RESEARCH ARTICLE

Glutathione S-Transferase P1 Gene Polymorphisms and Susceptibility to Coronary Artery Disease in North Indian Population sub-group

BHAT, M.A* AND GANDHI, G

Department of Human Genetics, Guru Nanak Dev University, Amritsar-143005, Punjab, India

Short Title: Association of GSTP1 gene polymorphisms with CAD

***Corresponding author:**

Dr. Mohd. Akbar Bhat
Presently at Multidisciplinary Research Unit
Government Medical College,
Amritsar-143001, Punjab, India
E-mail: akbargenetics@gmail.com

Abstract

Aims: The present study aimed to investigate the association of g.313A>G and g.341C>T polymorphisms of *GSTP1* with coronary artery disease (CAD) in a North Indian population sub-group. *Methods*: In the present case-control study, CAD patients (n=200) and age-, sex- and ethnicity-matched healthy controls (n=200) were genotyped for polymorphisms in *GSTP1* using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. *Results*: Genotype distribution of g.313A>G and g.341C>T polymorphisms of *GSTP1* gene were significantly different between cases and controls (p=0.005 and p=0.024, respectively). Binary logistic regression analysis showed significant association of A/G (OR: 1.6, 95% CI: 1.08-2.49, p=0.020), G/G (OR: 3.1, 95% CI: 1.41-6.71, p=0.005) genotypes of *GSTP1* g.313A>G and C/T (OR: 5.8, 95% CI: 1.26-26.34, p=0.024) genotype of *GSTP1* g.341C>T with CAD. The A/G and G/G genotypes of g.313A>G and C/T genotype of g.341C>T conferred 6.5-fold increased risk for CAD (OR: 6.5, 95% CI: 1.37-31.27, p=0.018). Moreover, the recessive model of *GSTP1* g.313A>G is the best fit inheritance model to predict the susceptible gene effect (OR: 2.3, 95% CI: 1.11-4.92, p=0.020). *Conclusions*: Statistically significant association of *GSTP1* g.313A>G (A/G, G/G) and g.341C>T (C/T) genotypes with CAD was observed.

Keywords

Glutathione S-transferase, *GSTP1*, Single nucleotide polymorphisms, Coronary artery disease

Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality world wide (Topol *et al*., 2006) and, has become a major public health burden in India. Complex interplay of environmental and genetic factors has been known to contribute to CAD pathophysiology (Cambien and Tiret, 2007). Traditional risk factors such as lipid rich diet, advanced age, smoking, hypertension, diabetes mellitus and dyslipidemia are associated with increased risk of CAD. In addition, oxidative stress has been regarded as one of the well established patho-physiological mechanisms that contribute in the pathogenesis and progression of CAD (Dhalla *et al*., 2000).

Glutathione S-transferases (GST) are phase-II detoxification enzymes present in the mitochondria and cytosol, that play an important role in conjugating electrophilic compounds (xenobiotics and endogeneously-produced products of oxidative stress) with glutathione, and in this manner mitigate oxidative stress and prevent cell injury (Li *et al*., 2000; Hayes *et al*., 2005). Increased vulnerability to oxidative stress can therefore result from decreased GST activity and increase susceptibility to inflammatory diseases including CAD (Doney *et al*., 2005; Bonomini *et al*., 2008; Turkanoglu *et al*., 2010). Therefore, GSTs are considered to be one of the most important defense mechanisms against the detrimental effects of oxidative stress.

Human GSTs are classified into eight classes: GST-alpha, GST-mu, GST-theta, GST-pi, GST-zeta, GST-sigma, GST-kappa and GST-omega (Lo and Ali-Osman, 2007). The glutathione S-transferase P1 (GSTP1) gene is 2.8kb long and maps on the long arm of chromosome 11 (11q13.3) and has seven exons. Genetic polymorphisms occur in exon 5 (rs1695) of *GSTP1*B* and in exon 6 (rs1138272) of *GSTP1*C* whereas *GSTP1*A* is the wild type. *GSTP1*B* results from A-G substitution at position 313 in exon 5 leading to the replacement of amino acid Isoleucine by Valine at 105 amino acid position (Ile105Val) whereas *GSTP1*C* results from C-T substitution at position 341 in exon 6 which causes replacement of Alanine by Valine at 114 amino acid position (Ala114Val) and these allelic variants have reduced enzyme activity and affinity for electrophilic substrates (Hayes *et al*., 2005; Ntais *et al*., 2005), and sequence variation in some *GST* genes have in fact shown association with CAD (Hayes *et al*., 2005). Genetic polymorphisms in the *GST* genes results in virtual absence of enzyme activity and consequently play an important role in individual susceptibility to CAD. Among these, the *GSTP1* g.313A>G polymorphism has been studied in some

ethnic groups (Nomani *et al*., 2011; Singh *et al*., 2011; Phulukdaree *et al*., 2012; Yeh *et al*., 2013) but no study regarding the *GSTP1* g.341C>T polymorphism and CAD has been reported so far, therefore this is the first study of its kind. Rather, no studies on stratified North-Indian sub-groups have been revealed on literature perusal. The state of Punjab represents diverse cultural and genetic heritage. The ethnicity, genetic makeup, dietary pattern and adoption of western life-style can greatly influence the onset and pathogenesis of CAD. As ethnic-group specific studies provide gainful insights on genetic determinants of disease, the present case-control study was carried out to investigate the association of g.313A>G and g.341C>T polymorphisms of *GSTP1* with CAD in North Indian population sub-group (*Jat Sikh*).

Material and Methods

Study participants

In this case-control study, 200 cases belonging to *Jat Sikh* population subgroup documented with CAD on the basis of electrocardiographic (ECG) changes, echocardiographic evidence of myocardial infarction, positive treadmill test were enrolled from A. P. Heart-Care Hospital, Amritsar and 200 age-, sex-and ethnicity-matched controls with no present or past family history of CAD or any other disease participated voluntary after written informed consent. Patients with lung, kidney, liver, thyroid disorders or malignancy were excluded. The study was conducted after approval from the Institutional Ethics Committee of Guru Nanak Dev University, Amritsar in accordance with the Declaration of Helsinki. Demographic, disease-specific information was recorded on pre-designed questionnaire and fasting venous blood samples (5 ml) for genotyping were obtained from each participant.

The *Jat-Sikhs* constitute the largest proportion (~35%) of Sikh community, are mostly agrarian and warriors, being endogamous at caste and exogamous at the sub-caste levels (Sidhu *et al*., 2003). They are descendents of the original Indo-Aryans and later of Indio-Scythian tribes (Dhillon, 1994). This ethnic sub-group has been investigated because of their food preferences (rich in high fat content and consumption of milk and dietary products), adoption of western life-style and lack of physical activity.

DNA isolation and detection of *GSTP1* (g.313A>G, g.341C>T) polymorphisms

Genomic DNA was extracted from peripheral blood cells by salting-out method (Miller *et al*., 1988). The g.313A>G (rs1695) (Vettriselvi *et al*., 2006) and g.341C>T (rs1138272) (Vedyakov and Tonevitskii, 2006) SNPs of the *GSTP1* were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primer pairs used to amplify the 176 bp of *GSTP1* were forward 5′-ACCCCAGGGCTCTATGGGAA-3′ and reverse 5′-TGAGGGCACAAGAAGCCCCT-3 and to amplify the 539 bp of *GSTP1* were forward 5′-CAGCAGAAGCAGCGTGTGTGC-3′ and reverse 5′-CCCACAATGAAGGTCTTGCCTCC-3′. Each reaction mixture (15 µl) contained 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 10 µM of each primer, *Taq* DNA polymerase (Bangalore Genei: 1.0 unit in g.313A>G, 1.25 units in g.341C>T) and 50 ng genomic DNA. The amplification conditions were initial denaturation at 95 ºC for 5 min followed by 35 cycles of denaturation at 95 ºC for 45 sec, annealing at 63 ºC for g.313A>G and 64 ºC for g.341C>T for 45 sec, extension at 72 ºC for 45 sec and final extension at 72 ºC for 10 min and, was carried out in Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany). The amplified products of g.313A>G and g.341C>T were digested with *Bsm*A1 and *Aci*1 restriction enzymes (New England Biolabs, USA) and the products were resolved on 3% and 2.5% agarose gels stained with ethidium bromide, respectively. The A/A genotype corresponded to 176 bp band, the A/G genotype showed 176 bp, 95 bp and 81 bp bands and the G/G genotype corresponded to 81 bp and 95 bp bands (Fig. 1a). The C/C genotype produced 365 bp, 120 bp and 54 bp fragments while the C/T genotype resulted in 485 bp, 365 bp, 120 bp and 54 bp fragments (Fig. 1b). For conformation, genotyping of 10% random samples with exploratory bi-directional DNA sequencing of few samples was done and the results were 100% concordant with out showing any discrepancy.

Statistical analysis

For analysis, the Statistical Package for the Social Sciences (SPSS, version 16.0 for Windows 7, Chicago, IL, USA) was used. Student's t-test, Chi-squared or Fisher's exact test were performed to compare demographic and clinical characteristics between patients and controls expressed as mean ± standard deviation (SD). Allele and genotype frequencies were determined by gene counting methods. Chi-squared test with Yates correction or Fisher's exact test were carried out to test genotype distribution expressed as frequency (n, %). Binary logistic regression analyses were used to calculate crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association of *GSTP1* (g.313A>G and g.341C>T) polymorphisms with CAD using age, gender, BMI, alcohol,

total cholesterol, hypertension and family history as potential covariates. Disease risk was also evaluated with different genetic models (dominant, co-dominant and recessive) using the Web-Assotest program (http://www.ekstroem.com). Hardy-Weinberg equilibrium was tested using the Court Lab Calculator (Court, 2008). SNPStats software was used to calculate linkage disequilibrium statistics and haplotype analysis (Sole *et al*., 2006). The corrections for multiple comparisons were done by Bonferroni method wherever required and there was no difference in statistical significance even after Bonferroni correction (i.e. reducing significance level to p= 0.025). Power calculations were performed by CaTS-Power Calculator (Skol *et al*., 2006) and the study had a statistical power of 85% to detect an association with an OR of 1.5 at p=0.05. *P*-values <0.05 were considered statistically significant.

Results

The characteristics of the study participants are as previously described (Bhat and Gandhi, 2016). The demographic and clinical characteristics of cases (CAD patients) and controls are presented in Table 1. The two groups were matched for age (59 .04± 0.75y for cases; 57.88±0.96y for controls), gender (36.50% males, 63.50% females in cases; 42.50% males, 57.50% females in controls) and waist-to-hip ratio (1.00±0.00 for cases; 0.99±0.00 for controls). Family history of CAD was observed in 15% of patients while hypertension was present only in 15.5% of patients. Smoking habits were lacking in both groups whereas alcohol consumption was present more in controls (18%) than in cases (6%).

Table 1. Demographic and clinical characteristics of the study participants

Variables		**Patients (n=200)**	**Controls (n=200)**	**p-value**
		Mean ±SD	**Mean ±SD**	
Age (years)		59.04±10.61	57.88±13.59	0.340[a]
Gender	Male	73 (36.50)	85 (42.50)	0.261[b]
	Female	127 (63.50)	115 (57.50)	
BMI (kg/m^2)	(<18.5) Underweight	-	16 (8.00)	0.000[c]
	(18.5-22.9) Normal	39 (19.50)	60 (30.00)	
	(23.0-24.9) Overweight	30 (15.00)	36 (18.00)	
	(≥25) Obese	131 (65.50)	88 (44.00)	
WHR	(Men ≥0.89; Women ≥0.81) Obese	199 (99.50)	194(97.00)	0.121[b]
	(Men <0.89; Women <0.81) Non-obese	01 (0.50)	06(3.00)	
WHtR	≥0.5 Obese	199(99.50)	181 (90.50)	0.000[c]
	<0.5 Non-obese	01(0.50)	19(9.50)	

≠Blood pressure categories	Normal	65 (32.50)	142 (71.00)	0.000[c]
	Pre-hypertension	92 (46.00)	58 (29.00)	
	Hypertension stage-I	39 (19.50)	-	
	Hypertension stage-II	04 (2.00)	-	
†Alcohol consumption		12 (6.00)	37 (18.50)	0.001[b]
Family history of CAD		30 (15.00)	0	0.001[c]

BMI: body mass index; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; † 2-3 times (40-50ml)/wk. [a]Students' t-test; [b]Chi-squared test; [c]Fisher's exact test. Figures in parentheses denote percentages. ≠ (Bhat and Gandhi, 2016).

The genotype distributions of *GSTP1* (g.313A>G, g.341C>T) SNPs among the cases and controls were consistent with Hardy-Weinberg equilibrium (cases: χ^2=3.641, p=0.060 and χ^2=0.160, p=0.689; controls: χ^2=3.646, p=0.060 and χ^2=0.005, p=0.943, respectively). Genotype and allele distributions of g.313A>G and g.341C>T polymorphisms of *GSTP1* were significantly different between cases and controls (p=0.05, p=0.003; p=0.024, p= 0.025, respectively) (Table 2). The A/G and G/G genotypes had frequency distributions of 54%, 12% and 46.5%, 5.5% in cases and controls, respectively. The frequency of G allele was found to be higher in cases (39%) than in controls (29%). For g.341C>T polymorphism, the frequency of C/T genotype was more in cases (5.5%) than in controls (1%). The frequency of T allele was tended to be more in cases than in controls (3% vs. 1%).

Table 2. Distribution of *GSTP1* g.313A>G and *GSTP1* g.341C>T genotypes and alleles in the study group

Genotype frequencies	**Patients n=200 (%)**	**Controls n=200 (%)**	**χ^2 p-value**	**Crude OR (95%CI)**	**p-value**	**Adjusted * OR 95%CI**	**p-value**
*GSTP1*g.313A>G							
A/A	68 (34.00)	96 (48.00)	0.005	Reference		Reference	
A/G	108 (54.00)	93 (46.50)		1.6 (1.08-2.49)	0.020	1.8 (0.79-4.23)	0.156
G/G	24 (12.00)	11 (5.50)		3.1 (1.41-6.71)	0.005	5.0 (1.09-23.01	0.039
Allele frequencies							
A	244 (61.00)	285 (71.00)	0.003				
G	156 (39.00)	115 (29.00)					
Hardy-Weinberg	p=0.06	p=0.060					

equilibrium	0						
Genetic models g.313A>G	Dominant (AG/GG vs. AA) OR: 1.8, 95% CI: 1.20-1.68, p=0.004						
	Co-dominant (AG vs. AA/GG) OR: 1.7, 95% CI: 1.23-2.35, p=0.001						
	Recessive (GG vs. AA/AG) OR: 2.3, 95% CI: 1.11-4.92, p=0.020						
*GSTP1*g.341C>T							
C/C	189 (94.50)	198 (99.00)	0.024	Reference		Reference	
C/T	11(5.50)	2 (1.00)		5.8 (1.26-26.34)	0.024	2.2 (0.23-20.51)	0.490
T/T	0	0					
Allele frequencies							
C	389 (97.00)	398 (99.00)	0.025				
T	11 (3.00)	2 (1.00)					
Hardy-Weinberg equilibrium	p=0.689	p=0.943					

χ^2- Chi-squared test, CI: confidence interval, OR: odds ratio
* Adjusted for age, gender, body mass index, alcohol intake, total cholesterol, hypertension and family history

On binary logistic regression analysis, significant association of G/G genotype of g.313A>G was observed with 3.1-fold increased risk for CAD (OR: 3.1, 95% CI: 1.41-6.71, p=0.005) and of A/G genotype with 1.6-fold (OR: 1.6, 95% CI: 1.08-2.49, p=0.020) (Table 2). Among the various models, the dominant model showed 1.8-fold (OR: 1.8, 95% CI: 1.20-1.68, p=0.004), co-dominant 1.7-fold (OR: 1.7, 95% CI: 1.23-2.35, p=0.001) and recessive 2.3-fold (OR: 2.3, 95% CI: 1.11-4.92, p=0.020) increased risk for developing CAD (Table 2). Therefore, the rs1695 G allele under the recessive model explained much higher 2.3-fold risk for CAD as compared to the dominant and co-dominant models. However, after adjustment for potential confounders such as age, gender, BMI, alcohol intake, total cholesterol, hypertension and family history of CAD, the increased risk of developing CAD increased to 5-fold (OR: 5.0, 95% CI: 1.09-23.01, p=0.039) in patients with the G/G genotype of *GSTP1* g.313A>G while the A/G genotype lost its risk (OR: 1.8, 95% CI: 0.79-4.23, p=0.156). The C/T genotype (g.341C>T) showed 5.8-fold increased risk for CAD (OR: 5.8, 95% CI: 1.26-26.34, p=0.024) but was not retained after adjustment (OR: 2.2, 95% CI: 0.23-20.51, p=0.490) (Table 2). However, combinations of *GSTP1* g.313 A/G and G/G genotypes together with *GSTP1* g.341 C/T genotype conferred 6.5-fold increased risk for the development of CAD (OR: 6.5, 95% CI: 1.37-31.27, p=0.018) while disease-risk was reduced (1.8-fold) on considering *GSTP1* g.313A/G and G/G

genotypes together with *GSTP1* g.341 C/C genotype (OR: 1.8, 95% CI: 1.16-2.64, p=0.007) (Table 3). Despite the close proximity of the two *GSTP1* SNPs, they were not in linkage disequilibrium (D':0.616 and r^2: 0.111). The haplotype structure (GC) comprising g.313 G and g.341 C revealed significant association with CAD (OR: 1.7, 95% CI: 1.20-2.32, p=0.002) which was higher for (GT) g.313G and g.341T with 5.5-fold (OR: 5.5, 95% CI: 1.07-27.76, p=0.042) (Table 4).

Table 3. Combined effects of *GSTP1* g.313A>G and *GSTP1* g.341C>T genotypes in the study group

Genotype interactions		Patients n=200 (%)	Controls n=200 (%)	OR (95% CI)	p-value
*GSTP1*g.313A>G	*GSTP1* g.341C>T				
AA	CC	66 (33.00)	96 (48.00)	Reference	
AG+GG	CT	9 (4.50)	2 (1.00)	6.5 (1.37-31.27)	0.018
AA	CT	2 (1.00)		-	-
AG+GG	CC	123 (61.50)	102 (51.00)	1.8 (1.16-2.64)	0.007

OR: odds ratio; CI: confidence interval

Table 4. The association between haplotypes in *GSTP1* gene with CAD

Haplotype*	g.313	g.341	Frequency	OR (95% CI)	p-value
1	A	C	0.657	1.00	-
2	G	C	0.326	1.7 (1.20 - 2.31)	0.002
3	G	T	0.012	5.5 (1.07 - 27.76)	0.042
Global haplotype association p-value**:** 0.0006					

*The SNP order defining the respective *GSTP1* haplotype structure is g.313 and g.341.
OR: odds ratio; CI: confidence interval

Discussion

The present study was carried out to investigate the association between *GSTP1* (g.313A>G, g.341C>T) polymorphisms and susceptibility to CAD in *Jat Sikh* population sub-group of North India. These observations showcase that both the SNPs associated with CAD in the *Jat Sikh* sub-group with 3.1-fold increased (p=0.005) disease-risk in cases with G/G and 1.6-fold increased risk (p=0.020) with A/G genotypes of *GSTP1* g.313A>G. On adjustment for G/G genotypes, the risk was increased with OR of 5.0 (p=0.039) implying that the genotype contributed more towards CAD susceptibility than traditional risk factors. Interestingly the C/T genotype of g.341C>T with CAD also conferred increased risk (5.8-fold; p=0.024) for disease-development. This association has been reported for the first time to the best of knowledge. On adjustment for confounding factors, the

relative risk has been reduced to 2.2-fold. Only limited studies on g.313A>G have been documented in literature but none on the *Jat Sikh* sub-group. Rather the present study results are inconsistent for g.313A>G wherein North Indians, G/G genotype was protective against CAD (Singh *et al*., 2011) while A/A genotype in young South African Indians associated with decreasing AMI-risk (Phulukdaree *et al*., 2012). Contrarily, no association was observed between g.313A>G polymorphism and CAD among Iranian (Nomani *et al*., 2011) and Taiwanese (Yeh *et al*., 2013) populations. As different sub-groups and/or populations have ethnic specificity and diverse genetic and environmental backgrounds, the variations in population structures may accord for such differences highlighting the value of ethnic-specific studies for population stratification.

Considering the g.341C>T SNP and CAD risk, the C/T genotype conferred 5.8-fold increased risk for developing CAD and has been observed as a first study in an ethnic-specific group. Not many studies on this SNP for disease-association have come to attention. However, C/T (5-fold risk) and T/T (11-fold risk) genotypes associated with esophageal cancer in South Africans (Li *et al*., 2010) which raises concern for persons with similar genotypes as present study patients for esophageal cancer risk, which may be further enhanced in those with g.313 A/G and G/G genotypes.

CAD is a complex polygenic disease and it is likely that the genetic susceptibility is influenced by several gene polymorphisms. Genetic polymorphisms in individual genes may impart to a small extent, and it is likely that the cumulative effect of many polymorphisms will be more important in its pathogenesis. Therefore, we analysed the g.313A>G and g.341C>T polymorphisms of *GSTP1* to determine whether the combined genotypes alter the CAD susceptibility. In combination, the g.313 A/G and G/G and g.341 C/C genotypes conferred 1.8-fold increased risk, while the risk was increased to 6.5-fold in those with g.313 A/G and G/G and g.341 C/T genotypes, indicating that individuals having more than one defective genotype would be therefore at greater risk for developing CAD. There is one more possibility that association between *GSTP1* gene polymorphisms and CAD in different ethnic groups could be due to different haplotype block, which may further increase the risk of disease.

In conclusion, the A/G and G/G genotypes of g.313A>G and C/T genotype of g.341C>T, alone and in combination, have shown increased susceptibility to CAD in *Jat Sikh* patients which may be potentiated or reduced on modifying the traditional risk factors. Such haplotype-based studies in

ethnic sub-groups with genetic diversity and unique cultural practices may be informative for identifying protective and/or susceptible SNPs for disease-development. However, more different ethnic studies with larger sample size are needed to corroborate the results of the present study.

Acknowledgements

Assistance of the cardiologist, Dr. Ajinder Pal Singh (A. P. Heart-Care Hospital, Amritsar) for patient diagnosis and participants for providing their blood samples and relevant information is gratefully acknowledged.

Funding

Grant under DRS-SAP. Mohd. Akbar Bhat is grateful to GNDU for providing UPE fellowship for Ph.D.

Conflict of interest: None declared.

References

1. Topol EJ, Smith J, Plow EF, Wang QK. 2006 Genetic susceptibility to myocardial infarction and coronary artery disease. *Hum Mol Genet* 15, R117-123.
2. Cambien F, Tiret L. 2007 Genetics of cardiovascular diseases: from single mutations to the whole genome. *Circulation* 116, 1714-1724.
3. Dhalla NS, Temsah R, Netticadan T. 2000 Role of oxidative stress in cardiovascular diseases. *J Hypertens* 18, 655-673.
4. Hayes JD, Flanagan JU, Jowsey IR. 2005 Glutathione transferase. *Annu Rev Pharmacol Toxicol* 45:, 51-88.
5. Li R, Boerwinkle E, Olshan AF, Chambless LE, Pankow JS, Tyroler HA. 2000 Glutathione S-transferase genotype as a susceptibility factor in smoking-related coronary heart disease. *Atherosclerosis* 149:, 451-462
6. Bonomini F, Tangattini S, Fabiano A, Bianchi R, Rezzani R. 2008 Atherosclerosis and oxidative stress. *Histol Histopathol* 23, 381-390.

7. Doney ASF, Lee S, Leese GP, Morris AD, Palmer CN. 2005 Increased cardiovascular morbidity and mortality in Type 2 diabetes is associated with the Glutathione S-Transferse theta-null genotype: A Go-Darts study. *Circulation* 111, 2927-2934.
8. Turkanoglu A, Demirdogen BC, Demirkaya S, Bek S, Adali O. 2010 Association analysis of GSTT1, GSTM1 genotype polymorphisms and serum total GST activity with ischemic stroke risk. *Neurol Sci* 31, 727-734.
9. Lo HW, Ali-Osman F. 2007 Genetic polymorphism and function of glutathioneS-transferase in tumor drug resistance. *Curr Opin.Pharmacol* 7, 367-374.
10. Ntais C, Polycarpou A, Loannidis JP. 2005 Association of GSTM1, GTTT1 and GSTP1 gene polymorphisms with the risk of prostate cancer: A meta-analysis. *Cancer Epidemiol Biomarkers Prev* 14, 176-181.
11. Nomani H, Mozafari H, Ghobadloo SM, Rahimi Z, Raygani, AV, Rahimi MA. 2011 The association between GSTT1, M1 and P1 polymorphisms with coronary artery disease in Western Iran. *Mol Cell Biochem* 354, 181-187.
12. Singh N, Sinha N, Kumar S, Pandey CM, Agrawal S. 2011 Glutathione S-transferase gene polymorphism as a susceptibility factor for acute myocardial infarction and smoking in the North Indian population. *Cardiology* 118, 16-21.
13. Phulukdaree A, Khan S, Moodley D, Chuturgoon AA. 2012 GST polymorphisms and early-onset coronary artery disease in young South African Indians. *S Afr Med J* 102, 627-630.
14. Yeh HL, Kuo LT, Sung FC, Chiang CW, Yeh CC. 2013 GSTM1, GSTT1, GSTP1 and GSTA1 genetic variants are not associated with coronary artery disease in Taiwan. *Gene* 523, 64-69.
15. Sidhu IS, Kaur K, Sarhadi VK, Bhanwer AJS. 2003 Study of genetic polymorphism at D21S11 and D21S215 loci in the Jat Sikh population of Punjab. *Int J Hum Genet* 3, 45-50.
16. Dhillon BS. (1994). *History and study of the Jats.* Beta Publishers, India.
17. Miller SA, Dykes DD, Polesky HF. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16, 1215.
18. Vettriselvi V, Vijayalakshmi K, Solomon FP. 2006 Genetic variation of GSTM1, GSTT1 and GSTP1 genes in South Indian population. *Asian Pac J Cancer Prev* 7, 325-328.
19. Vedyakov AM, Tonevitskii AG. 2006 Analysis of a series of significant genetic polymorphisms in athletes. *Human Physiol* 32, 204-208.

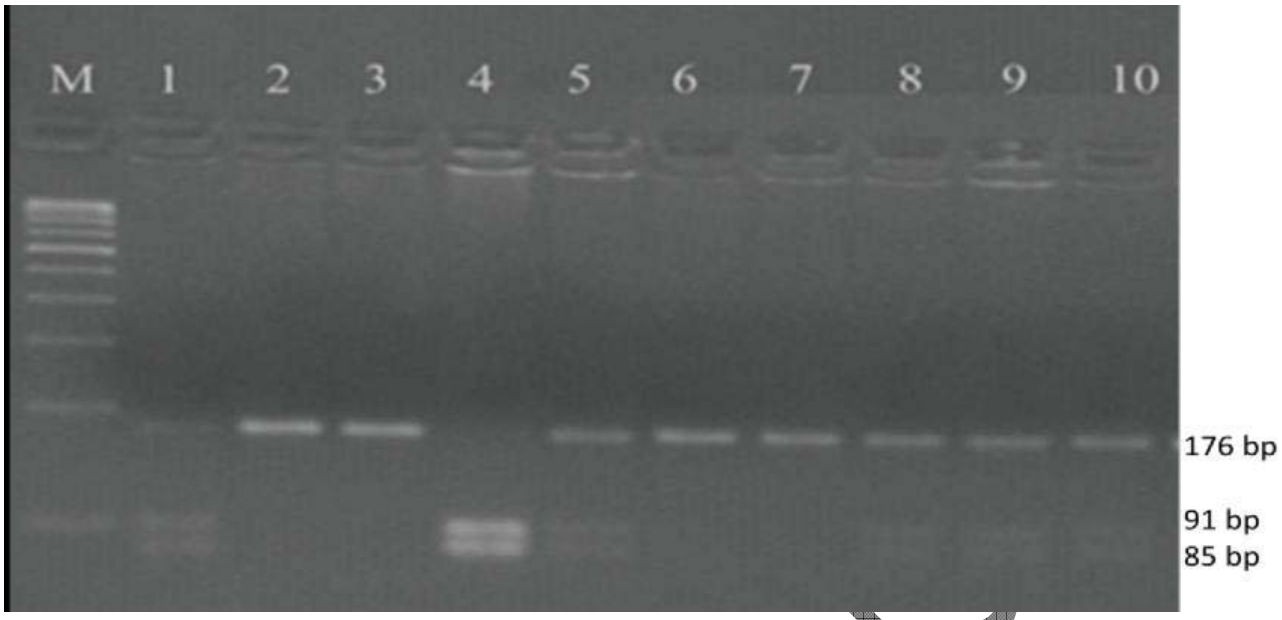
20. Court M. Court Lab Calculator. 2008; Available: http//www.tufts.edu/.
21. Sole X, Guino E, Valls J, Iniesta R, Moreno V. 2006 SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22, 1928-1929.
22. Skol AD, Scott LJ, Abecasis GR, Boehnke M. 2006 Joint analysis is more efficient than replication-based analysis for two stage genome wide association studies. *Nature Genetics* 38, 209-213.
23. Bhat MA, Gandhi G. 2016 Association of GSTT1 and GSTM1 gene polymorphisms with coronary artery disease in North Indian Punjabi population: a case-control study. *Postgrad Med J* 0, 1-6. doi:10.1136/postgradmedj-2015-133836.
24. Li D, Dandara C, Parker CMI. 2010 The 341C/T polymorphism in the GSTP1 gene is associated with increased risk of esophageal cancer. *BMC Genet* 11, 47. doi:10.1186/1471-11-47.

Received 30 November 2016, in revised form 11 February 2017; accepted 9 March 2017

Unedited version published online: 13 March 2017

Legends to figures

Fig1(a) Representative gel picture of *GSTP1* g.313A>G (rs1695). M: Molecular weight marker (100 bp). Lanes 3, 6, 7: Homozygous wild type (A/A), lanes 1, 5, 8, 9, 10: Heterozygous (A/G), lane 4: Homozygous mutant (G/G), and lane 2: undigested control.



Fig(b) A representative agarose gel of *GSTP1* g.341C>T (rs1138272). M: Molecular weight marker (100 bp). Lanes 1, 3-8, 10 represent homozygous wild type (C/C), lanes 2, 9, 11 represent heterozygous (C/T), and lane 12 represents undigested control.