Clin. Cardiol. 24, 281–284 (2001)

Clinical Investigations

Methylenetetrahydrofolate Reductase Gene Polymorphism and Risk of Premature Myocardial Infarction

SADI GÜLEÇ, M.D., ÖMER ARAS, M.D., ECE AKAR, M.D., ERALP TUTAR, M.D., KENAN ÖMÜRLÜ, M.D., FERIT AVCI, M.D., IREM DINÇER, M.D., NEJAT AKAR, M.D., DERVIS ORAL, M.D.

Medical School of Ankara University, Ankara, Turkey

Summary

Background: Elevated plasma homocysteine level is an independent risk factor for cardiovascular disease. A common mutation (nucleotid 677C-T) in the gene coding for methylenetetrahydrofolate reductase (MTHFR) has been reported to reduce the enzymatic activity of MTHFR and is associated with elevated plasma levels of homocysteine, especially in subjects with low folate intake.

Hypothesis: Methylenetetrahydrofolate reductase T/T genotype may be a risk factor for premature MI in Turkish population who are known to have low folate levels.

Methods: The study group was comprised of 96 men (aged < 45 years) with premature myocardial infarction (MI) and 100 age- and gender-matched controls who had no history or clinical evidence of coronary artery disease (CAD) and/or MI. DNA was extracted from peripheral blood and genotypes were determined by polymerase chain reaction, restriction mapping with HinfI, and gel electrophoresis. Conventional risk factors for CAD were prospectively documented.

Results: Allele and genotype frequencies among cases and control subjects were compatible with Hardy-Weinberg equilibrium. The frequencies of T/T, C/T, and C/C genotypes among patients with MI and control subjects were 15.6, 40.6, and 43.8%, and 5, 35, and 60%, respectively. Multivariate analyses identified smoking, MTHFR C/T polymorphism, diabetes mellitus, family history of CAD, and hypertension as the independent predictors of premature MI. Defining patients with non-T/T genotype (C/C and C/T combined) as reference, the relative risk of MI for subjects with T/T genotype was 5.94 (95% confidence interval: 1.96–18.02, p = 0.0016).

Conclusions: Our findings suggest that C677T transition in the MTHFR gene may be a risk factor for premature MI in Turkish men.

Key words: methylenetetrahydrofolate reductase gene, myocardial infarction

Introduction

There is firm evidence that an elevated total plasma homocysteine level is an independent risk factor for vascular disease including coronary artery disease (CAD) and myocardial infraction (MI).[1,2] A number of genetic or nutritional deficiencies can elevate the plasma levels of homocysteine.[3] The 5-10 metylenetetrahydrofolate reductase (MTHFR) is a recently described enzyme that catalyzes the reduction of 5,10 MTHF to 5 MTHF,[4,5] which is a cosubstrate in the remethylation of homocysteine to methionine. A functionally important and common mutation (nucleotide 677 C-T) in the gene coding for MTHFR has now been described.[6] This mutation has been reported to reduce the enzymatic activity of MTHFR and was associated with an elevated plasma level of homocysteine,[7] especially in subjects with low plasma folate levels.[8] It has been previously reported that in the Turkish population there is a high prevalence of CAD despite their having low cholesterol levels.[9] The Turkish population is also known to have low folate levels.[10] Taken together, the C677T polymorphism in the MTHFR gene may be a genetic risk factor for premature cardiovascular disease in the Turkish population. To test this hypothesis, we studied the prevalence of the C677T mutation in Turkish men with premature MI.

Methods

Study Population

The study group was comprised of 96 consecutive men (aged < 45 years) with premature MI and 100 age- and gender-matched healthy controls from the same geographic area. Myocardial infarction was diagnosed by the presence of two

Address for reprints:

Sadi Güleç, M.D.
Konutkent 2, A4 Blok No:30
06530 Çayyolu
Ankara, Turkey

Received: December 10, 1999
Accepted with revision: May 23, 2000

of three criteria for typical chest pain > 30 min, a rise in creatine kinase (CK)-MB, and ST elevation of at least 2 mm in two contiguous leads. None of the control subjects had a prior history of angina or MI. Each had a normal physical examination and normal electrocardiogram. Moreover, negative exercise testing was considered mandatory for control subjects. Conventional risk factors for CAD such as hypertension (blood pressure > 140/90 mmHg or prior therapy), diabetes mellitus (fasting blood glucose of > 140 mg/dl or prior therapy), hypercholesterolemia (total cholesterol > 220 mg/dl, or on cholesterol-lowering agents), family history of CAD, and smoking (current smokers) were prospectively documented. Informed consent was obtained from all subjects.

Methylenetetrahydrofolate Genotype Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedure, and mutation analysis was performed essentially as described by Frosst *et al.*[6] Electrophoresis in a 4% agarose gel followed by ethidium bromide staining and ultraviolet illumination allowed detection of mutated alleles.

Statistical Analysis

Statistical analyses were performed using SPSS (Statistical Package for Social Sciences) software package, version 8.0 for Windows (SPSS Inc., Chicago, Ill., USA). Data are expressed as numbers and percentages for discrete variables and as mean ± standard deviation (SD) for continuous variables. Alleles and genotype frequencies between cases and control subjects were counted and compared by chi-square test with Hardy-Weinberg predictions. Age, hypercholesterolemia, smoking, hypertension, family history of CAD, diabetes mellitus, and MTHFR genotypes were selected as potential risk factors for MI. Independent predictors of MI were determined by using multiple stepwise regression analyses. Odds ratios (OR) with two-tailed p values and 95% confidence intervals (CIs) were calculated as a measure of the association of the MTHFR C/T genotype with MI, assuming a recessive model of inheritance (T/T vs. C/T and C/C combined). All probability values are two-tailed and a value of $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of patients with MI and controls are summarized in Table I. All conventional risk factors for CAD were significantly more prevalent among patients with MI. Table II indicates the MTHFR genotype frequencies of patients and controls. Genotype frequencies of patients and controls were compatible with Hardy-Weinberg equilibrium. The T/T genotype was more frequently observed among patients with MI than among controls (15.6 vs. 5%, $p = 0.016$). The association between conventional risk factors for CAD and MTHFR genotypes is indicated in Table III. Family history of CAD was significantly more prevalent among patients with T/T genotype than among those with non-T/T genotype (40 vs. 25%, $p = 0.017$). Multiple stepwise logistic regression analysis identified smoking, MTHFR C/T polymorphism, diabetes mellitus, family history of CAD, and hypertension as the independent predictors of MI (Table IV). Defining patients with non-T/T genotype (C/T and C/C combined) as reference, the relative risk of MI for patients with T/T genotype was 5.94 (95% CI: 1.96–18.02, $p = 0.0016$.

TABLE I Characteristics of patients with myocardial infaction and controls

	MI (n = 96)	Controls (n = 100)	p Value
Age, years	38 ± 7	37 ± 5	NS
Diabetes mellitus, n (%)	17 (17.8)	5 (5)	0.005
Hypertension, n (%)	29 (30.2)	13 (13)	0.003
Family history of CAD, n (%)	33 (34.4)	19 (19)	0.015
Smoking, n (%)	60 (62.5)	35 (35)	< 0.001
Hypercholesterolemia, n (%)	28 (29.2)	16 (16)	0.027

Abbreviations: MI = myocardial infarction, CAD = coronary artery disease, n = number of patients.

Discussion

In this population of Turkish men with premature MI, we observed a significantly higher prevalence of T/T genotype than in the age- and gender-matched controls. This might be attributed to the relatively lower prevalence of T/T genotype in

TABLE II Genotype frequencies of patients with myocardial infaction (MI) and normal controls

Genotypes	T/T	C/T	C/C
MI (n = 96) (%)	15 (15.6)	39 (40.6)	42 (43.8)
Controls (n = 100) (%)	5 (5)	35 (35)	60 (60)

TABLE III The association between conventional risk factors for CAD and MTHFR genotypes

Genotype	T/T (n = 15)	C/T (n = 39)	C/C (n = 42)	p Value
Diabetes mellitus, n	1	7	9	NS
Hypertension, n	1	12	16	NS
Family history of CAD, n	6	19	8	0.017
Smoking, n	7	27	26	NS
Hypercholesterolemia, n	3	8	17	NS

Abbreviations: MTHFR = methylenetetrahydrofolate reductase, CAD = coronary artery disease, n = number of patients.

TABLE IV Independent predictors of myocardial infarction for the study population

	OR (95% CI)	p Value
Diabetes mellitus	3.52 (1.19–10.42)	0.0226
Smoking	1.54 (1.54–5.53)	0.0010
Family history of CAD	2.27 (1.10–4.71)	0.0266
Hypertension	2.88 (1.30–6.34)	0.0085
MTHFR T/T genotype	5.94 (1.96–18.02)	0.0016[a]

[a] T/T vs. non T/T (C/T+C/C)

Abbreviations: OR = odds ratio, CI = confidence interval. Other abbreviations as in Table III.

our control subjects (5%) than that found in other western populations. However, our results are in line with a recent study of the Turkish population in which T/T genotype frequency in control subjects was 5.2%.[10] Although an increased prevalence of thermolabile MTHFR has been previously reported among patients with CAD,[11–13] this has not been confirmed in several others.[14, 15] Unlike in our study, these studies mostly consisted of patients who had CAD, but not necessarily MI. Although there is, of course, a relationship between angiographic CAD and MI, the two are not synonymous and each has distinct determinants. This is likely to extend to genetic causes. Indeed, analysis of different phenotypes may explain at least some of the discrepancies that have been reported in relation to the thermolabile MTHR mutation. However, studies that also investigated the association between MI and MTHFR C677T polymorphism obtained different results. Kluijtmans *et al.*[16] found a three-fold increased risk of premature cardiovascular disease among men with T/T genotype in 60 patients (32 with cerebrovascular disease, 18 with peripheral arterial disease, and 10 with MI). Similarly Morita *et al.*[17] reported that T/T genotype of MTHFR was significantly associated with occurrence of MI. In contrast, Ma *et al.*[18] failed to find an association between MTHFR polymorphism and risk of MI in U.S. physicians. However, subgroup analysis showed a trend toward an increase in MI risk among younger men with T/T genotype who had low folate levels. Anderson *et al.*[19] also noted a nonsignificant trend toward the T/T genotype with MI risk in younger patients, especially in those with relative folate deficiency. Authors suggested that individuals with homozygous mutation in the MTHFR gene may be able to compensate for the genetic disturbance in tHcy metabolism through increasing folate intake. Accordingly, they stressed the importance of determining the impact of this polymorphism in a general population sample with lower intake of folate and among young individuals. In the current study, we did not study the folate levels; however, previous studies demonstrated that the folate level in the Turkish population is lower than that of well-nourished populations.[10] Furthermore, prevalence of congenital neural tube defects, due to folate deficiency, is known to be significantly higher in Turkey than in other countries.[20, 21] Therefore, our study provides the opportunity for determining the role of MTHFR mutation as a risk factor for MI in young individuals from a community with low folate intake. Considering the ethnic differences between populations, the higher prevalence of homozygous MTHFR gene in our population with MI is not necessarily in conflict with other studies suggesting that MTHFR gene polymorphism is not associated with risk of MI.

There have been many studies, including one from Turkey, declaring that individuals homozygous for the C677T mutation of the MTHFR gene have mild to moderate hyperhomocysteinemia due to reduced MTHFR enzyme activity.[7, 10, 22] Moreover, it has been shown that among individuals homozygous for the C677T mutation, the plasma homocysteine level was 24% greater than in those who had plasma folate concentration of < 15.4 mmol/l compared with those who had plasma folate concentration > 15.4 mmol/l.[8] As we did not measure the plasma homocysteine level, our study does not provide the opportunity to determine the relationship between the C677T mutation and homocysteine concentrations. However, on the basis of previous data, it would be logical to speculate that the increased risk of premature MI in our subjects with TT genotype is at least partly due to hyperhomocysteinemia, which is known to have atherothrombotic effects.[23] In addition, lower folate concentrations of the population might be considered to be an environmental factor that potentates the effect of this common mutation on homocysteine concentrations.[8]

Conclusion

We have demonstrated that MTHFR C677T mutation is significantly associated with risk of premature MI in Turkish men. Relatively lower folate concentrations of this population support the hypothesis that, in individuals with insufficient folate intake, this mutation may heighten cardiovascular risk, whereas in well-nourished populations it might be tolerated without consequences on clinical phenotype. Accordingly, folic acid supplementation, especially in communities with lower dietary folate intake and higher prevalence of TT genotype, may helpful in reducing the cardiovascular risk by overcoming the enzyme defect.[6, 24] However, there are still insufficient data to justify such recommendations fully.

References

1. Genest JJ, McNamara JR, Salem DN, Wilson PW, Schaefer EJ, Malinow MR: Plasma homocysteinemia levels in men with premature coronary artery disease. *J Am Coll Cardiol* 1990;16: 1114–1119
2. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH: A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. *J Am Med Assoc* 1992;268:877–881
3. Mayer EL, Jacobsen DW, Robinson K: Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol* 1996;27:517–527
4. Daubner SC, Matthews RG: Purification and properties of methylenetetrahydrofolate reductase from pig liver. *J Biol Chem* 1982;257:140–145

5. Goyette P, Summer JS, Milos R Duncan AM, Rosenblatt DS, Matthews RG, Rozen R: Human methylenetetrahydrofolate reductase: Isolation of cDNA, mapping and mutation identification. *Nat Genet* 1994;7:195–200
6. Frosst P, Blom HJ, Milos R Gotette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Hevvel LP: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10: 111–113
7. Engbersen AM, Franken DG, Boers GH, Stevens EM, Trijbel FJ, Blom HJ: Thermolabile 5, 10 methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995; 56:142–150
8. Jacques PF, Bostom AG, Williams RR Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R: Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996;913:7–9
9. Onat A, Senocak M: Relative risk of factors for coronary heart disease in a population with low cholesterol levels. *Int J Cardiol* 1994;43:51–60
10. Tokgözoğlu SL, Alikaşifoğlu M, Ünsal İ, Atalar E, Aytemir K, Ozer N, Ovunc K, Usal O, Kes S, Tuncbilek E: Methylenetetrahydrofolate reductase genotype and the risk and extent of coronary artery disease in a population with low plasma folate. *Heart* 1999;81: 518–522
11. Gallagher PM, Meleady R, Shields DC, Tan S, Naughten E, Kraus JP, Sellar GC, Mc Connell, DJ, Graham I, Whitehead AS: Homocysteine and risk of premature coronary heart disease: Evidence for a common gene mutation *Circulation* 1996;94:2154–2158
12. Kang SS, Wong PW, Susmano A, Sora J, Norisis M, Ruggie N: Thermolabile methylenetetrahydrofolate reductase: An inherited risk factor for coronary artery disease. *Am J Hum Genet* 1991; 48:536–545
13. 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
14. Verhoef P, Rimm EB, Hunter DJ, Chen J, Willett WC, Kelsey K, Stampfer MJ: A common mutation in the methylenetetrahydrofolate reductase gene and risk of coronary heart disease: Results among U.S. men. *J Am Coll Cardiol* 1998;32:353–359
15. Narang R, Callahan G, Haider AW, Davies GJ, Tuddenham EG: Methylenetetrahydrofolate reductase mutation and coronary artery disease. *Circulation* 1996;94:2322–2323
16. Kluijtmans LA, Van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oast BA, den Heijer M, Trijbels FJ, Rozen R, Blom HJ: Molecular genetic analysis in mild hyperhomocysteinemia: A common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Human Genet* 1996;58:35–41
17. Morita H, Taguchi J, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, Maemura K, Shindo T, Minamuno T, Ohno M, Yamaoki K, Ogasawara K, Aizawa T, Suziki S, Yazaki Y: Genetic polymorphism of 5,10 methylenetetrahydrofolate reductase as a risk factor for coronary artery disease. *Circulation* 1997;95:2032–2036
18. Ma J, Stampfer MJ, Hennekens CH, Frosst P, Selhub J, Horsford J, Malinow MR, Willett WC, Rozen R: Methylenetetrahydrofolate reductase poymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation* 1996;94: 2410–2416
19. Anderson JL, King GJ, Thomson MJ, Todd M, Bair TL, Muhlestein JB, Carlquist JF: A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. *J Am Coll Cardiol* 1997; 30:1206–1211
20. Boduroglu K, Alikasifoglu M, Anar B, Tuncbilek E: The 677 CT mutation of the MTHFR gene is not a risk factor for neural tube defects in the Turkish population. *Arch Dis Child Fetal Neonatal Ed* 1998;78:F235
21. Tunçbilek E, Alikaşifoğlu M, Akdalı B: *Türkiye'de konjenital malformasyon sıklığı, dağılımı, risk faktörleri ve yenidoğanların antropometik değerlendirmesi*. Ankara: Tubitak Matbaası, 1996
22. Kluijtmans LA, Kastelein JP, Lindemans J, Boers GH, Heil SG, Bruschke AU, Jukema JW, van den Heuvel LP, Trijbels FJ, Boerma GJ, Verheught FW, Willems F, Blom HJ: Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. *Circulation* 1997;96:2573–2577
23. Rees MM, Rodgers GM: Homocysteinemia: Association of a metabolic disorder with vascular disease and thrombosis. *Thromb Res* 1993;71:337–359
24. Kang SS, Wong PW, Norusis M: Homocysteinemia due to folate deficiency. *Metabolism* 1987;36:458–462
25. Franken DG, Boers GH, Blom HJ Cruysberg JR, Trijbels FJ, Hamel BC: Treatment of mild hyperhomocysteinemia in vascular disease patients. *Arterioscler Thromb* 1994;14:465–470