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RESEARCH ARTICLE

Glutathione *S*-transferase P1 gene polymorphisms and susceptibility to coronary artery disease in a subgroup of north Indian population

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Abstract. 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 subgroup of north Indian population. In the present case–control study, CAD patients ($n = 200$) and age-matched, sex-matched and ethnicity-matched healthy controls ($n = 200$) were genotyped for polymorphisms in *GSTP1* using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Genotype distribution of g.313A>G and g.341C>T polymorphisms of *GSTP1* gene was significantly different between cases and controls ($P = 0.005$ and 0.024, respectively). Binary logistic regression analysis showed significant association of A/G (odds ratio (OR): 1.6, 95% CI: 1.08–2.49, $P = 0.020$) and 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, $P = 0.020$). In conclusion, statistically significant associations of *GSTP1* g.313A>G (A/G, G/G) and g.341C>T (C/T) genotypes with CAD were observed.

Keywords. glutathione *S*-transferase; *GSTP1* gene; single-nucleotide polymorphisms; coronary artery disease.

Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality worldwide (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 to 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 endogenously-produced products of oxidative stress) with glutathione, and, by doing so 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 2.8-kb 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 indeed shown association with CAD (Hayes *et al.* 2005). Genetic polymorphisms in the *GST* genes result 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 is studied in some ethnic groups (Nomani *et al.* 2011; Singh *et al.* 2011; Phulukdaree *et al.* 2012; Yeh *et al.* 2013), but study regarding the *GSTP1* g.341C>T polymorphism and CAD is not reported so far, therefore, this is the first study of its kind. No studies on stratified north Indian subgroups were 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 subgroup (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-matched, sex-matched and ethnicity-matched controls with no present or past family history of CAD or any other disease participated voluntarily 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 predesigned 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, were mostly agrarian and warriors, being endogamous at caste and exogamous at the subcaste levels (Sidhu *et al.* 2003). They are descendents of the original Indo-Aryans and later of Indio-Scythian tribes (Dhillon 1994). This ethnic subgroup was investigated because of their food preferences (rich in high-fat content and consumption of milk and dietary products), adoption to 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′-CAGCAGAAGCA GCGTGTGTGC-3′ and reverse 5′-CCCACAATGAAGG TCTTGCCTCC-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°C for 5 min followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 63°C for g.313A>G and 64°C for g.341C>T for 45 s, extension at 72°C for 45 s and a final extension at 72°C 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, respectively, stained with ethidium bromide. The A/A genotype corresponded to 176 bp band, the A/G genotype showed 176, 95 and 81 bp bands and the G/G genotype corresponded to 81 and 95 bp bands (figure 1a). The C/C genotype produced 365, 120 and 54 bp fragments, while the C/T genotype resulted in 485, 365, 120 and 54 bp fragments (figure 1b). For confirmation, genotyping of 10% random samples with exploratory bidirectional DNA sequencing of a few samples was done and the results were 100% concordant without showing any discrepancy.

Statistical analysis

For analysis, the Statistical Package for the Social Sciences (SPSS, ver. 16.0 for Windows 7, Chicago, USA) was used. Student's *t*-test, χ^2 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. χ^2 Test with Yates correction or Fisher's exact test was 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

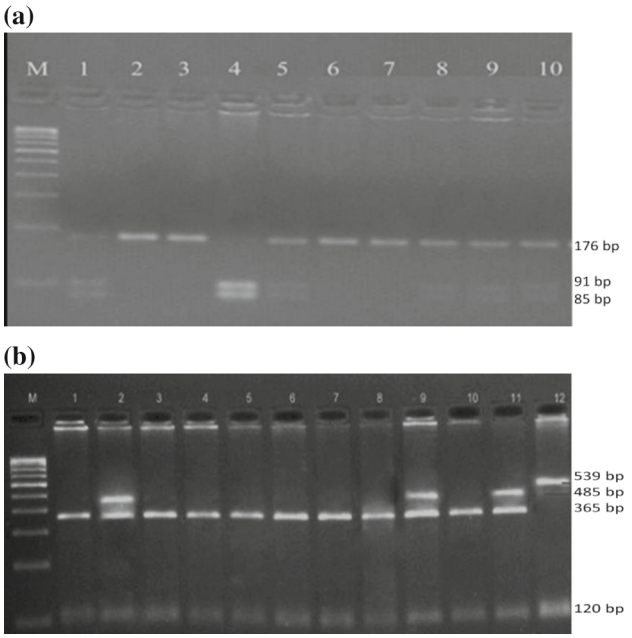

Figure 1. Representative gel picture of (a) *GSTP1* g.313A>G (rs1695) and (b) *GSTP1* g.341C>T (rs1138272). In both gels, the lane marked M has the molecular weight marker (100 bp). In (a), lanes 3, 6, and 7 have homozygous wild type (A/A), lanes 1, 5, 8, 9, and 10 have heterozygous (A/G) genotypes, lane 4 has a homozygous mutant (G/G), and lane 2 has an undigested control. In (b), lanes 1, 3–8, and 10 represent homozygous wild type (C/C), lanes 2, 9, and 11 represent heterozygous (C/T) genotype, and lane 12 represents undigested control.

history as potential covariates. Disease risk was also evaluated with different genetic models (dominant, codominant and recessive) using the Web-Asso test program (http://www.ekstroem.com). Hardy–Weinberg equilibrium was tested using the Court Lab Calculator (Court 2008, https://www.tufts.edu/). 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 ± 0.75 yr for cases; 57.88 ± 0.96 yr 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%).

The genotype distributions of *GSTP1* (g.313A>G, g.341C>T) SNPs among the cases and controls were consistent with Hardy–Weinberg equilibrium (cases:

Table 1. Demographic and clinical characteristics of the study participants.

Variable		Patient ($n = 200$) Mean ± SD	Control ($n = 200$) Mean ± SD	P
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)	Underweight (<18.5)	–	16 (8.00)	0.000[c]
	Normal (18.5–22.9)	39 (19.50)	60 (30.00)	
	Overweight (23.0–24.9)	30 (15.00)	36 (18.00)	
	Obese (≥25)	131 (65.50)	88 (44.00)	
WHR	Obese (Men ≥ 0.89; Women ≥ 0.81)	199 (99.50)	194 (97.00)	0.121[b]
	Nonobese (Men < 0.89; Women < 0.81)	01 (0.50)	06 (3.00)	
WHtR	Obese (≥0.5)	199 (99.50)	181 (90.50)	0.000[c]
	Nonobese (<0.5)	01 (0.50)	19 (9.50)	
Blood pressure categories**	Normal	65 (32.50)	142 (71.00)	0.000[c]
	Prehypertension	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; *two to three times (40–50 mL) per week. [a] Student's t-test; [b] χ^2 test; [c] Fisher's exact test. Figures in parentheses denote percentages. **Bhat and Gandhi (2016).

$\chi^2 = 3.641$, $P = 0.060$ and $\chi^2 = 0.160$, $P = 0.689$; controls: $\chi^2 = 3.646$, $P = 0.060$ and $\chi^2 = 0.005$, $P = 0.943$, respectively). Genotype and allele distributions of g.313A>G and g.341C>T polymorphisms of *GSTP1* were significantly different between cases and controls ($P = 0.05, 0.003$; $P = 0.024, 0.025$; 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 the 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 tended to be more in cases than in controls (3% versus 1%).

On binary logistic regression analysis, significant association of G/G genotype of g.313A>G was observed with a 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, $P = 0.004$), codominant 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 rs1695G allele under the recessive model, explained much higher (2.3-fold) risk for CAD as compared to the dominant and codominant 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.313A/G and G/G genotypes together with *GSTP1* g.341C/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.341C/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.313G and g.341C 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).

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 subgroup of north India. These observations showcase that both the SNPs were associated with CAD in the Jat Sikh subgroup, with a 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 higher, with an 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 our knowledge. On adjustment for confounding factors, the relative risk was reduced to 2.2-fold. Only limited studies on g.313A>G have been documented in literature, but none on the Jat Sikh subgroup. Relatively, the present study results are inconsistent for g.313A>G in north Indians, G/G genotype was protective against CAD (Singh *et al.* 2011), while the A/A genotype in young South African Indians was 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 subgroups and/or populations have ethnic specificity and diverse genetic and environmental backgrounds, the variations in population structures may account 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 a 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 were noticed. However, C/T (5-fold risk) and T/T (11-fold risk) genotypes are associated with oesophageal cancer in South Africans (Li *et al.* 2010), which raises concern for persons with similar genotypes as the present study patients for oesophageal cancer risk, which may be further enhanced in those with g.313A/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 in disease causation, 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.313A/G and G/G and g.341C/C genotypes conferred 1.8-fold increased risk, while the risk was increased to 6.5-fold in those with g.313A/G and G/G and g.341C/T genotypes, indicating that individuals with more than one defective genotype would be at greater risk for developing CAD. There is one more possibility, that association between *GSTP1* gene polymorphisms and CAD in

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 $n = 200$ (%)	$\chi^2 P$ value	Crude OR (95% CI)	*P*	Adjusted* OR 95% CI	*P*
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 equilibrium	$P = 0.060$	$P = 0.060$					
Genetic models g.313A>G	Dominant (AG/GG versus AA): OR, 1.8; 95% CI, 1.20–1.68; $P = 0.004$						
	Codominant (AG versus AA/GG): OR, 1.7; 95% CI, 1.23–2.35; $P = 0.001$						
	Recessive (GG versus 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.

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*
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*
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.

different ethnic groups could be due to different haplotype blocks, 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 subgroups 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.

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References

Bhat M. A. and 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.* **92**, 701–706.

Bonomini F., Tangattini S., Fabiano A., Bianchi R. and Rezzani R. 2008 Atherosclerosis and oxidative stress. *Histol. Histopathol.* **23**, 381–390.

Cambien F. and Tiret L. 2007 Genetics of cardiovascular diseases: from single mutations to the whole genome. *Circulation* **116**, 1714–1724.

Dhalla N. S., Temsah R. and Netticadan T. 2000 Role of oxidative stress in cardiovascular diseases. *J. Hypertens.* **18**, 655–673.

Dhillon B. S. 1994 *History and study of the Jats.* Beta Publishers, India.

Doney A. S. F., Lee S., Leese G. P., Morris A. D. and Palmer C. N. 2005 Increased cardiovascular morbidity and mortality in Type 2 diabetes is associated with the glutathione S-Transferase theta-null genotype: A Go-Darts study. *Circulation* **111**, 2927–2934.

Hayes J. D., Flanagan J. U. and Jowsey I. R. 2005 Glutathione transferase. *Annu. Rev. Pharmacol. Toxicol.* **45**, 51–88.

Li D., Dandara C. and Parker C. M. I. 2010 The 341C/T polymorphism in the GSTP1 gene is associated with increased risk of esophageal cancer. *BMC Genet.* **11**, 47.

Li R., Boerwinkle E., Olshan A. F., Chambless L. E., Pankow J. S., Tyroler H. A. *et al*. 2000 Glutathione S-transferase genotype as a susceptibility factor in smoking-related coronary heart disease. *Atherosclerosis* **149**, 451–462.

Lo H. W. and Ali-Osman F. 2007 Genetic polymorphism and function of glutathione S-transferase in tumor drug resistance. *Curr. Opin. Pharmacol.* **7**, 367–374.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.

Nomani H., Mozafari H., Ghobadloo S. M., Rahimi Z., Raygani A. V., Rahimi M. A. *et al.* 2011 The association between GSTT1, M1 and P1 polymorphisms with coronary artery disease in Western Iran. *Mol. Cell. Biochem.* **354**, 181–187.

Ntais C., Polycarpou A. and Loannidis J. P. 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.

Phulukdaree A., Khan S., Moodley D. and Chuturgoon A. A. 2012 GST polymorphisms and early-onset coronary artery disease in young South African Indians. *S. Afr. Med. J.* **102**, 627–630.

Sidhu I. S., Kaur K., Sarhadi V. K. and Bhanwer A. J. S. 2003 Study of genetic polymorphism at D21S11 and D21S215 loci in the Jat Sikh population of Punjab. *Int. J. Hum. Genet.* **3**, 45–50.

Singh N., Sinha N., Kumar S., Pandey C. M. and 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.

Skol A. D., Scott L. J., Abecasis G. R. and Boehnke M. 2006 Joint analysis is more efficient than replication-based analysis for two stage genome wide association studies. *Nat. Genet.* **38**, 209–213.

Sole X., Guino E., Valls J., Iniesta R. and Moreno V. 2006 SNPStats: a web tool for the analysis of association studies. *Bioinformatics* **22**, 1928–1929.

Topol E. J., Smith J., Plow E. F. and Wang Q. K. 2006 Genetic susceptibility to myocardial infarction and coronary artery disease. *Hum. Mol. Genet.* **15**, 117–123.

Turkanoglu A., Demirdogen B. C., Demirkaya S., Bek S. and Adali O. 2010 Association analysis of GSTT1, GSTM1 genotype polymorphisms and serum total GST activity with ischemic stroke risk. *Neurol. Sci.* **31**, 727–734.

Vedyakov A. M. and Tonevitskii A. G. 2006 Analysis of a series of significant genetic polymorphisms in athletes. *Hum. Physiol.* **32**, 204–208.

Vettriselvi V., Vijayalakshmi K. and Solomon F. P. 2006 Genetic variation of GSTM1, GSTT1 and GSTP1 genes in South Indian population. *Asian Pac. J. Cancer Prev.* **7**, 325–328.

Yeh H. L., Kuo L. T., Sung F. C., Chiang C. W. and Yeh C. C. 2013 GSTM1, GSTT1, GSTP1 and GSTA1 genetic variants are not associated with coronary artery disease in Taiwan. *Gene* **523**, 64–69.

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