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CARDIOVASCULAR DISORDERS | RESEARCH ARTICLE

Association of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms with coronary artery disease (CAD) in a North Indian population

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Abstract: There is significant variation in reported associations of the MTHFR C677T (rs1801133) and A1298C (rs1801131) polymorphisms and coronary artery disease (CAD) in different global populations. This study aims to identify any individual or combined associations between the 1298 and 677 loci of MTHFR and CAD in a North Indian population. A total of 159 patients and 166 controls were genotyped using validated TaqMan assays. Odds ratio analysis identified associations at crude level and multiple logistic regression controlled for confounding variables. Linkage disequilibrium between the loci was assessed along with haplotype association analysis. At the C677T locus, homozygosity of the T allele identified a significantly protective association (OR = 0.38, CI: 0.24–0.60). For the A1298C locus the AC genotype had a protective effect in codominant model (OR = 0.53, CI: 0.32–0.85) and CC genotype showed a susceptible association in recessive model when controlled for age, sex and lipids (OR = 2.70, CI: 1.27–5.77). This study identified that, independently, both heterozygous genotypes show a protective association with CAD. In addition the CC genotype of A1298C in recessive model was a susceptible genotype. The combined associations of MTHFR are protective (primarily due to the effects of C677T locus) suggesting an interaction between the loci and their associations with CAD within this sample.

Subjects: Bioinformatics; Genetics; Human Biology; Molecular Biology; Medicine; Medical Genetics; Cardiology; Clinical Nutrition

ABOUT THE AUTHORS

Stephen Butler and Aaron Young were undergraduate students who carried out laboratory based genetic analysis which were supervised by Sarabjit Mastana, Senior Lecturer in Human Genetics, and Elizabeth C. Akam, Lecturer in Biochemistry, who together planned and carried out these analyses.

Nakul Sinha is Professor of Cardiology and did clinical evaluation and recruitment of participants.

Suraksha Agrawal is Professor of Medical Genetics and who planned and coordinated this research.

PUBLIC INTEREST STATEMENT

This is a first report regarding role of MTHFR polymorphisms in coronary artery disease (CAD) in a single centre, well characterised sample. CAD is responsible for nearly half of the deaths at global level and Indians are more prone to early heart disease. Our understanding of its genomic biology is still limited. There are only limited number of studies on role of MTHFR in CAD among Indian populations and none from this region. In this study, we observed that individual and combination of polymorphisms do decrease the CAD risk which needs further confirmation using larger, multi-centric opportunities for resolving the genetics of CAD in India.

Keywords: methylenetetrahydrofolate reductase; CAD; polymorphism

1. Introduction

Cardiovascular diseases (CVDs) are currently the world's number one killer, causing 17.7 million deaths in 2015 and accounting for almost 31% of global deaths (World Health Organisation [WHO], 2017). It encompasses a variety of circulatory diseases, most notably coronary artery disease (CAD), which causes approximately 7 million deaths worldwide each year (Sharma et al., 2014). Characterised by the progressive build-up of atherosclerotic plaques within the coronary arteries, the disease is a result of myocardial ischemia causing the death of cardiac muscle tissue and resulting in cardiac arrest and possible mortality. It has been estimated that the prevalence of CAD within the population in India is currently around 10%. CAD is multifactorial and polygenic with a range of genetic polymorphisms and their products contribute to disease inception, progression and severity.

Homocysteine is a homologue of the amino acid cysteine and is synthesised from methionine via a multi-step process and has two primary fates, the conversion via tetrahydrofolate (THF) back into methionine or conversion to cysteine. Homocysteine is involved in the promotion of platelet activation, hypercoagulability, oxidative stress, endothelial dysfunction, smooth muscle proliferation and oxidation and peroxidation of lipids, all of which are associated with CVD via the atherosclerotic pathway (Refsum & Ueland, 1998). However, the full mechanisms for vascular damage induced by hyperhomocysteinemia still remain unclear.

Methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes in the metabolism of homocysteine that catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body and a carbon donor for the conversion of homocysteine to methionine in the remethylation pathway (Bailey and Gregory 1999). With the impaired function of the enzyme, plasma total homocysteine (tHcy) is able to increase to a point at which it becomes an independent risk factor for CAD. Reduced activity of the enzyme can lead to increased levels of plasma tHcy, resulting in hyperhomocysteinemia (HHcy). The association between HHcy levels and CAD has been found to vary in different populations following different dietary practices, smoking, and inhabiting different environmental conditions. There was no significant association identified in Southern (Deepa et al., 2001; Sastry et al., 2001) or Western India (Nair, Nair, Ashavaid, Dalal, & Eghlim, 2002), whereas a study from the UK (Chambers et al., 2000) and Northern India (Khare, Ghosh, Shetty, Kulkarni, & Mohanty, 2004; Puri et al., 2003) found HHcy to be significantly associated with CAD. Furthermore, evidence suggests that a 10% increase in homocysteine levels can be matched by the increased risk of CAD (Varga, Sturm, Misita, & Moll, 2005).

The MTHFR gene situated on the short branch of chromosome 1 (1p36.3) encodes for MTHFR enzyme and consists of ~17 kb, which include 11 exons spanning 2.2 kb (Goyette et al., 1998). A total of 34 rare mutations in the MTHFR gene, as well as a total of 9 common variants have been reported in studies examining MTHFR deficiency (Goyette et al., 1998). The common variants include: C677T (rs1801133), A1298C (rs1801131) and ARG184TER (rs121434294).

The MTHFR C677T transition is a missense mutation in the exon 4 of this gene. This converts an alanine to a valine at codon 222 in the N-terminal catalytic domain of the protein leading to a thermolabile protein, with decreased enzymatic activity (Unfried et al., 2002). The T allele modulates the levels of hyperhomocysteinemia and cardiovascular risk in different populations. Some studies have reported direct effect of this allele on homocysteine levels in European and other populations (Gudnason et al., 1998; Huang et al., 2011; Pitsavos et al., 2006) while others report no associations (Chambers et al., 2000) primarily due to the influence of various dietary and environmental factors. Chambers et al. (2000) found that homocysteine levels among Indians living in the UK, showed no association with MTHFR-T allele, which was partially explained by low folate and

vitamin B12 levels, highlighting that MTHFR-homocysteine-CAD associations may be influences by various dietary, environmental, ethnic and other factors, including gene-gene, gene-environment interactions. Some studies have documented the T allele to be significantly higher in CAD patients than controls (Dhar et al., 2010; Ezzat et al., 2014; Falchi et al., 2005; Ghazouani et al., 2009; Sinha et al., 2010; Vinukonda, Mohammad, Jain, Chintakindi, & Akella, 2009; Yu et al., 2014). Homozygosity of the T allele at the 677 locus has been found to be causal in some studies, while many studies identified heterozygosity to be significantly causal (Dhar et al., 2010; Ezzat et al., 2014; Freitas et al., 2008; Ghazouani et al., 2009; Sinha et al., 2010).

The second mutation in MTHFR A1298C is a point mutation in exon 7. This transversion causes a glutamate to alanine substitution at codon 429, within the C-terminal regulatory domain of the protein (Ananth et al., 2007). This polymorphism also causes a reduction in MTHFR activity, although its effect is considered to be less than that conferred by the C677T transition (Le Marchand, Wilkens, Kolonel, & Henderson, 2005). The differences in levels of attenuation conferred by the different polymorphisms have been investigated in some studies, which postulate that the C677T and A1298C polymorphisms reduce the activity of MTHFR by 60% and 35%, respectively (Sinha et al., 2010).

The A1298C polymorphism is less well documented, with variable association results in different populations. Much of the research into the area suggests that the variant "C" allele is associated with higher levels of CAD (Kumar et al., 2005; Sinha et al., 2010; Szczeklik et al., 2001). However, other studies have found 1298AA genotype (Freitas et al., 2008) and 1298AC genotype (Laraqui et al., 2007; Sinha et al., 2010) to be associated with CAD. Few studies have combined the two loci; however, it has been shown that the combined additive effect of homozygosity in both the C677T and A1298C loci leading to significantly higher levels of plasma tHcy in the Portuguese population (Castro et al., 2004). Heterozygosity at both loci was found to be significantly causal in Portuguese (Freitas et al., 2008) and Tunisian (Ghazouani et al., 2009) populations.

The aims of the current study were to document the genetic variation of MTHFR C677T and A1298C loci and evaluate genetic associations with CAD in a well characterised North Indian population.

2. Methods

The study population consisted of 166 healthy controls and 159 patients with diagnosed CAD, from Uttar Pradesh, North India. Patients were classified on the basis of at least 50% or more stenosis in one or more coronary arteries, verified through coronary angiography. The healthy controls had no known history of ischemic heart disease, hypertension, diabetes, endocrine or metabolic disorders and were selected after administering a treadmill test to exclude the possibility of the patients having an underlying CAD (Rai, Sinha, Finn, Agrawal, & Mastana, 2016; Rai et al., 2012). The DNA samples were collected with full written consent and study protocol was approved by SGPGIMS Lucknow ethics committee and Loughborough University. Clinical data was gathered on: age, gender, diet (vegetarian vs. non-vegetarian), smoking-status and lipid profiles (plasma total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL) and apolipoprotein B (ApoB)). DNA extraction was done through the use of organic methods, as detailed in Rai et al. (2012).

MTHFR C677T (rs1801133) and A1298C (rs1801131) polymorphisms were genotyped by sensitive TaqMan® SNP Genotyping kits (Assay IDs C_1202883_20 and C_850486_20 respectively) on StepOne Plus QPCR machine (Applied Biosystems).

Statistical analysis was carried out using the computer packages EXCEL and SPSS (version 21.0). The patient and control databases were both assessed for Hardy–Weinberg Equilibrium (HWE) by calculating a chi-square value. Association statistics like odds ratios (OR) associated confidence intervals and *p* values were calculated using online programme called SNPSTATs

(http://bioinfo.iconcologia.net/SNPstats) (Solé, Guinó, Valls, Iniesta, & Moreno, 2006). Clinical and life style parameters were analysed using a Kruskal-Wallis test. A *P* value <0.05 was considered statistically significant (for the analysis of two SNPs, significant *p* value of 0.025 was used). SNPstats programme was also used for adjustments for various confounding parameters and calculation of linkage disequilibrium and construction of haplotypes.

Sample size was calculated using Quanto' software (http://biostats.usc.edu/Quanto.html), which allowed an estimation of a required sample size of 153 patients and 153 controls to achieve an OR of 1.5 and above at 80% power. As the genotyping was blinded, 19 (6 patients and 13 controls) extra samples were analysed to allow for any failures or missing data points. Overall success rate for both loci was 96.7%.

3. Results

Analysis of the clinical data through a Kruskal-Wallis test (Table 1) identified a significant difference in age and all lipid parameters. To control for these differences adjusted OR were calculated in addition to crude OR. HWE was violated in controls for C677T locus ($P < 0.001$), therefore these results should be considered with caution. Allele frequencies and genotype distribution of controls were compared with 1000 genome and Indian populations (IGSR;Saraswathy et al., 2012; Sinha et al., 2010). At C677T locus, T allele in current control group (33%) is higher than observed in 1,000 Genome South Asian populations (8.3–15%, average 12%) but comparable to many European populations (27–46%). At A1298C locus (rs1801131), genotype and allele frequencies showed no differences (40% vs. 42%).

At the C677T locus (rs1801133), significant differences were noticed in heterozygosity when comparing controls versus patients (56.0% and 34.6%, respectively). TT genotype frequency was low in both groups, while CC genotype was more frequent in patients (63.5%) compared to controls (39.2%). Overall, there was a significant difference between patients and controls (chi-square 19.69, 2df, $P< 0.001$). At the A1298C locus (rs1801131), again, there was lower level of heterozygosity in patients compared to controls (35.9% vs. 53.0%) and higher frequency of both homozygotes. This led to significant differences between the two groups (10.03, 2df, $P< 0.01$).

The ORs were calculated using codominant, recessive and log-additive (allelic) models at crude level and with adjustments for clinical parameters (age, sex and lipids) and the results are given in Table 2. At the C677T locus, CT and TT genotypes in codominant mode show statistically significant protective effects (OR = 0.38 (CI 0.24–0.60, $P < 0.001$) and OR = 0.24 (CI 0.06–0.94, $P < 0.001$)) and these effects were stronger when adjustments were made for age, sex and lipid parameters. A similar protective effect is observed in log additive/allelic model but the recessive model showed a non-significant effect. Caution is warranted as the number of TT genotypes observed in the study is relatively low and the control population is not in HWE.

At A1298C locus, it is evident that heterozygosity provides a significant protection from CAD with an OR of 0.53 (CI: 0.32–0.85, $P < 0.01$) when compared to an AA genotype, which remains significant after adjustments (OR = 0.49, CI:0.25–0.93, $P < 0.01$). The variant CC genotype shows susceptible effect with odd ratios of 1.71 (CI: 0.95–3.09, $P = 0.07$) in crude recessive model and is statistically significant when adjusted for age, sex and lipids (OR = 2.70 (CI: 1.27–5.77, $P = 0.008$)).

Haplotypes were constructed using SNPStats programme; patients had a relatively higher frequency of C/C haplotype (C677T/A1298C) (44.7% vs. 31.0%) (Table 1) while controls had a higher frequency of T/A haplotype (29.9% vs. 16.7%) leading to protective associations in all combinations (Tables 1 and 2). Linkage disequilibrium D′ statistics was of medium range (D′ = 0.684) but statistically significant ($P < 0.001$).

Table 1. Descriptive statistics of clinical data and summary of genotyping results

Parameter (SI)	Patients *n* = 159 (Mean ± SD)	Controls *n* = 166 (Mean ± SD)	P Value
Age (Years)	48.72 ± 11.87	44.55 ± 13.79	0.004*
Gender (Male/Female)	137/22	137/29	0.55
Smokers (Yes/No)	98/61	90/76	0.15
Diet (Non Veg/Veg	91/68	85/81	0.22
S.TC (mg/dL)	175.50 ± 53.11	136.05 ± 31.41	<0.001*
S.TG (mg/dL)	201.71 ± 91.42	138.49 ± 59.12	<0.001*
S.HDL (mg/dL)	31.78 ± 9.29	27.10 ± 9.75	<0.001*
S.LDL (mg/dL)	103.26 ± 46.90	82.08 ± 24.53	<0.001*
S.VLDL (mg/dL)	41.63 ± 16.95	28.55 ± 12.16	<0.001*
ApoB (mg/dL)	147.97 ± 46.70	104.28 ± 44.93	<0.001*
C677T Genotype Frequencies (n (%))			
CC	101 (63.5%)	65 (39.2%)	
CT	55 (34.6%)	93 (56.0%)	
TT	3 (1.9%)	8 (4.8%)	
HWE p value	0.15	0.001^	
Allele Frequencies ± SE			
C	0.81 ± 0.02	0.67 ± 0.03	
T	0.19 ± 0.03	0.33 ± 0.03	
Patients vs. Controls: Chi-square and p value: 19.69, 2DF, P value <0.001			
A1298C Genotype Frequencies (n (%))			
AA	69 (43.4%)	56 (33.7%)	
CA	57 (35.9%)	88 (53.0%)	
CC	33 (20.8%)	22 (13.3%)	
HWE p value	0.002^	0.17	
Allele Frequencies ± SE			
A	0.61 ± 0.03	0.60 ± 0.03	
C	0.39 ± 0.03	0.40 ± 0.03	
Patients vs. Controls: Chi-square and p value: 10.03, 2DF, Pvalue = 0.007			
Observed Haplotype Frequencies C677T/A1298C %			
C/A	44.7	31.0	
C/C	36.2	36.2	
T/A	16.7	29.3	
T/C	2.5	3.6	

D′ statistics = 0.684; *p* value <0.001.
**T*-test or non-parametric test *p* values. All *P* values are quoted to a maximum of 3 d.p.
^Significant departure from Hardy-Weinberg equilibrium.

Genotype-wise analysis of lipids and other parameters did not reveal any significant pattern for both loci (results not shown) but MTHFR genotype combinations (Diplotypes) were also evaluated for genetic associations in crude model and results are given in Table 3. It is clear from Table 3 that combination of heterozygous genotypes, 677CT/1298CA, protects against CAD in this sample (OR = 0.14, CI: 0.06–0.32, $P < 0.001$). Further analysis using a Kruskal-Wallis test identified that the presence of a CT/CA diplotype versus a CC/AA diplotype was associated with a significantly lower HDL ($P = 0.003$), LDL ($P = 0.027$) and TC ($P = 0.046$) levels.

Table 2. Crude and adjusted odds ratios for MTHFR C677T and A1298C loci among CAD patients and controls

		Odds ratios	
Model	**Genotype/Allele**	**Crude**	**Adjusted for age, sex and lipids**
C677T (n = 325)			
	CC	1 (Ref)	1 (Ref)
Codominant	CT	0.38 (0.24–0.60)*	0.34 (0.19–0.61)*
	TT	0.24 (0.06–0.94)*	0.16 (0.03–.92)*
	P Value	<0.001*	0.001*
Recessive	CC+CT	1 (Ref)	1 (Ref)
	TT	0.38 (0.10–1.46)	0.26 (0.05–1.49)
	P value	0.14	0.09
Log additive	*T**	0.41 (0.27–0.61)*	0.35 (0.21–0.60)*
	P value	<0.001*	0.001*
A1298C (n = 325)			
	AA	1 (Ref)	1 (Ref)
Codominant	AC	0.53 (0.32–0.85)*	0.49 (0.25–0.93)*
	CC	1.22 (0.64–2.32)	1.84 (0.80–4.22)
	P value	0.01*	0.003*
Recessive	AA-AC	1 (Ref)	1 (Ref)
	CC	1.71 (0.95–3.09)	2.70 (1.27–5.77)*
	P value	0.07	0.008*
Log additive	C*	0.96 (0.71–1.30)	1.15 (0.77–1.70)
	P value	0.78	0.50
Haplotype association analysis C677T and A1298C (n = 325)			
	C/A	1 (Ref)	1 (Ref)
	C/C	0.71 (0.49–1.05)	0.80 (0.49–1.31)
	T/A	0.34 (0.21–0.56)*	0.29 (0.16–0.56)*
	T/C	0.36 (0.11–1.18)	0.35 (0.08–1.48)
P value		<0.001	<0.001
	Global Haplotype association *P* value	<0.001*	<0.001*

*Chi-square *P* values, quoted to a maximum of 3 d.p.

4. Discussion

The dissimilarity in allele frequencies between the patients (19%) and controls (33%) for the variant T allele, at position 677 (rs1801133), suggests this allele may have a CAD protective effect. This finding was further confirmed by the OR and associated confidence intervals in various combinations (crude and adjusted) where T allele and CT and TT genotypes are observed to be protective with OR values lower than 1. This conclusion differs from some previous studies proposing the T allele as causal (Dhar et al., 2010; Ezzat et al., 2014; Falchi et al., 2005; Ghazouani et al., 2009; Sinha et al., 2010; Vinukonda et al., 2009; Yu et al., 2014). One possibility for this apparent divergence could be due to the low number of TT genotypes observed in this study and departure from HWE in controls, so caution is required in interpretations here. That said, the association of the T allele (C677T (rs1801133)) with CAD is currently inconsistently reported with both causal and protective roles proposed (Wald, Law, & Morris, 2002; Zee et al., 2007). A meta-analysis of 80 studies has given an estimate of a 14% greater risk of CAD associated with the CC genotype (Lewis, Ebrahim, & Davey Smith, 2005), which supports our study illustrating an

Table 3. Combined analysis of MTHFR C677T and A1298C genotypes

Genotype combinations (C677T/A1298C)	Odds ratio	95% confidence intervals	Chi-square	*P*-Value*
CC/AA	1.00	Reference		
CC/AC	0.38	0.17–0.83	5.12	0.024
CC/CC	0.69	0.28–1.69	0.33	0.564
CT/AA	0.33	0.15–0.73	6.65	0.010
CT/CA	0.14	0.06–0.32	22.23	<0.001
CT/CC	0.27	0.07–0.99	2.93	0.087
TT/AA	0.14	0.03–0.61	6.24	0.012
TT/AC	NA			
TT/CC	NA			

*Chi-square *P* value, quoted to 3 d.p.

increased suseptibility with the CC genotype (OR = 4.14 (CI = 1.06–16.19)) and the ancestral C allele (OR = 2.09 (CI = 1.44–2.96)).

The overall low frequency of the TT genotype (C677T (rs1801133)) in this study (patients 1.9% and controls 4.8%) is comparable to results from 23 populations from different parts of India where TT genotype frequency and T allele frequency are also low (Saraswathy et al., 2012). A significantly higher incidence of the TT genotype has been reported in an Eastern Indian study (patients 26.7% and controls 12.9%; Dhar et al., 2010), yet it is anticipated that this simply reflects geographical variation (Saraswathy et al., 2012). The TT genotype would be expected to be more frequent among patients as it is likely to be more deleterious (Sinha et al., 2010), however, the current study shows a slightly higher frequency among controls. This may be due to the small sample size but Sinha et al. (2010) suggest that the control individuals with TT genotypes coupled with favourable biochemical markers are able to survive, whereas TT patients are in survival disadvantage with unfavourable biochemical markers (high TG and LDL) and thus being selected against.

The similarity in allele frequencies for MTHFR A1298C (rs1801131) between the patients and controls suggests that in this population there is no significant CAD association. This is further reinforced by the crude OR analysis with an OR close to 1 being observed (0.96 (CI: 0.71–1.31) refer Table 2). The overall spectrum of observed variation for the C allele was similar to other Indian and 1,000 genome populations (1,000 genome; Saraswathy et al., 2012), but it should be noted that these were higher than observed in European populations (Freitas et al., 2008; Kölling et al., 2004).

Genotype frequencies for MTHFR A1298C (rs1801131) differed between cases and controls, with key differences being in the higher proportion of homozygosity in patient's (Table 1). The observed genotypes identified in this study differ from previous studies, which report lower proportions of homozygosity at MTHFR A1298C (rs1801131) in different populations (Freitas et al., 2008; Kölling et al., 2004; Sinha et al., 2010). The observed homozygosity of the MTHFR A1298C variant allele appeared to increase the CAD susceptibility (Table 2) but this association did not attain statistical significance in the codominant model for crude or adjusted analyses. In the recessive model (CC vs. AA + AC), the effect was higher in crude analysis (OR = 1.71, CI 0.95–3.09), and remained statistically significant when adjusted for age, sex and lipids (OR = 2.57, CI 1.22–5.45). The heterozygous genotype AC showed a CAD protective effect in all analyses (Table 2) that would support the recessive nature of homocystinuria (Mandava & Kent, 2017). The key loci effect apears to lie in heterozygosity, Freitas et al.

(2008) and Janošíková et al. (2003) have also identified CAD protective implications of heterozygosity in Portuguese and Czech populations respectively. Yet, no clear trend in the effect of the locus on the activity of MTHFR is seen in areas, such as Tunisia (Ghazouani et al., 2009), Poland (Szczeklik et al., 2001) and Delhi (Sinha et al., 2010) where heterozygosity appears to have a potentially causal effect.

Independently, both C677T and A1298C polymorphisms displayed heterozygosity as significantly protective (Table 3). It is proposed that with the addition of the heterozygous 677CT genotype to each of the 1298 genotypes the nature of the combined genotype becomes increasingly protective (Table 3). The heterozygous combination, CT/CA, presented the highest significance with a protective nature ($p < 0.001$) due to a potentially interactive relationship however; combining the two most causal genotypes did not have the same effect. This would suggest that the allelic composition, in particular the 677 alleles, influences the overall interaction of the loci and their association with CAD. Kruskal-Wallis testing identified that the presence of a CT/CA genotype versus a CC/AA was associated with a significantly lower HDL ($P = 0.003$), LDL ($P = 0.027$) and TC ($P = 0.046$). This suggests that the pathogenesis of the combined genotypes; resulting in protective and causal natures, involve HDL, LDL and TC, which are extremely important risk factors for CAD. This points towards a relation of other potential confounding variables (diet, dietary fats, smoking, etc.) with causation of CAD and thus it could be considered that the MTHFR polymorphisms are not independent risk factors for CAD in the present population.

It has been suggested that the C677T mutation can decrease the enzymatic activity of MTHFR enzyme by almost 60% and that the A1298C mutation could decrease the enzymatic activity by about 35%. There is a highly significant ($P < 0.001$) protective association for the T/A haplotype at crude (OR = 0.34, CI: 0.21–0.56) and adjusted models (OR = 0.30, CI: 0.16–0.57, $P < 0.001$) (Table 2). Comparably, Sinha et al. (2010) showed the T/A haplotype to be protective with a similar OR suggesting that the C677T mutation when combined with the wild-type 1298A allele is protective within an Indian population. However, in the Portuguese (Freitas et al., 2008) and Tunisian (Ghazouani et al., 2009) populations, a significant causal association was identified for the T/A haplotype with an OR of 1.24 (CI: 1.00–1.54) and 2.35 (CI: 1.62–3.38), respectively, illustrating ethnic differences.

Few recent studies have attempted to compare C677T and A1298C genotype distributions between CAD patients and controls (Alam et al., 2007; Bennouar et al., 2007; Freitas et al., 2008). Different samples sizes and different ethnic backgrounds mean the studies are non-contiguous, however these studies agree when reporting a significant association between 677TT genotype and hyperhomocysteinemia. However, only one of the studies found a significant association with CAD (Bennouar et al., 2007), potentially proposing population specificity of the CAD hyperhomocysteinemia association. It is reported that the expression of MTHFR mutations is both Vitamin B12 and folate dependent (Yajnik et al., 2006), so, it could be suggested that the C677T polymorphism is only associated with an increased risk of CAD when compounded by low Vitamin B12 and/or folate (Klerk et al., 2002). Folic acid deficiency may be relatively uncommon in Indians compared to the western world mainly due to their principally vegetarian diet but a high prevalence of Vitamin B12 deficiency is reported among Indians (Sinha et al., 2010; Yajnik et al., 2006). As Vitamin B12 is responsible for metabolising folic acid, its deficiency in the diet alone is likely to cause hyperhomocysteinemia due to unmetabolised folic acid, thereby increasing the risk of CAD (Sinha et al., 2010). So, the differing associations found between C677T polymorphism and CAD are potentially mediated through Vitamin B12 deficiency, thus, supplementation could be recommended for those individuals carrying causal alleles as a protective step predisposing to CAD.

In conclusion, further study with a larger sample size, including the assessment of vitamin status, amongst other confounding variables, such as hypertension and plasma homocysteine levels, to better clarify the relationship between MTHFR genotypes and CAD in various Indian populations is warranted.

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