Frequency of methylenetetrahydrofolate reductase C677T polymorphism in patients with cardiovascular disease in Eastern Saudi Arabia

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ABSTRACT

Objective: Homozygosity for the C677T mutation in the gene of the thermolabile enzyme 5,10 methylenetetrahydrofolate reductase (MTHFR) associates with reduced enzyme activity, leading to mild hyperhomocysteinemia. We now know that an elevated level of homocysteine is an important risk factor for cardiovascular disease (CVD). The objective of this study was to determine the prevalence of the C677T mutation in Saudi patients diagnosed with CVD.

Methods: Over a period of 2 years (2003-2004) in a case control study, we determined the prevalence of the C677T mutation in 83 CVD patients and in 40 age and gender-matched controls in the Eastern Province of Saudi Arabia. We determined the MTHFR genotype by restriction fragment length polymorphism and allele specific hybridization procedures.

Results: The CVD group showed over representation of the C677T allele frequencies (20.5%) compared with unaffected controls (15%) (p=0.3). Furthermore, the genotypic data indicated that the prevalence of homozygosity for the C677T mutation was dramatically higher in the CVD patients (10.8%) when compared with normal (0%) (p=0.058).

Conclusion: These results suggest that the MTHFR C677T variant mildly influences CVD. However, we require further investigation in large independent samples.

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Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in the developing and developed worlds.[1,2] A number of genetic and environmental risk factors increase the predisposition to CVD.[3,4] One of the genetic factors reported to be associated with CVD is the C677T polymorphism in the thermolabile enzyme methylenetetrahydrofolate (MTHFR).[5,6] The enzyme MTHFR participates in one carbon metabolism, the product of which is 5-methyltetrahydrofolate. We require the latter to remethylate homocysteine into methionine, which reduces plasma homocysteine. Subjects with homozygosity for the mutation (TT genotype) exhibit reduced activity of the enzyme, which leads to hyperhomocysteinemia. Clinical and epidemiological studies show that even a mild increase in total plasma homocysteine associates with an increased risk of CVD.[7,8] There are several studies concerning this association. Studies of European and North American populations found that TT genotype is present in 12.5% of CVD patients, and 11.9% of control subjects, giving an

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odds ratio for CVD of 1.15, which is not significant.[9] However, 3 Japanese studies showed a significant increased risk for CVD in those with the TT genotype.[10-12] These conflicting reports prompted us to investigate this relationship of genotype and phenotype in our population. The current study determined the prevalence of this mutation in subjects with confirmed CVD and in age and gender-matched controls among the population of the Eastern Province of Saudi Arabia.

Methods. Over a period of 2 years, and after obtaining written informed consent, 83 patients attending the cardiology clinic of King Fahad University Hospital, Dammam were recruited. Patients presenting with hypertension, myocardial infarction, unstable angina or peripheral artery disease were included in the study. Control subjects (n=40) were recruited from the same age group and geographical area. The sole exclusion criterion for control subjects was a past, present or family history of CVD. Blood samples were collected in EDTA tubes and were stored frozen until analysis. A DNA sample was extracted from 300 µl whole blood by standard procedures. The MTHFR genotypes were determined by restriction fragment length polymorphism (RFLP) procedures according to Frosst et al.[5] The procedures encompassed the amplification of a specific DNA fragment by polymerase chain reaction (PCR) using specific primers as previously described. The PCR product was then digested with Hinf1 to distinguish C677T and wild type alleles. The results were routinely confirmed by a reverse-hybridization assay developed by Vienna laboratory. In this method, the relevant gene sequences are simultaneously in vitro amplified and biotin labeled in a single amplification reaction. The amplified products are selectively hybridized to a test strip that contains allele specific oligonucleotide probes immobilized as an array of parallel lines. Allele frequencies were calculated by allele counting. The expected genotype frequencies were calculated according to Hardy-Weinberg law and compared to observed genotype.

Results. **Table 1** shows the genotype and allele frequencies for the C677T variant of MTHFR and the results of χ^2 analysis. The data show that the prevalence of the T allele and homozygous TT genotype in the control group to be 15% and 0%. These data are lower than those reported for this variant in other populations.[13] The frequency of T allele is over represented in the CVD group by 5.5% when compared to the control group (CVD 20.5%, control 15%). The data also show that the distribution of genotypes between the 2 groups is different with frequencies of heterozygote and homozygote for the T allele at 30% and 0% in the control group compared to 19.3% and 10.8% in the CVD group. However, these observed allele and genotype differences were not significant at α-level of 0.05 (p=0.3 and p=0.058).

Discussion. Cardiovascular disease is the leading cause of morbidity and mortality in the developing and developed worlds.[2] Clinical and epidemiological studies show that environmental and genetic factors play an important role in the development of CVD.[4] Among these factors, a mild elevation in serum homocysteine level associates with an increased risk for CVD.[7,11] Certain mutations in the gene encoding MTHFR, involved in the metabolism of homocysteine, may lead to decreased enzyme activity.[5,6] This decrease associates with an elevated homocysteine level.[9] Homozygous individuals for the C677T mutation had a significantly elevated plasma homocysteine level and consequently an increased risk for CVD.[5] However, a meta-analysis study in UK patients with CVD found no association of the MTHFR genotype with CVD.[9] The association between the genotype and elevated homocysteine levels was only true in

Table 1 - Distribution of methylenetetrahydrofolate reductase C677T frequencies in cardiovascular disease (CVD) and control groups.

Group	Number of genotypes (%)			Total genotypes	Number of alleles (%)	
	C/C n (%)	C/T n (%)	T/T n (%)		C n (%)	T n (%)
CVD	58 (69.9)	16 (19.3)	9 (10.8)	**83**	132 (79.5)	34 (20.5)
Control	28 (70)	12 (30)	0	**40**	68 (85)	12 (15)
Allele comparison χ^2 = 1.07, p=0.3; Genotype comparison χ^2 = 5.7 , p=0.058						

patients with low serum folate levels below the population medium. Al-Nuaim,[14,15] reported a progressive increase in the incidence of CVD in the Mediterranean region including Saudi Arabia.[16,17] They attribute this to the tremendous changes in socioeconomic factors and life style of these populations. This is the first report on the possible interaction of MTHFR polymorphism and an increased risk for CVD in this population. Our investigation shows that C677T mutation in the MTHFR gene is present in our population, as also reported in the neighboring regions.[18] The allele frequencies and genotype distribution among CVD patients and normal controls in the Eastern Province population are slightly lower than those reported for other populations.[13,17] We can explain this difference in view of the fact that the T allele frequency and genotype distribution differ substantially among different ethnic populations.[18] The results also show an increased prevalence of the T allele and its composite genotype in the CVD group compared with the normal control group. This is consistent with previously reported data for other populations.[10,17] The present results agree with other studies which indicated that the presence of the C677T allele confers a modest increased risk for CVD. It is also reasonable to assume that a combination of other genetic or environmental factors, or both, with the MTHFR mutation presents an increase in the CVD risk factors. Nevertheless, our results provide evidence that the MTHFR C677T allele, which causes mild hyperhomocysteinanemia, may be an independent risk factor for CVD. However, we require further investigation in large independent samples.

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References

1. NHLBI morbidity and mortality chartbook, 2002. Bethesda (MD): National Heart, Lung and Blood Instit. May 2002. Available from URL: http://www.nhlbi.nih.gov/resources/cht-book
2. American Heart Association. International Cardiovascular Disease Statistic. USA: American Heart Organization; 2002.
3. Brass LM, Shaker LA. Family history in patients with transient ischemic attacks. ***Stroke*** 1991; 22: 837-872.
4. Nabel EG. Cardiovascular disease. ***N Engl J Med*** 2003; 349: 60-72.
5. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Mathews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylene-tetrahydrofolate reductase. ***Nat Genet*** 1995; 10: 111-113.
6. Kang SS, Wong PWK, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. ***Am J Hum Genet*** 1991; 48: 536-545.
7. Nygard O, Vollset SE, Refsum H, Brattstorm L, Veland PM. Total homocysteine and cardiovascular disease. ***J Intern Med*** 1999; 246: 425-454.
8. Guba SC, Fink LM, Fonseca V. Hyperhomocysteinemia an emerging and important risk factor for thromboembolic and cardiovascular disease. ***Am J Clin Pathol*** 1996; 106: 709-722.
9. Prattstorm L, Wilcken DEL, Ohrvik J, Brudin L. The common MTHFR gene mutation leads to hyperhomocysteinemia but not to vascular disease-the results of meta-analysis. ***Circulation*** 1998; 98: 2520-2526.
10. Izumi M, Iwai N, Ohmichi N, Nakamura Y, Shimoike H, Kinoshita M. Molecular variant of 5,10-methylenetetrahydrofolate reductase is a risk factor of ischemic heart disease in the Japanese population. ***Atherosclerosis*** 1996; 121: 293-294.
11. Morita H, Taguchi J, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, et al. Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase as a risk factor for coronary artery disease. ***Circulation*** 1997; 95: 2032-2036.
12. Ou T, Yamakawa-Kobayashi K, Arinami T, Ameniya H, Fujiwara H, Kawata K, et al. Methylenetetrahydrofolate reductase and apolipoprotein E polymorphism are independent risk factors for coronary heart disease in Japanese: a case control study. ***Atherosclerosis*** 1998; 137: 23-28.
13. Scholtz CL, Odendaal HJ, Thiart R, Loubser L, Hillermann R, Delport R, et al. Analysis of two mutations in the MTHFR gene associated with mild hyperhomocysteinemia-heterogeneous distribution in the South African population. ***S Afr Med J*** 2002; 92: 464-467.
14. Al-Nuaim AR. High prevalence of metabolic risk factors for CVD among Saudi population, aged 30-64 years. ***Int J Cardiol*** 1997a; 19: 227-235.
15. Al-Nuaim AR. Serum total and fractionated cholesterol distribution and prevalence of hypercholesterolemia in urban and rural communities in Saudi Arabia. ***Int J Cardiol*** 1997; 58: 141-149.
16. Almawi WY. C677T and A1298C single nucleotide polymorphism in the methylenetetrahydrofolate reductase gene among Bahraini Arabs. ***Thromb Haemaost*** 2004; 91: 843-845.
17. Heux S, Morin F, Lea RA, Ovcaric M, Tajouri L, Griffiths LR. The methylenetetrahydrofolate reductase gene variant (C677T) as a risk factor for essential hypertension in Caucasians. ***Hypertens Res*** 2004; 27: 663-667.
18. Pollak RD, Friedlander Y, Polllak A, Idelso M, Bejarano A, Blumenfeld A. Ethnic differences in the frequency of the C677T mutation in the methylenetetrahydrofolate reductase gene in healthy Israeli populations. ***Genet Test*** 2000; 4: 309-311.