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REGULAR ARTICLE

Methylenetetrahydrofolate reductase gene, homocysteine and coronary artery disease: The A1298C polymorphism does matter. Inferences from a case study (Madeira, Portugal)

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Abstract

Elevated levels of plasma homocysteine, an independent risk factor and a strong predictor of mortality in patients with coronary artery disease (CAD), can result from nutritional deficiencies or genetic errors, including methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms. The contribution of these polymorphisms in the development of CAD remains controversial. We analysed the impact of MTHFR C677T and A1298C on fasting homocysteine and CAD in 298 CAD patients proved by angiography and 510 control subjects from the Island of Madeira (Portugal). After adjustment for other risk factors, plasma homocysteine remained independently correlated with CAD. Serum homocysteine was significantly higher in individuals with 677TT and 1298AA genotypes. There was no difference in the distribution of MTHFR677 genotypes between cases and controls but a significant increase in 1298AA prevalence was found in CAD patients. In spite of the clear effect of C677T mutation on elevated homocysteine levels we only found an association between 1298AA genotype and CAD in this population. The simultaneous presence of 677CT and 1298AA genotypes provides a significant risk of developing the disease, while the 1298AC genotype, combined with 677CC, shows a significant trend towards a decrease in CAD occurrence. The data shows an independent association between

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elevated levels of homocysteine and CAD. Both MTHFR polymorphisms are associated with increased fasting homocysteine (677TT and 1298AA genotypes), but only the 1298AA variant shows an increased prevalence in CAD group. Odds ratio seem to indicate that individuals with the MTHFR 1298AA genotype and the 677CT/1298AA compound genotype had a 1.6-fold increased risk for developing CAD suggesting a possible association of MTHFR polymorphisms with the risk of CAD in Madeira population.

Introduction

Fasting plasma homocysteine (Hcy) is a graded and independent risk factor for coronary and other forms of vascular disease [1–3]. Individual variation in Hcy levels is related to several biological traits and is strongly influenced by dietary habits, specifically the intake of vitamins such as folate, vitamin B6 and B12 [4]. Deficiencies in these vitamins, which are essential coenzymes in homocysteine metabolism, elevate plasma Hcy levels [5,6].

The methylation of homocysteine into methionine is catalysed by the 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme. Gene polymorphisms of those enzymes involved in the Hcy metabolism, particularly MTHFR, are well known and some are the cause of mild increases in the levels of circulating Hcy [7]. MTHFR gene polymorphisms C677T and A1298C are common in human populations. The C677T mutation (Alanine→Valine), resulting in a thermolabile enzyme, is associated with mild hyperhomocysteinemia and possibly with an increased risk of CAD [8]. Some studies showed positive associations [8–10] between MTHFR TT homozygotes and risk of CAD, but other studies have failed to demonstrate such a relationship [11–13]. A recent meta-analysis concluded that individuals with the 677TT genotype had 16% higher odds of CAD compared with individuals with the CC genotype [14]. However, the results were heterogeneous, being significant in European populations but non significant in North Americans, probably as a consequence of the interaction between the MTHFR polymorphism and folate status. The apparent discrepancy between the risk attributable to plasma Hcy and to the MTHFR C677T polymorphism may therefore be partly explained by the complexity of the nutritional/genetic interaction underlying the homocysteine metabolism [4]. In brief, most prospective and retrospective studies to date have indicated that mildly elevated plasma Hcy is independently associated with CAD, and that the MTHFR TT genotype is associated with elevated Hcy. Paradoxically, most studies have not demonstrated an association between CAD and TT genotype.

Another polymorphism in MTHFR, 1298 A→C mutation, results in an amino acid change of glutamate to alanine in the regulatory C-terminal domain of the enzyme [15]. In vitro, 1298C carriers showed a decreased enzyme activity, suggesting a functional importance of the A1298C polymorphism although through an unknown molecular mechanism [16]. Nevertheless, it appears that the A1298C polymorphism alone does not significantly affect plasma Hcy but may be determinant when combined with the 677T variant [17].

The main focus of the present study was to analyse two markers belonging to the MTHFR gene, alone or combined in haplotypes and clarify their potential association with Hcy values and the presence of CAD related conditions in a series of patients who underwent coronary angiography.

Materials and methods

The total population of this study consisted of 808 Caucasian individuals (aged between 18 and 70 years old) divided in two groups; 510 subjects without a history of cardiovascular disease (CAD), randomly selected from the electoral rows, who participated as controls, plus 298 individuals recruited from patients admitted to the Cardiology Care Unit of the main hospital of Funchal (Hospital Central do Funchal, Madeira Island, Portugal). All subjects gave informed consent to participate in this research and the work was approved by the hospital ethics committee. Recruitment of cases satisfied the following criteria: stable coronary disease suggested by clinical analysis and proved by a coronary angiographic exam ("we considered the existence of a significant coronary lesion when over 50% of the left main or over 75% of one or more of the major coronary arteries and their branches, right coronary artery, left anterior descendent or circumflex were obstructed.") and occurrence of acute myocardial infarction. To inquire the family clinical history, the subjects were questioned about the existence of diabetes, essential hypertension or coronary disease in first degree relatives (parents and brothers or sisters). Premature death related to any of these causes was also registered. Cases and controls filled in a questionnaire about their personal histories (age, sex, essential hypertension, diabetes mellitus, smoking habits, dyslipidaemia, overweight, sedentary habits, alcohol ingestion and family history and provided blood samples for genotype analysis and biochemical measurements.

Genetic and biochemical analysis

Genomic DNA was extracted from an 80 µl aliquot of whole blood collected in EDTA using standard phenol/chloroform methodologies with ethanol precipitation. The extracted DNA was stored at −20 °C until analysis.

MTHFR C677T polymorphism was assayed by PCR amplification using primers and conditions previously described [18]. 10 µl of PCR product was digested overnight at 37 °C with 1 unit of HinfI in a final volume of 20 µl according to the manufacturers' instructions (New England Biolabs). This reaction yielded fragments of 198 base pairs in the presence of the C allele, and 175 and 23 base pairs in the presence of the T allele. Digestion results were submitted to electrophoresis in T9C5 polyacrilamide gels and visualised after silver staining. The A1298C mutation of the MTHFR gene was amplified by PCR as previously described [19], sequenced using BigDye terminator kit (Applied Biosystems) and visualised in an ABIPrism 310 Automatic Sequencer.

Fasting plasma homocysteine was determined by HPLC. Serum total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were measured using a Hitachi 911 Automatic Analyser.

Statistical analyses

Basic genetic parameters such as allele and genotype frequencies at each locus, proportion of individual heterozygous samples (direct count heterozygosity, H_o, as well as the unbiased estimate, H_e) and population differentiation were calculated using Genepop v3.1d [20] and Arlequin [21]. Haplotype frequencies were calculated using the PHASE 2.0 software [22]. The overall genetic diversity (H_T), within each group diversity (H_S) and the amount among groups (D_{ST}) were also calculated. Deviation from Hardy–Weinberg equilibrium per population and locus was calculated according to F_{IS} estimator [23]. The significance value of F_{IS} was evaluated through a bootstrap technique based on random permutation of the original dataset, as implemented in FSTAT v.2.9.3 [24]. This program implements a Bonferroni correction for all significance levels [25]. Within all groups of subjects, distribution of allele and genotype frequencies and their differences were calculated

Table 1 Baseline characteristic, clinical data of conventional risk factors and distribution of genotypes in cases and control subjects [adapted [26]]

		Controls	Cases	*P*
N		510	298	
Gender (Male/Female, %)		57.84/42.16	78.86/31.82	***
Age (years, mean)		47.47 ± 12.55	54.96 ± 10.40	***
Sedentarism (%)		61.96	58.39	NS
Alcohol (g/day)		35.32 ± 81.94	56.80 ± 87.01	***
Smoking habit (%)		26.86	38.93	***
Familiar CAD history (%)		12.16	56.04	***
Systolic blood pressure (mm Hg)		127.54 ± 17.43	134.63 ± 20.43	***
Diastolic blood pressure (mm Hg)		75.83 ± 10.69	79.10 ± 10.46	***
Arterial hypertension [a] (%)		22.35	58.72	***
Cardiac frequency		72.04 ± 8.62	68.90 ± 12.75	***
PWV (m/s)		8.80 ± 1.89	10.26 ± 2.13	***
Body mass index (kg/m^2)		26.41	27.80	***
Glycaemia (mg/dl)		97.96 ± 24.47	119.94 ± 50.60	***
Diabetes mellitus [b] (%)		3.14	23.15	***
Total cholesterol (mg/dl)		217.72 ± 44.73	205.39 ± 48.14	**
HDL (mg/dl)		57.15 ± 17.04	39.74 ± 9.75	***
LDL (mg/dl)		114.70 ± 37.27	119.02 ± 44.08	NS
Triglycerides (mg/dl)		130.73 ± 85.71	192.97 ± 142.96	***
Dyslipidaemia [c] (%)		11.76	70.81	***
APO B (mg/dl)		103.52 ± 28.37	103.47 ± 27.53	NS
Homocysteine (mmol/l)		9.72 ± 3.58	11.70 ± 3.72	***
Fibrinogen (mg/dl)		274.78 ± 61.18	335.95 ± 95.85	***
MTHFR C677T	CC	262 (51.37%)	130 (43.62)	NS[d]
	CT	200 (39.22%)	136 (45.53%)	
	TT	48 (9.41%)	32 (10.75%)	
MTHFR A1298C	AA	222 (43.53%)	158 (53.02%)	*[d]
	AC	259 (50.78%)	123 (41.28%)	
	CC	29 (5.69%)	17 (5.70%)	

*P<0.05, **P<0.005, ***P<0.0001, NS not significant P>0.05.

PWV, pulse wave velocity; HDL high-density lipoprotein; LDL low-density lipoprotein; APO B apolipoprotein B.

[a] medicated against hypertension/arterial tension≥139/89 mm Hg.

[b] medicated with anti-diabetic drugs/glycaemia≥126 mg/dl.

[c] total cholesterol≤200 mg/dl, triglycerides≥150 mg/dl, LDL≥130 mg/dl and HDL≤40 mg/dl.

[d] χ^2 (2df) was performed for the overall distribution of genotypes among cases and controls.

Table 2 Allele frequencies and population parameters related to MTHFR polymorphisms

	Alleles	Control group	Cases	Fis Control group/cases	Ho	Hs	Gis
MTHFR677	C	0.710	0.665	0.049/−0.022	0.424	0.430	0.012
	T	0.290	0.335				
MTHFR1298	A	0.689	0.747	−0.185**/0.052	0.460	0.409	−0.126
	C	0.311	0.253				
Overall				−0.040/−0.070	0.442	0.419	−0.055

**$P<0.005$.
Gis is an estimator of Fis based on Nei's (1987) statistic [27] and is estimated for each locus and overall. Ho is the observed proportion of heterozygotes, Hs is the within sample gene diversity.

using χ^2 tests. Associated probabilities (P) were calculated applying Fisher's exact test adjusted for multiple comparisons of associated genotypes. To test the significance of association between genotypes at pairs of loci in each sample we used a log-likelihood ratio G-statistic as implemented in Genepop. The relative odds ratio (OR) and 95% confidence interval of relative risk of CAD for any of the genetic polymorphisms and biochemical and behaviour markers, was assayed by multiple logistic regressions using the SPSS package.

Results

The baseline demographic and clinical characteristics of the study population are listed in Table 1. As expected, both the control and patient groups show differences in the biochemical markers and other conventional risk factors analysed (Table 1). Systolic and diastolic blood pressure, dyslipidaemia, arterial hypertension, diabetes mellitus, triglycerides and fibrinogen were significantly higher in CAD patients then in controls.

Table 3 Variation of homocysteine concentrations among cases and controls according to MTHFR alleles, genotypes and haplotypes

		Homocysteine (mmol/l)	
		Cases	Controls
Allele			
MTHFR677	C/T	11.53±3.43/13.12±5.45*	9.57±3.03/11.20±6.78***
MTHFR1298	A/C	11.74±3.76/11.17±2.96	9.73±3.64/9.70±2.26
Genotype			
MTHFR677	CC/nCC	11.25±2.82/12.05±4.26**	9.42±2.78/10.05±4.24**
	CT/nCT	11.80±3.92/11.62±3.56	9.77±3.33/9.70±3.74
	TT/nTT	13.12±5.45/11.53±3.43*	11.20±9.73/9.57±3.03***
MTHFR1298	AA/nAA	12.35±4.25/10.97±2.85*	9.99±4.17/9.53±3.03*
	AC/nAC	10.94±2.84/12.24±4.15**	9.50±3.11/9.95±3.99
	CC/nCC	11.17±2.96/11.74±3.76	9.70±2.26/9.73±3.64
Haplotype			
MTHFR 677-1298	C–A/nC–A	11.56±3.46/12.44±4.80	9.57±3.08/10.63±5.55**
	T–A/nT–A	12.08±4.26/11.22±2.84**	10.04±4.25/9.44±2.79**
	C–C/nC–C	11.02±2.87/12.27±4.22*	9.43±2.87/10.08±4.25*
	T–C/nT–C	10.63±2.90/11.95±3.85	9.82±3.08/9.70±3.70
Combined genotype			
MTHFR 677-1298	CC–AA/nCC–AA	11.35±2.87/11.77±3.85	9.57±2.22/9.75±3.78*
	CC–AC/nCC–AC	11.14±2.82/11.87±3.94*	9.32±3.14/9.90±3.74
	CC–CC/nCC–CC	11.44±2.83/11.72±3.77	9.57±2.19/9.73±3.64
	CT–AA/nCT–AA	12.44±4.27/11.41±3.44*	9.95±3.87/9.67±3.50
	CT–AC/nCT–AC	10.82±2.95/11.88±3.84	9.54±2.60/9.77±3.77
	CT–CC [a]/nCT–CC	–	–
	TT–AA/nTT–AA	13.79±5.65/11.50±3.42**	10.98±7.17/9.63±3.12***
	TT–AC/nTT–AC	9.54±1.95/11.74±3.73	11.92±5.47/9.68±3.52*
	TT–CC [b]/nTT–CC	–	–

The values obtained for each single genotype, haplotype and combined genotype are compared to the combined set of all other genotypes, haplotypes and combined haplotypes found in our population (ex.: CC/nCC means that homocyteine values are being compared between individuals carrying CC genotype and those carrying CT or TT genotypes).
Values presentation: mean±SD; *$P<0.05$, **$P<0.005$, ***$P<0.0001$.
[a] Only one control subject and one patient presented the CT–CC combination.
[b] Combined genotype TT–CC was not observed.

Although LDL values were not statistically different among patients and the control group, HDL and total cholesterol were significantly lower in the former group. Plasma total homocysteine levels were significantly higher in cases when compared with subjects without CAD.

Table 1 also presents the distribution of genotypes for the 2 loci in controls and cases. In contrast to MTHFR677, genotypes at MTHFR A1298C locus show statistically different distributions between cases and controls: there is a significant heterogeneity in the genotype distribution when comparing patients versus controls, certainly due to an increase in MTHFR1298 AA in CAD patients (53.02%) compared to healthy subjects (43.53%). While the prevalence of homozygous 1298CC was similar in both groups, higher number of heterozygous AC carriers was seen among controls vs. cases (50.78 vs. 41.28%). The distribution of both MTHFR mutations among CAD patients and controls with respect to age groups and gender was investigated, but no significant results were obtained. All further analyses were also performed taking into account these population subdivisions but, since no statistical relevance was obtained, results are not shown.

Only the group of patients is in Hardy–Weinberg equilibrium (HWE) at each locus and overall (Table 2). The control group is not at HWE at locus MTHFR A1298C ($P<0.005$). As expected, both mutations at MTHFR locus show strong significant linkage disequilibrium ($P<0.00001$). Thus taking in consideration the two loci combined, both populations are significantly different in which concerns the genic differentiation ($\chi^2_{df4}=11.559$, $P=0.02$).

Individuals with MTHFR 677T allele showed significantly higher levels of serum Hcy than those carrying the 677C allele, both in CAD patients and controls (Table 3). There was a grade increase in plasma Hcy concentrations from CC to TT genotypes of MTHFR677 polymorphism, both in individuals with and without CAD. For cases and controls, none of the MTHFR1298 alleles seemed to be associated with Hcy plasmatic levels, but those subjects presenting the 1298AA genotype showed significantly higher values of serum Hcy than those carrying the 1298AC and CC genotypes. On the other hand, 1298AC heterozygous patients presented the lowest observed Hcy levels. The haplotype 677T/1298A was found to be significantly associated with an increase in Hcy values in cases and controls, while 677C/1298C haplotype carriers had significantly lower Hcy levels when compared to non 677C/1298C. In controls, the haplotype 677C/1298A also corresponded to significantly lower mean plasmatic Hcy values, when compared to non 677C/1298A individuals. When combining genotypes from both polymorphisms, we found that 677CC/1298AA corresponds to a significant increase in Hcy levels for both cases and controls, while there was a divergence concerning the genotype combination corresponding to lower Hcy medium values: a significant decrease was obtained for the CC/AC combination in cases and CC/AA in controls.

Both the T allele of MTHFR677 and the A allele of MTHFR1298 showed a significant association with hyperhomocysteinemia but not with CAD (Table 4). While both 677TT and 1298AA genotypes were found to be related with hyperhomocysteinemia, only the 1298AA showed significant association with the development of

Table 4 Allele, genotype and haplotype association between MTHFR polymorphisms, hyperhomocysteinemia and CAD

		HyperHcy [a]	CAD
Allele			
MTHFR677	C	NS	NS
	T	2.271(1.56–3.30)***	NS
MTHFR1298	A	1.822(1.14–2.91)*	NS
	C	NS	NS
Genotype			
MTHFR677	CC	NS	NS
	CT	NS	NS
	TT	2.862(1.47–5.56)***	NS
MTHFR1298	AA	2.056(1.19–3.54)*	1.476(1.10–1.96)**
	AC	NS	NS
	CC	NS	NS
Haplotype			
MTHFR 677-1298	C–A	NS	NS
	T–A	2.224(1.53–3.24)***	1.241(1.00–1.54)*
	C–C	0.513(0.32–0.83)**	0.798(0.64–1.00)*
	T–C	NS	NS
Combined genotype			
MTHFR 677-1298	CC–AA	NS	NS
	CC–AC	0.506(0.26–0.99)*	0.688(0.49–0.96)*
	CC–CC	NS	NS
	CT–AA	2.148(1.27–3.64)**	1.558(1.12–2.17)*
	CT–AC	NS	NS
	CT–CC	NS	NS
	TT–AA	3.323(1.70–6.49)**	NS
	TT–AC	NS	NS
	TT–CC [b]	–	–

Values presentation: OR(95% CI); *$P<0.05$, **$P<0.005$, ***$P<0.0001$, NS not significant $P>0.05$.

[a] for Hcy levels>15 μmol/l.

[b] Combined genotype TT–CC was not observed.

CAD. On the other hand, the haplotype associations showed the same tendencies both for hyperhomocysteinemia and CAD: the 677T-1298A provided an increased risk, while the 677C-1298A haplotype corresponded to the lowest significant odds value. The individuals carrying the combined MTHFR genotype 677CC-1298AC had the lowest risk of hyperhomocysteinemia and CAD, vs. individuals 677CT-1298AA with the highest risk of CAD and individuals 677TT-1298AA with the highest risk of hyperhomocysteinemia.

Multiple logistic regression was used to test for independent correlates of the presence of CAD. Included in the model were most of the variants included in Table 1. A Family history of CAD, arterial hypertension, PWV, Diabetes mellitus, dyslipidaemia and plasma Hcy (OR = 1.149 95% CI: 1.06–1.21, $P = 0.002$) were found to be independent correlates of the presence of CAD.

Discussion

Biochemical and behavioural factors such as arterial hypertension, dyslipidaemia, diabetes, obesity, cigarette smoking and alcohol ingestion were found to be significantly different in cases versus controls. Nevertheless, total cholesterol values were lower among cases, most probably due to the therapy nearly all of them follow after being diagnosed for CAD. Our study showed that patients with documented CAD by angiography had significantly higher levels of Homocysteine (Hcy) than those without CAD. Plasma Hcy level remained an independent risk factor for CAD, even after multivariate logistic regression analysis. These data support a hypothetical connection between mild hyperhomocysteinemia and CAD. Some studies support our findings, showing a strong association between Hcy and CAD [28]. Others have reported an elimination of this association after adjusting for cardiovascular risk factors claiming that hyperhomocysteinemia may be an effect of vascular disease rather than its cause [4,29,30]. Even in the midst of conflicting data, a consensus emerges considering mild hyperhomocysteinemia as an independent risk factor for cardiovascular disease [1,31].

Few recent studies have tried to compare MTHFR 677 and/or 1298 genotype distributions between CAD patients and controls, their influence in circulating Hcy, and also the role of hyperhomocysteinemia in the aetiology of CAD [32–35]. Different sample sizes and a different genetic ethnic background, are probably on the basis of discrepancies between these studies: all are in agreement with the association between MTHFR C677T-TT genotype and higher Hcy plasmatic levels [32–35], but only one found a significant genotypic association with CAD [33]. Regarding the A1298C variant, results tend to greatly disagree: among Moroccans MTHFR 1298 was found to influence Hcy levels and act as an independent risk factor for CAD [34]; but no association whatsoever with Hcy levels or CAD were found in Caucasians [35]. Three out of four studies [32–34] obtained a significant and independent association between HyperHcy and CAD.

Mechanisms by which hyperhomocysteinemia promotes the development of CAD are still not fully understood. Supposed mechanisms of Hcy induced atherosclerosis include impaired production of endothelium-derived nitric oxide, stimulated proliferation of smooth muscle cells, endothelial cell growth inhibition and effects on platelets and coagulation [36,37]. Increased production of free radicals may also be involved in Hcy-mediated damage [38]. Several studies have also shown that elevated levels of this aminoacid may induce DNA damage [39,40]. Hyperhomocysteinemia may result from either genetic or nutritional causes. If a genetic variant influences the plasma level of a potential causal risk factor, a similar association would be expected between the variant and disease, as between the plasma level and disease. The most common genetic defects of Hcy metabolism are two mutations in the gene encoding for the enzyme MTHFR: MTHFR C677T, originating a thermolabile form of the enzyme and MTHFR A1298C. Our results show that the thermolabile MTHFR variant has a marked impact on plasma Hcy level which is significantly higher in the presence of the T allele and TT genotype. We also found the same pattern of influence with the A allele and AA genotype of the 1298 polymorphism. Thus, the presence of haplotype 677C/1298C resulted in significantly lower mean levels of Hcy, while the individuals with haplotype 677T/1298A showed a significant increase in plasmatic Hcy. Homozygosity for the C677T MTHFR mutation has been extensively associated with intermediate and mild hyperhomocysteinemia [11,41,42], while most studies have failed to find an association between the MTHFR A1298C and Hcy levels [43,44].

Despite the clear effect of the thermolabile variant on elevated tHcy levels, we did not observe any association between C677T alleles or genotypes and the risk of CAD in our population. Our study is in the same line with previous ones in which no association between C677T variants and angiographically diagnosed CAD was found [13, 45–47]. It seems that this polymorphism is only associated with an increased risk of CAD under low-folate conditions, varying between different populations according to characteristic folate intake [14]. On the other hand, our results show that the 1298AA genotype promotes a significantly increased risk of CAD. Similar results were found in a study evolving a Corsica island population [48]. The 1298A allele association with CAD provides significance to haplotype and combined genotype association with the disease. The haplotypes 677T/1298A and 677C/1298C provide the highest and lowest relative CAD risk, respectively.

While individuals with 677CT/1298AA genotype combination have a significant increase in the odds of becoming CAD patients, 677CC/1298AA corresponds to a significant trend towards a decrease in CAD incidence.

While our patient subpopulation was in Hardy–Weinberg equilibrium for A1298C polymorphism of MTHFR gene, the control subpopulation was not, due to an excess of heterozygotes AC. Similar results have previously been reported for other human genes, including ACE I/D polymorphism, also commonly associated with CAD [49,50]. This may be interpreted as a case of heterosis, or even confirm the higher risk homozygotes AA face in the development of CAD.

In agreement with previous reports, our results demonstrated that the two variants 677T and 1298C are in complete linkage disequilibrium [12,15]. Notably, the combination of a 677TT and 1298CC genotype did not occur in our population, and the number of individuals with 677TT/ 1298AC and 677CT/1298CC genotype combinations was very low. Various studies have also examined the compound genotype distribution of the two MTHFR polymorphisms, and reported that the 677T and 1298C alleles never or rarely occur in cis configuration [15,48,51]. Presumably, the two polymorphisms arose separately on different alleles, and because of the small distance separating them on the chromosome, little crossing over has occurred. The prevalence of C677T and A1298C polymorphisms in neonatal and foetal tissue samples reported that although the 677TT/1298CC genotype was present in foetal tissue samples, it was absent in the neonatal group [52]. The authors surmised that this genotype with four mutant alleles in the MTHFR gene may impair the viability of the foetus.

Our study made a contribution to the assessment of MTHFR genotype and haplotype influence on circulating Hcy levels in a Portuguese population, confirming these observations in patients with angiographic CAD and healthy controls. These findings suggested that the AA genotype of MTHFR1298, which can cause a predisposition to increased plasma Hcy levels, may itself be a genetic risk factor for CAD. On the other hand, the MTHFR677 gene polymorphism is related to homocysteine metabolism but does not predict the risk of CAD as a susceptible genetic marker in our population.

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