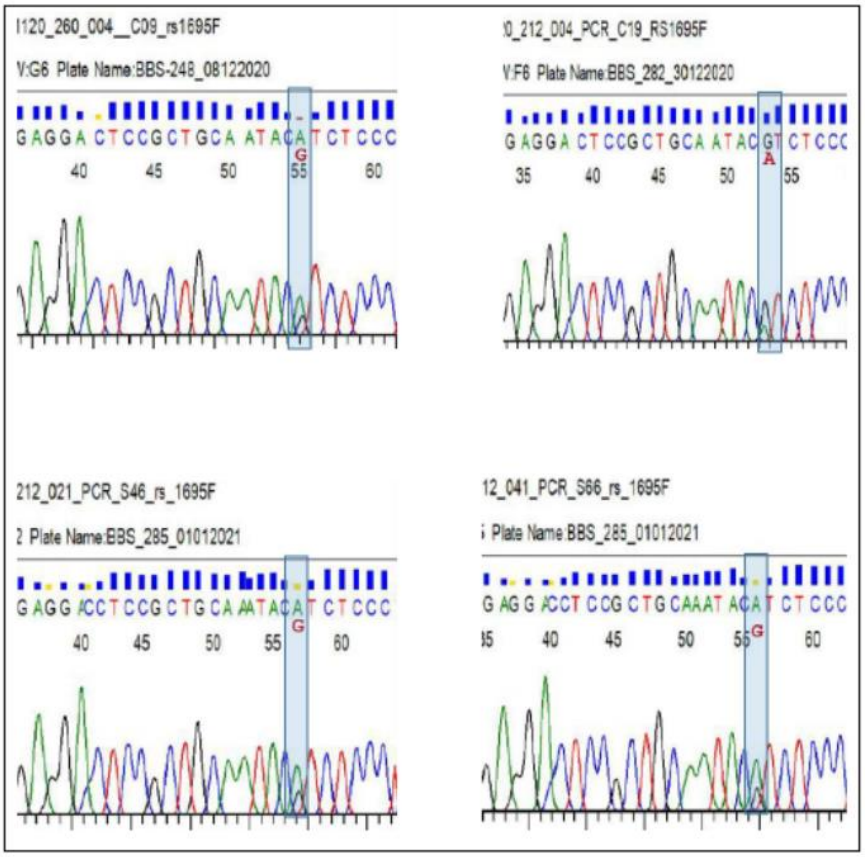
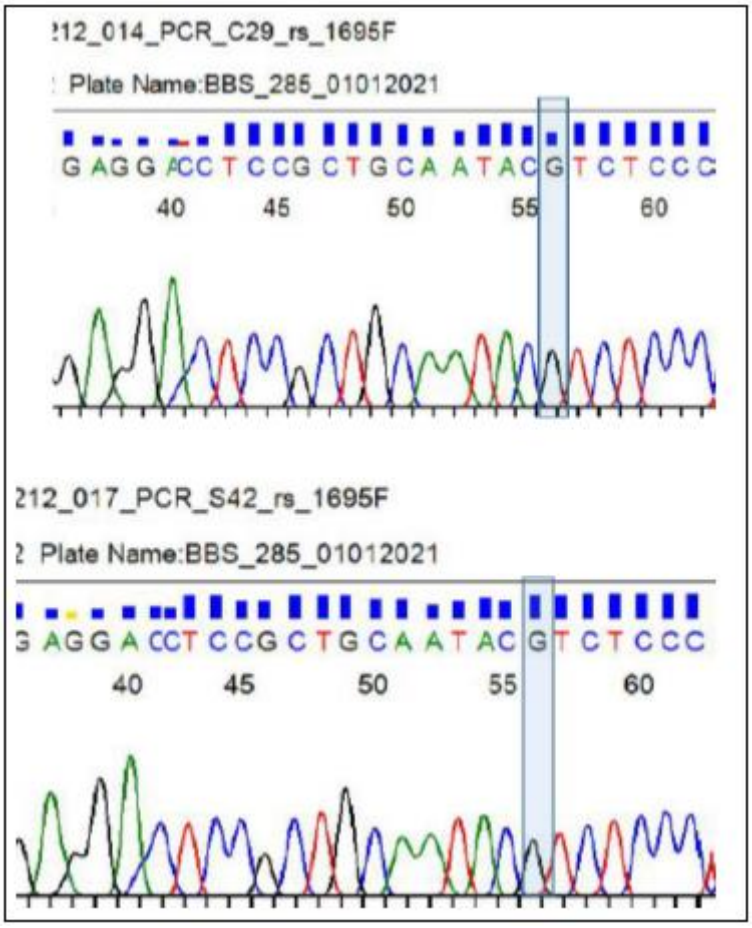


Figure 3. The genotype 'AG' was shown by sequencing in four representative samples.

Figure 4. The genotype 'GG' is shown by sequencing. This genotype is seen in only two samples.



Results

Relationship of typical risk components and oxidative stress with CAD

Association of conventional risk factors of CAD such as age more than 50 years, smoking, alcoholism, Family History of CAD, Diabetic Mellitus and hypertension with CAD were analyzed. The results are given in table no 1. Smoking, alcoholism, Family History (FH) of CAD, Diabetic Mellitus and hypertension were found to be significantly associated with CAD, whereas age more than 50 years was found to be not associated with CAD.

Table 1. Association of conventional risk factors of CAD with CAD

Risk factors		Control (N=30)	Test (N=50)	Chi-Square	Df	P-Value
Age	<=50 Yrs	19	24	1.773	1	0.183
	>50 Yrs	11	26			
Smoking	No	26	28			
	Yes	4	22	8.038	1	0.005
Alcoholism	No	24	27	5.485	1	0.019

	Yes	6	23			
Family History of CAD	No	30	29			
	YES	0	21	17.085	1	0.000
Diabetic	No	30	27			
	YES	0	23	17.085	1	0.000
Hypertension	No	24	16			
	YES	6	34	7.11	1	0.008

Association of oxidative stress marker MDA and antioxidants like SOD, GST and Vitamin E with CAD was calculated using chi-square test, and the results are given in table no 2. High levels of MDA more than 4.65 nmol/mL, low levels of SOD activity that is less than 165 U/L, low levels of GST activity that is less than 11.4 ng/ml and low levels of Vitamin E less than 17 nmol/mL were found to be significantly ($p < 0.05$) associated with CAD.

Table 2. Association of MDA, SOD, GST and Vitamin E with CAD

Parameter		Control (N=30)	Test (N=30)	Chi-Square	Df	P-Value
MDA(nmol/ml)	<=4.65	23	0	53.801	1	0.00
	>4.65	7	50			
SOD(U/L)	<=165	17	48	19.041	1	0.00
	>165	13	2			
GST (ng/ml)	<=11.4	1	12	5.884	1	0.015
	>11.4	29	38			
VITAMIN E(nmol/ml)	<=17	1	45	57.63	1	0.00
	>17	29	5			

Genotype Study of rs1695 polymorphism

Of the 50 patient samples checked for rs1695 polymorphism, 28 were found to be homozygous with 'AA' genotype; 21 were heterozygous 'AG', and only 1 was 'GG'. Among the 30 control samples, there were 18 'AA', 11'AG' and 1 'GG' genotypes. The genotype and allele frequencies are given in Table-3.

Table 3- Genotype and allele frequencies (in brackets) of rs1695 SNP polymorphism in case and control groups.

rs1695	N	Genotypes			Alleles	
		AA	AG	GG	A	G
Control	*30*	18 (0.60)	11 (0.37)	1 (0.03)	47 (0.78)	13 (0.22)
Cases	*50*	28 (0.56)	21 (0.42)	1 (0.02)	77 (0.77)	23 (0.23)
Total	*80*	46	32	2	124	36
		-0.58	-0.4	-0.02	-0.78	-0.22

However, there was no substantial difference between allele frequencies (p= 0.85) and genotype frequencies (p= 0.85) between cases and control groups studied. In SNP polymorphism rs1695, when we consider all the studied population worldwide, 'G' is the minor allele with a frequency of 0.35 as per the 1000 Genomes Project, a collaborative multinational endeavour to create the most comprehensive record of human genetic variation. Present data of the South-Indian Tamil population agrees with the allele and genotype frequencies of the other South Asian people. The closest one showed a similar allele and genotype frequency among world populations as the present Tamil population is the Bengali population (Table- 4).

Table- 4. Allele and genotype frequencies of rs1695 in Tamil population, in comparison with the other world populations. The closest population was found to be Bengali.

Population	Allele Frequency		Genotype Frequency		
	A	G	AA	AG	GG
African	0.52	0.48	0.27	0.51	0.22
Latin American	0.52	0.48	0.28	0.48	0.24
East Asian	0.82	0.18	0.67	0.3	0.03
European	0.67	0.33	0.45	0.45	0.1
South Asian	0.7	0.3	0.51	0.39	0.1
Bengali	0.78	0.22	0.61	0.35	0.04
Tamil (Present study)	0.78	0.22	0.58	0.4	0.02

Discussion

This study analyzed the association of conventional risk factors of CAD, oxidative stress and antioxidants with CAD. The essential element in developing coronary heart disease and death once coronary atherosclerosis has manifested is age [7]. "Pathways involving oxidative stress and mitochondrial function, genomic stability and epigenetic modifications, lipid metabolism, extracellular matrix, coagulation/hemostasis, inflammation, and endothelial homeostasis are thought to influence vascular ageing" [8]. There was no significant connection observed between ages more than 50 years with CAD in this research.

Several possible mechanisms seem to have a role to play in the effects of smoking on atherosclerosis progression. Inflammation, endothelial dysfunction, decreased insulin sensitivity, and lipid abnormalities are some of the mechanisms involved. "A pro-oxidative environment is created by free radicals and oxidants contained in cigarette smoke, as well as endogenously produced oxidants and radicals (coming from the smoke chemical-induced modification in the cellular redox system" [9]. "Diabetes mellitus is linked to a higher risk of cardiovascular death and the occurrence of cardiovascular illnesses such as coronary artery disease (CAD) and congestive heart failure" [10]. One of the most well-known risk factors, hypertension, has been linked to an elevated risk of coronary artery disease in various groups.

Cigarette smoking, hypertension, a familial history of CAD, and diabetes have been highly associated with CAD, and our results corroborate those of previous research. [11 & 12]. When antioxidant capacity is insufficient to eliminate reactive oxygen species and other free radicals, oxidative stress in the cardiovascular system might ensue. The aetiology of atherosclerosis and incident coronary artery disease has been linked to oxidant stress. "Several studies reported an increased level of MDA in CAD patients compared with healthy control groups" [13]. "Higher consumption of vitamin E intake from food or supplements was linked to a lower risk of cardiovascular disease in several observational studies" [14]. Superoxide dismutase gene expressions were decreased in CAD patients compared to controls; "GST is a large family of detoxifying enzymes that catalyzes the conjugation of glutathione to a wide variety of chemicals, therefore reducing oxidative stress" [15].

The current investigation examined the GSTP1 single nucleotide polymorphism rs1695 and its relation with CAD in the South Indian population. In this study, there was no measurable difference in allele and genotype frequencies between patients and controls. We also attempted to compare the present data of the South-Indian Tamil population with the allele and genotype frequencies of the other world populations. Current data of the South-Indian Tamil population agrees with the allele and genotype frequencies of the other South Asian people.

Endothelial cells produce more ROS when they are uncovered to risk factors for atherosclerosis and coronary artery disease. GSTP1 is an antioxidant and a glutathionylation enzyme that plays a crucial function in the cellular redox state's maintenance. GSTP1 is required to activate peroxiredoxin VI (Prdx6), a member of the antioxidant enzyme family that catalyzes lipid peroxide detoxification, particularly in biological membranes. "GSTP1 is a gene found on chromosome 11q13 that is expressed in normal epithelial cells, such as those

found in the cardiovascular system" [16]. "An A to G transition in codon 105 of GSTP1 enzyme leads to the substitution of isoleucine (Ile) to valine (Val) amino acids (rs 1625)" [17]. When compared to the wild-type, this mutation affects enzyme activity. GSTP1 catalytic activity is influenced by amino acid 105, located near the enzyme's active site.

"Changes in GSTP1 catalytic activity may hasten disease progression in carriers of the GSTP1 variant genotype, resulting in various cellular responses such as DNA synthesis, transcription factor activation, and protein expression changes" [18]. A study in heart failure (HF) patients due to CAD found that "GSTP1 allele carriers might have a higher antioxidant potential to provide a favourable environment for a better prognosis. GSTP1 Val allele might significantly contribute to the decreased antioxidant capacity of HF patients" [19]. In this present study, we could not observe a significant association of *GSTP1* gene SNP polymorphism with coronary artery disease and with any of the conventional risk factors of CAD.

There could be a few reasons for the lack of association of *GSTP1* gene SNP polymorphism with coronary artery disease in the present study. At first, the disease could be multifactorial, and the causes may exist very individually. The *GSTP1* related pathways could be only one reason for the illness. The causes could be several others in our samples. Secondly, the genetic risk factors could be population specific. Positive findings in one population may not get replicated in another. Finally, our sample size selected for the study (30 control and 50 cases) was relatively minor. This may not be enough to attain sufficient statistical power. A more detailed analysis with higher sample size is required to obtain better and conclusive results.

Conclusion

The present study observed that the well-known CAD risk components such as smoking, HTN, family history of CAD and diabetes were strongly correlated with CAD. The present study could not observe a significant association of *GSTP1* gene SNP polymorphism with coronary artery disease and any of the conventional risk factors of CAD. Current data of the South-Indian Tamil population agrees with the allele and genotype frequencies of the other South Asian people.

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