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Association of angiotensin-converting enzyme, methylene tetrahydrofolate reductase and paraoxonase gene polymorphism and coronary artery disease in an Indian population

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Abstract

Background: *Coronary artery disease (CAD) and cancer remain the leading causes of death in most developed countries. Elucidating the genetic components that contribute to their pathogenesis is challenging. In this case-control association study, we examine the association of single nucleotide polymorphisms (SNPs) in paraoxonase 573 A/G genes, methylene tetrahydrofolate reductase (MTHFR) 677 C/T and angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism with CAD independently, as well as synergistically, in a north Indian population.*

Methods and results: *Patients with at least 50% stenosis of at least one major coronary artery were classified as cases. The controls had no myocardial infarction. Polymerase chain reactions (PCR) followed by restriction fragment length polymorphism (RFLP) analyses were carried out to determine the SNPs. No significant association of the polymorphisms of the ACE or MTHFR genes with the risk of CAD was observed. However, the allele frequencies of the 573 A/G polymorphism of the paraoxonase gene differed significantly among cases and controls before and after controlling for confounding factors. The frequencies of AG* vs *AA genotypes and GG+AG* vs *AA genotypes also differed significantly in the two groups ($p = 0.0002$). The interaction of paraoxanase with both MTHFR and ACE independently showed significant positive associations*

Conclusions: *The identification of 'at risk' individuals by genetic mapping of susceptible genes for effective control of other host factors will be a very effective and practical approach for prevention, as well as the development of improved therapy for patients.* (Cardiol J 2011; 18, 4: 385–394)

Key words: paraoxanase, gene, polymorphism, coronary artery disease

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Introduction

Coronary artery disease (CAD) and cancer remain the leading causes of death in most developed nations. In developing countries like India, an epidemiological transition has taken place, and CAD and other chronic diseases are now coming to the fore [1, 2]. According to estimates by the World Health Organization (WHO), nearly seven million people worldwide die of CAD every year, with most of these deaths occurring in developing countries [3]. More than 80% of sudden cardiac deaths are caused by atherosclerotic CAD, with the remainder caused by other diseases [4].

Genetics has traditionally been viewed through the window of relatively rare single-gene diseases. It is, however, increasingly apparent that virtually every medical condition, apart perhaps from simple trauma, has a genetic component. As is often evident from a patient's family history, many common disorders such as hypertension, heart disease, asthma, diabetes mellitus, and mental illnesses are significantly influenced by the genetic background. These polygenic or multifactorial (complex) disorders involve the contributions of many different genes, as well as environmental and host factors that can modify disease risk. A major current challenge is therefore to elucidate the genetic components that contribute to the pathogenesis of complex disorders [5]. Many of our commonest diseases are complex disorders that run in families, but lack the simple inheritance patterns characteristic of single gene disorders. Complex diseases have a low heritability compared to single gene disorders.

Understanding the genetic factors underlying these complex disorders is therefore challenging but vital [6]. There has been spectacular success in identifying the genes responsible for Mendelian disorders, but the quest for the susceptibility genes involved in multifactorial diseases such as CAD has been a struggle [7]. The task of finding a particular disease-associated gene or genes within this haystack is daunting. Genetic testing for susceptibility to chronic disease is therefore being increasingly integrated into the practice of medicine.

Inflammation plays a crucial role in the pathogenesis of CAD [8]. A number of candidate genes and loci have recently been identified as being associated with susceptibility to myocardial infarction (MI; the most acute form of CAD). Mostly, the gene products are implicated in the processes of inflammation [9].

In the present case-control association study, we examined the association of single nucleotide polymorphisms (SNPs) in three genes: paraoxonase 573 A/G genes, methylene tetrahydrofolate reductase (MTHFR) 677 C/T, and angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism with CAD independently, as well as synergistically, in a north Indian population. Independent studies have been undertaken into the role and mechanism of the association of the SNPs of these genes with CAD, but no definitive conclusions have yet been reached, because of the ethnic divergence of gene polymorphisms. It is therefore important to examine polymorphisms related to CAD in low- or high-risk individuals of each ethnic group. Association studies on the polymorphisms in these genes have been rare in India. This study was therefore carried out in an Indian population to study the ethnic variations, and their association with CAD.

Methods

Study design

The study sample included 203 sporadic CAD cases and 212 unrelated healthy control subjects. The cases were recruited between July 2006 and July 2007 from the patients, belonging primarily to north India, who had had MI or angina and were being treated at the Department of Cardiology, GSVM Medical College, Kanpur, India. All patients (age range 28 to 80 years) were diagnosed using electrocardiography (ECG) and angiography. Patients who had more than 50% stenosis of at least one major coronary artery, demonstrated via coronary angiography, were categorized as CAD patients. The controls were unrelated individuals recruited from the Outpatient Department of Cardiology. Their clinical histories were reviewed in an interview. They had vascular risk factors such as hypertension, diabetes mellitus, smoking, and hypercholesterolemia, but no history or occurrence of MI. Group matching of cases and controls was done for age and sex.

Arterial hypertension was defined as having had a previous diagnosis of hypertension or if systolic or diastolic blood pressure was more than 140 mm Hg or more than 90 mm Hg respectively, or both, on at least two different occasions.

Subjects were classified as having diabetes mellitus if their fasting blood glucose level was more than 126 mg/dL or if they had a history of being diagnosed with the disease.

Hypercholesterolemia was defined as having > 200 mg/dL total cholesterol in the blood.

Subjects previously or currently smoking tobacco were defined as smokers, whereas nonsmokers had no history of smoking.

Table 1. Genotype and allele distribution of single nucleotide polymorphisms of angiotensin-converting enzyme (ACE), methylene tetrahydrofolate reductase (MTHFR) and paraoxonase genes in cases and controls.

ACE I/D		
Genotype	**Cases (n = 203)**	**Controls (n = 212)**
DD	54 (26.6%)	73 (34.4%)
ID	88 (43.3%)	80 (37.7%)
II	61 (30.0%)	59 (27.8%)
Allele	**Cases (n = 406)**	**Controls (n = 424)**
D	196 (48.3%)	226 (53.3%)
I	210 (51.7%)	198 (46.7%)
MTHFR 677 C/T		
Genotype	**Cases (n = 203)**	**Controls (n = 212)**
TT	9 (4.4%)	3 (1.4%)
CT	50 (24.6%)	53 (25.0%)
CC	144 (70.9%)	156 (%)
Allele	**Cases (n = 406)**	**Controls (n = 424)**
T	68 (16.7%)	59 (13.9%)
C	338 (83.3%)	365 (86.1%)
Paraoxonase 573 A/G		
Genotype	**Cases (n = 203)**	**Controls (n = 212)**
GG	12 (5.9%)	10 (4.7%)
AG	108 (53.2%)	77 (36.3%)
AA	83 (40.9%)	125 (59.0%)
Allele	**Cases (n = 406)**	**Controls (n = 424)**
G	132 (32.5%)	97 (22.9%)
A	274 (67.5%)	327 (77.1%)

The study protocol was approved by the Ethical Committee for Human Genetics Research of the Indian Institute of Technology, Kanpur. Informed written consent was obtained from all individuals after a full explanation of the study.

Genotype determination

Genomic DNA was extracted from the blood samples of cases and controls by the 'salting out' method. Using the isolated genomic DNA as a template, polymerase chain reactions (PCR) were carried out, followed by restriction fragment length polymorphism (RFLP) analyses to determine the SNPs. Detailed information on the PCR conditions, primer sequences, restriction enzymes etc is set out in Table 1.

Statistical analysis

Data was analyzed using Stats Direct (version 2.5.7) software. Discrete data was reported as frequencies and analyzed using Pearson's χ^2 test for normal distribution. Continuous data was reported as means with standard deviations and analyzed using *t* test. Two-tailed p value was considered, and values < 0.05 were considered statistically significant. Deviation from Hardy-Weinberg equilibrium proportions was tested for each genetic marker. To assess the independent association of the genotypes with CAD, multivariate logistic regression analysis was performed controlling for the confounding effects of age, sex, hypertension, smoking and diabetes mellitus. For each odds ratio (OR), 95% confidence intervals (CIs) were calculated. Synergistic effects of the gene polymorphisms considering a combination of two or more genes at a time were assessed using χ^2 test.

Results

Characteristics of cases and control subjects

Group matching revealed that cases and controls were similar with regards to most characteristics. As expected, patients with CAD were older males and were more frequently diabetic. However, the controls had higher mean levels of total cholesterol and low-density lipoproteins (LDL). More controls had serum cholesterol, serum triglyceride and LDL values higher than the cut-off values. However, more cases had high-density lipoprotein (HDL) values lower than the cut-off values (Table 2).

Genotype frequencies

The genotype frequencies for the ACE, MTHFR and paraoxonase polymorphisms in the control groups were in Hardy-Weinberg equilibrium. The D, T and G allelic frequencies in the control groups were 53.3%, 13.9% and 22.9% respectively.

Association between ACE I/D polymorphism and coronary artery disease

The overall distribution of genotypes did not differ significantly between the cases and controls ($p = 0.21$). A lower frequency of the DD genotype was observed among the cases than in controls (26.6% *vs* 34.4%, $p = 0.08$). Frequencies of the II genotype were similar in both the groups (30% *vs* 27.8%), whereas the D allele in combination with

Table 2. Clinical characteristics of the study population.

Determinant	Cases (n = 203)	Controls (n = 212)	P
Age (years)	56.6 ± 12.1	54.5 ± 10.7	0.06
Age range	28–92	20–84	
Sex (male/female)	160/43	144/68	0.01
Hypertension	74 (36%)	83 (39%)	0.57
Diabetes	65 (32%)	30 (14%)	< 0.0001
Smoking	52 (26%)	40 (19%)	0.09
Serum cholesterol [mg/dL]	174.36 ± 36.17	185.68 ± 41.88	0.003
Serum cholesterol (≥ 200 mg/dL)	44 (22%)	69 (33%)	0.01
Serum triglyceride [mg/dL]	167.64 ± 53.0	170.55 ± 56.11	0.58
Serum triglyceride (> 200 mg/dL)	44 (22%)	69 (33%)	0.01
HDL [mg/dL]	41.56 ± 11.36	41.48 ± 9.26	0.93
HDL < 40 mg/dL	99 (49%)	50 (24%)	0.0000001
LDL [mg/dL]	100.60 ± 31.38	112.32 ± 31.05	0.0001
LDL ≥ 100 mg/dL	97 (48%)	138 (65%)	0.0003
VLDL [mg/dL]	33.95 ± 11.05	34.06 ± 11.94	0.92

I allele occurred somewhat more frequently in cases than in controls (43.3% *vs* 37.7%, p = 0.24).

Comparison of relative frequencies revealed that D allelic frequency tended to be enriched in those without CAD (53.3%) compared to the CAD patients (48.3%), although the difference did not achieve significance (p = 0.14).

We also tested a dominant model that compared risk of disease (CAD) with the DD+ID *vs* II genotypes; a significant difference was not found (OR 0.90, p = 0.61). A homozygous comparison (DD *vs* II) revealed a trend towards an increased risk of II (OR 0.72), although it did not achieve significance (p = 0.18). ID also conferred an increased risk for CAD, although it did not achieve significance (OR 1.06 for ID *vs* II, p = 0.79).

Association between MTHFR 677 C/T polymorphism and coronary artery disease

The overall distribution of genotypes was similar in the cases and controls (p = 0.18). The frequency of TT genotype was greater in cases than the controls (4.4% *vs* 1.4%, p = 0.06). The frequency of CT genotype was similar in both groups, whereas CC genotypes were more abundant in controls (73.6% *vs* 70.9%, p = 0.54). These differences however did not reach significance level.

The C allelic frequency was found to be enriched in the controls, while T was found to be more prevalent in the cases. A homozygous comparison (TT *vs* CC) showed an increased risk of CAD with TT genotype. The odds of cases having the TT genotype, as compared to the CT genotype, were three times higher than the controls.

Association between paraoxonase 573 A/G polymorphism and coronary artery disease

An overall distribution of the genotypes of paraoxonase 573 A/G polymorphism showed significant differences between cases and controls (p = 0.001). The frequencies of AG *vs* AA genotypes and GG+AG *vs* AA genotypes also differed significantly in the two groups (p = 0.0002) with an OR of approximately 2. The comparison of GG *vs* non GG genotypes also revealed an odds ratio of greater than 1, but was not found to be significant. Similarly, a homozygous comparison of the genotypes revealed a greater risk with GG genotype while AA genotype seemed to be protective.

The G allelic frequency increased in patients with CAD, whereas A allele was more abundant in the controls (p = 0.001, OR 1.62, 95% CI 1.18–2.23; Table 3).

Synergistic effects of gene polymorphisms on coronary artery disease

To evaluate gene interactions (gene-gene) in the etiology of CAD, cumulative effects of different gene polymorphisms were analyzed by combining alleles of two or three genes and comparing their frequencies in the cases and control groups.

Synergistic effect of polymorphisms in two genes. Interaction of polymorphisms in ACE and

Table 3. Odds ratio of genotype and allele of single nucleotide polymorphisms of angiotensin-converting enzyme (ACE), methylene tetrahydrofolate reductase (MTHFR) and paraoxonase genes.

Genotype comparison	OR	χ^2	P	95% CI
ACE I/D				
DD *vs* non DD	0.69	3.00	0.08	0.44–1.07
II *vs* non II	0.90	0.25	0.61	0.57–1.40
ID *vs* non ID	1.26	1.36	0.24	0.84–1.91
ID *vs* II	1.06	0.07	0.79	0.65–1.75
DD *vs* II	0.72	1.71	0.19	0.42–1.22
Allele comparisons				
D *vs* I	0.82	2.10	0.14	0.62–1.08
MTHFR 677 C/T				
TT *vs* non TT	3.23	3.36	0.06	0.79–18.78
TT+CT *vs* CC	1.14	0.36	0.54	0.73–1.80
TT *vs* CT	3.18	3.01	0.08	0.73–19.11
CT *vs* CC	1.02	0.01	0.92	0.64–1.64
TT *vs* CC	3.25	3.37	0.06	0.79–18.96
Allele comparisons				
C *vs* T	1.24	1.29	0.25	0.84–1.85
Paraoxonase 573 A/G				
GG *vs* non GG	1.27	0.29	0.58	0.50–3.25
GG+AG *vs* AA	2.08	13.55	0.0002	1.38–3.13
AG *vs* AA	2.11	13.38	0.0002	1.38–3.23
GG *vs* AA	1.81	1.76	0.18	0.69–4.76
Allele comparisons				
G *vs* A	1.62	9.64	0.001	1.18–2.23

MTHFR genes did not seem to confer an increased risk, with I+/T+ combination being nearly significant (OR 1.57, p = 0.06). The interaction of paraoxanase with both MTHFR and ACE independently showed significant positive associations. The presence of T–/G+ combination was 1.8 times higher in the cases, indicating a positive association with CAD. On the other hand, the T–/G+ combination was found to be protective leading to about a 60% reduction of risk.

Similarly, evaluation of ACE and paraoxanase synergism revealed that I+/G+ was significantly more abundant in the cases. On the other hand, I+/G– combination and I–/G– combination seemed to be preventive against CAD. The reduction of risk with I+/G– and I–/G– combinations were 40% and 90% respectively. Alone, neither ACE nor MTHFR had shown any significant association.

We also looked for the synergistic effect of all three genes combined. We observed that I+/T+/G+ combination of the alleles in either homozygous or heterozygous forms had two times greater odds than D+/C+/A+ and non I+/T+/G+ combinations. It also had about four times greater odds than I–/T–/G– combination of alleles (Table 4).

After controlling for the confounding effects of age, sex, hypertension, diabetes and smoking by multivariate logistic regression analysis, we observed that the 573 A/G polymorphism of the paraoxonase gene showed significant association with CAD. The GG+AG was associated with a two times greater odds of CAD (OR 2.03, 95% CI 1.35––3.04) compared to individuals with the AA genotype. The TT genotype in MTHFR 677 C/T polymorphism also demonstrated approximately four times greater odds as compared to the CT+CC genotype (Table 5).

Discussion

Natural variations, known as SNPs, in the four bases from which DNA and genes are composed lead to most of the genetic differences between individual people. Association studies at the population level help in the fine mapping of alleles. These studies examine the frequency of specific DNA variants (alleles) in groups of unrelated individuals with disease and unaffected controls.

Since the commonest and most important pathological changes in ischemic heart disease are atherosclerosis and thrombogenesis, and since genetic traits contribute significantly to the risk of CAD, several studies have investigated whether the genetic polymorphisms in inflammatory markers increase the risk of these diseases [9]. The corresponding information in the Indian population was indequate. We therefore tried to examine in this case-control association study, whether the gene variants that are involved in enhancing the risk for CAD through their contribution to the inflammatory processes and atherosclerosis are also independent risk factors for CAD in the study population.

ACE, located in endothelial cells, causes the conversion of physiologically inactive angiotensin I to a potent vasoconstrictor angiotensin II. ACE also inactivates bradykinin, a vasodilator. Both angiotensin II and bradykinin play an important role in cardiovascular regulation through various mechanisms [10–14]. Studies into the association of ACE I/D polymorphism and MI have shown variable results. A recent meta-analysis of 15 studies into the association between the ACE I/D polymorphism and MI in male dominated populations found a mean OR of 1.26 for MI of the DD *vs* ID+II genotypes (95% CI 1.15–1.39, p = 0.001) [15]. Another meta-analy-

Table 4. Synergistic effects of combination of single nucleotide polymorphisms of angiotensin-converting enzyme (ACE), methylene tetrahydrofolate reductase (MTHFR) and paraoxonase genes.

Alleles	Cases (n = 203)	Controls (n = 212)	χ^2	P	OR	95% CI
ACE and MTHFR						
I+/T+	48 (23.6%)	35 (16.5%)	3.30	0.06	1.57	0.94–2.62
I+/T–	101 (49.7%)	104 (49.0%)	0.02	0.88	1.03	0.69–1.54
I–/T+	11 (5.4%)	21 (9.9%)	2.93	0.08	0.52	0.23–1.17
I–/T–	43 (21.2%)	52 (24.5%)	0.66	0.41	0.83	0.51–1.34
MTHFR and paraoxonase						
T+/G+	34 (16.7%)	26 (12.3%)	1.69	0.19	1.44	0.80–2.59
T+/G–	25 (12.3%)	30 (14.1%)	0.30	0.58	0.85	0.46–1.56
T–/G+	86 (42.4%)	61 (28.8%)	8.37	0.003	1.82	1.19–2.79
T–/G–	58 (28.6%)	95 (44.8%)	11.75	0.0006	0.42	0.32–0.76
ACE and paraoxonase						
I+/G+	91 (44.8%)	58 (27.3%)	13.75	0.0002	2.16	1.40–3.32
I+/G–	58 (28.6%)	81 (38.2%)	4.32	0.03	0.65	0.42–1.00
I–/G+	29 (14.3%)	29 (13.7%)	0.03	0.85	1.05	0.58–1.90
I–/G–	25 (12.3%)	44 (20.8%)	59.14	0.00000	0.11	0.06–0.21
ACE, MTHFR and paraoxonase						
I+/T+/G+ *vs* D+/C+/A+			5.84	0.01	2.24	1.10–4.59
I+/T+/G+ *vs* I–/T–/G–			9.91	0.001	3.78	1.50–9.63
I+/T+/G+ *vs* non I+/T+/G+			6.36	0.01	2.28	1.14–4.61

Table 5. Multivariate logistic regression analysis of single nucleotide polymorphisms of angiotensin-converting enzyme (ACE), methylene tetrahydrofolate reductase (MTHFR) and paraoxonase genes (adjusted for age, sex, hypertension, diabetes, and smoking).

Genes and polymorphisms	Genotype contrasts	OR	95% CI	P
ACE I/D	DD *vs* ID+II	0.71	0.46–1.10	0.13
	DD+ID *vs* II	1.04	0.66–1.62	0.87
MTHFR 677 C/T	TT *vs* CT+CC	3.87	0.99–15.18	0.05
	TT+CT *vs* CC	1.09	0.69–1.71	0.70
Paraoxonase 573 A/G	GG *vs* AG+AA	1.37	0.57–3.33	0.48
	GG+AG *vs* AA	2.03	1.35–3.04	0.0007

sis, and several other studies, have suggested a positive association [16, 17]. However, a number of other studies have reported negative results [18, 19]. Notable among them is the cohort study by Lindpaintner et al. [20], which found no association between the ACE I/D polymorphism and the risk of CAD. Another meta-analysis reported a mean OR of 1.26 for MI in DD homozygotes, which was later found to be due to the confounding effect of publication bias [15].

We did not observe any significant genotypic frequency differences for the ACE I/D polymorphism between the cases and controls in our study. The frequency distribution of the genotypes (DD 26.6%, I/D 43.3%, II 30%) was different compared to most other studies [21–23].

The D allele frequency was found to be lower in cases than controls, which contradicts observations made by others [18]. Studies in the Indian population are too scarce to make comparisons, and differences in the ethnicity of the population studied may lead to differences in the observed genotypic frequencies.

Homocystinuria was first associated with cerebrovascular disease in 1962 [24, 25]. It has since been observed that even moderate levels may be associated with atherosclerosis [26–29]. Genetic defects of the enzymes or dietary deficiency of

B-vitamin cofactors involved in this metabolism result in elevated homocysteine levels, which has been associated with an increased risk of coronary heart disease (CHD). Whether this association is causal remains uncertain [30, 31].

Observational studies have shown that individuals with low folate levels or intake have a higher risk of CHD, and it is possible that these associations may be independent of homocysteine [32–36]. A common polymorphism exists for the gene that encodes the MTHFR enzyme, which converts 5,10--methylene tetrahydrofolate to 5-methyltetrahydrofolate, required for the conversion of homocysteine to methionine.

Individuals who have a C-to-T substitution at base 677 of the gene have reduced enzyme activity and higher homocysteine and lower folate levels than those without this substitution [37–42]. A meta-analysis of 40 studies demonstrated that individuals with the MTHFR 677 TT genotype have a 16% higher odds of CHD compared to individuals with the CC genotype. The results support the hypothesis that impaired folate metabolism, resulting in high homocysteine concentrations, plays a causal role in the occurrence of CHD. The MTHFR gene has been cloned and sequenced, and disease-associated mutations have been identified [43]. In particular, a prevalent C-T polymorphism at nucleotide position 677 results in a conservative Ala to Val (A223V) replacement. This polymorphism is characterized by reduced enzyme activity and thermolability (tMTHFR) [44] and is likely to be an important genetic factor contributing to the variation in total plasma homocysteine (tHcy), which is recognized as an independent predictor of atherosclerotic disease, including stroke, MI, and peripheral vascular disease [45]. Elevated total homocysteine induces oxidative injury to vascular endothelial cells and impairs the production of nitric oxide, a strong vascular relaxing factor, from the endothelium. Hyperhomocysteinemia also enhances platelet adhesion to endothelial cells, promotes growth of vascular smooth muscle cells, and is associated with higher levels of prothrombotic factors such as thromboglobulin, tissue plasminogen activator and factor VIIIa.

The association between the MTHFR C677T polymorphism, the strongest genetic determinant of moderate hyperhomocysteinemia, which has been found to be a marker of CAD, has been studied in different settings. The strongest associations have been reported in European and Asian studies, whereas a similar trend among North American studies did not reach statistical significance [46]. The T allele in the present study was not found to be associated with higher CAD risk. Association of T allele with CAD risk has been reported variably in different studies [46–50].

A recent meta-analysis of 80 studies has given an estimate of a 14% (95% CI 5–24%) greater risk of CAD associated with the MTHFR CC genotype [48]. We however observed a slight predominance of CC genotypes in the controls. Differences in the ethnicity of the study population could explain the differences observed.

Mechanisms underlying the protective effect of HDL cholesterol (HDL-C) against CHD are not fully understood. One plausible theory is that the mechanism is related to the antioxidant properties of HDL-C, which in turn is determined by its enzymes, in particular paraoxonase 1 (PON1) [51–55]. These findings have led to the suggestion that PON1 activity has a role in susceptibility to atherosclerotic disease [52–55]. Recent studies indicate that PON is one of the enzymes responsible for the antioxidative and anti-inflammatory properties of the HDL [56, 57].

In vitro studies indicate that PON can significantly reduce lipid peroxide generation during LDL oxidation, and thus may provide HDL-associated protection against atherosclerosis. Several case--control studies have assessed the association of the *PON1* G192A polymorphism with CHD. A strong association between a polymorphism in the *PON1* gene at position 192 and PON1 activity has been found [58, 59]. A recent review and meta-analysis of these studies found a weak overall effect, such that there was no effect when results were pooled for studies including 500 or more cases [60].

In the present study, we evaluated the role of PON1-573 polymorphism and found it to be associated with an increased CAD risk before and after controlling for confounding factors. Other studies have already analyzed the role of *PON1-192* and *PON2-311* polymorphisms in CAD risk, finding variable results [52, 61–65].

The AA genotype was found to yield protection against the CAD as compared to the non AA genotypes as well as the AG genotype. The odds of having the CAD were higher with the GG genotype but the association was not found to be significant. The G allele was associated with a significantly increased risk as compared to the A allele.

In the present study, polymorphism in ACE and MTHFR genes individually did not show any association with CAD. However, lack of an individual association for each of these polymorphisms does not rule them out as risk factors. These alleles might still confer susceptibility to CAD through

gene-gene interaction, or gene-environment interaction modulated through the involvement of multiple physiological pathways. This is quite evident from the associations observed after evaluating for the synergistic effects of these genes in the present study. Although our study results must be validated in larger population-based studies, the synergistic effects observed are quite intriguing.

It is also possible that the examined polymorphisms may not be risk factors themselves, but might be in linkage disequilibrium with another genetic variant with a definite risk. A comprehensive analysis of all SNPs for each gene with an independent positive correlation or in combination with another gene would assist in the identification of the causal gene variants associated with disease.

The role of the environment and other host factors in contributing to the susceptibility of multifactorial diseases in individuals with one or more predisposing allele is well-known [66]. An environmental vulnerability always persists for genetically susceptible individuals. Evasion of these factors, even in the presence of genetic susceptibility, can therefore postpone, or even prevent, the development of the disease. Since CAD, like most other chronic diseases, is mostly an irreversible process, prevention is always better than cure.

Identifying 'at risk' individuals by genetic mapping of susceptible genes for effective control of other host factors will therefore be a very effective and practical approach. Apart from their preventive role, these studies also assist in the development of improved therapy for patients. Our study suggests the existence of heterogeneity in the genetic risk factors that could be population-specific, and calls for rigorous approaches involving a larger study group for an in-depth analysis.

Limitations of the study

The study is limited by the fact that only CAD patients who arrived at hospital alive were recruited; therefore, a possible survival bias should be taken into account. Nevertheless, this limitation is inherent in all case-control studies. Another limitation is that serum values could only be determined for those CAD patients who survived for at least six months after the event. On the other hand, the other variables of interest were measured during hospital stay.

Conclusions

The association of paraoxanase with CAD, as well as the effect of synergism of two and three genes on CAD, is an interesting observation. Continued observations in larger, well-defined populations, with prospective follow-up and control for other genetic and environmental factors of relevance, would lead to a better understanding of the genetically determined risk of MI.

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