Public Health Nutrition: 4(2B), 493–497 DOI: 10.1079/PHN2001159

MTHFR gene polymorphism, homocysteine and cardiovascular disease

Claudio Cortese[1]* and Corradino Motti[2]
[1]Department of Internal Medicine, University of Tor Vergata, Via di Tor Vergata 135, 00133 Rome, Italy:
[2]Institute of Biochemistry and Molecular Biology, University of Teramo, Italy

Abstract

Homocysteine is an emerging new risk factor for cardiovascular disease. It is a thiol compound derived from methionine and involved in two main metabolic pathways: the cycle of activated methyl groups, requiring folate and vitamin B12 as cofactors, and the transsulfuration pathway to cystathionine and cysteine requiring vitamin B6 as cofactor. The homocysteine metabolism represents an interesting model of gene-environment interaction. Elevations in homocysteine may be caused by genetic defects in enzymes involved in its metabolism or by deficiencies in cofactor levels. A common polymorphism in the gene coding for the 5,10-methylene tetrahydrofolate reductase (MTHFR) (C677T, Ala → Val) is associated with a decreased activity of the enzyme due to thermolability. In case of homozygosity for the Val allele, a relative deficiency in the remethylation process of homocysteine into methionine leads to a mild-to-moderate hyperhomocysteinemia, a condition recognized as an independent risk factor for atherosclerosis. The genetic influence of the MTHFR polymorphism on homocysteine levels is attenuated in females in premenopausal age and is not significant in subjects who exhibit serum levels of folate and/or vitamin B12 above the 50th percentile of distribution in the general population. The prevalence of the Val/Val genotype varies among different ethnic groups. It is very low in African populations, whereas in Europe and North America it ranges between 5% and 15%. In Italy an even higher prevalence has been reported in some regions. The question whether the MTHFR polymorphism might be *per se* an independent contributor to cardiovascular risk is debated. The interaction between this or other genetic factors and environmental/nutritional conditions (i.e. intake of vitamins such as folate) is a key determinant for homocysteine concentrations in healthy conditions as well as in some disease (i.e. in renal disorders). Another example of gene/environment interaction in the field of atherosclerosis is given by the apolipoprotein E polymorphism and its influence in response to diet. The presence of a high prevalence of risk-related allelic variants of such candidate genes within a certain population could serve to locally reinforce the recommendations concerning nutrient intake.

Keywords
Homocysteine
Folate
Cardiovascular disease
Methylene Tetrahydrofolate reductase
Polymorphism
Apolipoprotein E

There is accumulating evidence that adequate intake of folate may protect from the risk of developing atherothrombotic disease and its complications. The most likely mechanism involves the pivotal role that folate exerts on homocysteine (Hcy) metabolism, being a co-substrate in Hcy remethylation to methionine[1]. Hcy is a putatively atherothrombotic sulfur aminoacid produced during methionine metabolism. Most probably Hcy exerts its atherogenic influence by interfering with several physiological conditions (activation of coagulation cascade and inhibition of fibrinolysis, impairment of endothelial function, lipoprotein modification and oxidation, etc). In conditions of excess of methionine Hcy enters the so-called transsulfuration pathway which eventually leads to the formation of cysteine. The initial reaction of this pathway is catalized by cystathionine β-synthase, an enzyme whose total deficiency is the cause of homocystinuria, a pathological condition which is characterized by elevated circulating levels of Hcy as high as 100–500 μmol/L and clinically by thromboembolic episodes often at a young age. Alternatively Hcy enters a remethylation cycle. 5,10-methylenetetrahydrofolate reductase (MTHFR) is a folic acid-related enzyme crucial for the remethylation of Hcy to methionine. It catalyzes the formation of 5-methyl tetrahydrofolate, which is the methyl donor in the reaction catalyzed by methionine synthase in presence of cobalamin (vitamin B12) as essential cofactor. A homozygous deficiency of MTHFR is

*Corresponding author: Email cortese@med.uniroma2.it

a rare condition associated with severe hyperhomocysteinemia, developmental delay, neurological abnormalities and vascular complications. Kang *et al.* in 1988 identified a thermolabile variant of MTHFR which correlated with mildly elevated plasma Hcy concentrations[2]. Recently this variant has been demonstrated to be caused by a point mutation (C677T) in the coding region of the MTHFR gene, leading to the substitution of valine (Val) for alanine (Ala)[3]. Because of the increasing interest in cardiovascular risk and mild elevation of circulating Hcy, many studies in recent years have attempted to establish the relevance of the MTHFR mutation to coronary risk. The MTHFR polymorphism could represent another example of a diet-responsive genetic factor contributing to atherosclerosis, as in the case of the genetic polymorphism of apoliprotein E which influences the lipoprotein metabolism.

Influence of MTHFR gene polymorphism on Hcy levels

The Val/Val genotype is associated with increased levels of Hcy, compared to Ala/Val and Val/Val. The MTHFR genotype-related increase in Hcy levels, however, is not evident when restricting the analysis to subsets of subjects exhibiting plasma folate or vitamin B12 at or above the median value in a given population. This suggests that higher levels of these cofactors counteract the negative effect of MTHFR thermolability on the efficiency of the remethylation process. As a corollary, individuals who are homozygous for the Val allele have an exaggerated elevation in fasting Hcy levels in response to the depletion of folic acid or even to suboptimal circulating levels of folate[4]. The same phenomenon is not evident when Hcy is measured after methionine load. As well, the influence of the MTHFR polymorphism on fasting Hcy levels is evident at all ages in the male sex but is attenuated in women in premenopausal age, probably because of their hormonal status. Thus, the MTHFR C677T (Ala/Val) polymorphism represents the best example of how a genetic polymorphism might affect nutrient requirements in a population.

Differences in the prevalence of thermolabile MTHFR

The prevalence of thermolabile MTHFR shows macroheterogeneity in different human groups. Homozygote frequencies of 5% to 16% have been quoted for healthy control populations. It may be reasonable to conclude that for white populations in the US and Australia the thermolabile MTHFR homozygote frequency is about 11.5%. However, in a critical evaluation of the many results reported in literature, the size and appropriateness of control populations investigated is of crucial importance.

In a recent meta-analysis of studies on the association between the MTHFR polymorphism and arteriosclerotic vascular disease, much attention has been focused on the population specificity of MTHFR genotype frequencies[5]. Most of these studies have been performed in countries in which subjects are predominantly of white European descent. Among different populations of white Caucasians, a Val/Val homozygote frequency of about 16.3% in an Italian study should be compared with the frequency of 5.4% in a Dutch study. The limited data available on other populations suggest that here too the Val/Val homozygote frequency varies dramatically; 11.0% for a Japanese population should be compared with 0% for Black Americans.

The heterogeneity in world distribution of the thermolabile MTHFR has been investigated very recently[6]. The frequency of the Val allele resulted very high (between 0.40 and 0.50) in the Mediterranean regions of Europe (South Italy and Spain) with an increasing north-to-south cline in the continent. A similar situation is evident in Asians with higher Val allele frequency in Japanese and Chinese (0.32) and a very low value for Indonesians (less than 0.05). Very low Val frequencies were found in Subsaharian and Ethiopian populations (between 0.05 and 0.07), as well as in African Americans. In hybrid populations of South America (Colombians and Brazilians), the reported Val frequency is very close to that of European populations (about 0.40).

A study on the impact of the MTHFR polymorphism on plasma Hcy levels in 12 different population based samples across Europe has been conducted by Gudnasson and colleagues on behalf of the European Atherosclerosis Research Study (EARSII)[7]. The study participants were male students aged between 18 and 28 years with either a paternal history of myocardial infarction ($n = 386$) or with no such history ($n = 402$). In the analysis of results, subjects were grouped into four European regions (Baltic, United Kingdom, Middle and South). No significant difference between the cases and controls in Hcy levels and MTHFR allele distribution was evidenced in any of the subgroups, or in the sample overall. Subjects from the South region (Portugal, Spain, Italy and Greece) had on average higher Hcy values than those from other regions of Europe (12.2 versus 10.3 μmol/l, $P < 0.001$). The prevalence of the Val allele was highest in South region (0.39). It was significantly lower in the Baltic region (Finland and Estonia) than in the rest of Europe (0.23) and this is in accordance with the narrower range of plasma Hcy concentration observed in prospective studies in Finland. A highly significant recessive effect of the MTHFR polymorphism on plasma Hcy levels was evident throughout all Europe regions; however, among the Val/Val carriers, those from the South region showed the highest Hcy concentrations. This regional difference can be attributable to other genetic or environmental factors. It has been suggested that the dietary intake of

https://doi.org/10.1079/PHN2001159 Published online by Cambridge University Press

folate may be lower in South Europe, probably because of peculiar dietary habits such as consumption of coffee. Evidence of an effect of the consumption of caffeinated filtered coffee on the increase in Hcy levels has been reported in a large study conducted in Norway[8]. Furthermore, an independent inverse dose-response relation between dietary protein intake and serum Hcy has been described in a recent study carried out in the Baltimore metropolitan area[9], suggesting that an increased protein intake may reduce total Hcy and the related risk for atherosclerosis. On the other hand, the recent policy enacted by the U.S. Food and Drug Administration in 1996, aimed at increasing folate intake in women of childbearing age to reduce the risk of neural tube defect by enriching with folic acid flour breads, rice, pasta,cornmeal and other cereal grain products, was associated with a substantial improvement in folate status and homocysteine levels in a population of middle-aged and older adults[10,11]; one cannot exclude that this might have influenced the nutrient intake particularly in Northern Europe in the last years. The high frequency of Val allele observed in Europe and particularly in the Mediterranean area argues per se against a possible role of this mutation as a single factor involved in the pathogenesis of coronary artery disease.

A microheterogeneity within the same population is also evident. An example is reported here regarding the published data on MTHFR Val/Val genotype prevalences in different regions of Italy (Table 1).

Reasons for the increased frequency of Val/Val in Italy and in general in South Europe remain obscure. An intriguing hypothesis could lie in a putative favorable effect of the MTHFR thermolabile variant in the presence of other genetic disorders that are prevalent in the Mediterranean area, such as the deficiencies in erythrocyte metabolism.

In our study on a population of healthy young subjects from Rome, we found that uric acid levels in plasma were strongly and independently correlated with Hcy levels[12]. The association between these two biochemical variables was indeed strong in the presence of homozygosity for the Val allele. This could suggest that, in presence of a defective MTHFR, a greater proportion of its natural substrate (5,10-methylene tetrahydrofolate) is shunted toward alternative metabolic pathways, such as the de novo synthesis of purines with consequent overproduction of uric acid. Otherwise, adenosine deriving from S-adenosyl-homocysteine (a precursor of Hcy) may represent the link between the metabolic pathways of Hcy and uric acid.

Hcy, folate,and MTHFR polymorphism in renal patients

The influence of folate, vitamin B6 and vitamin B12 intake on plasma Hcy concentration and the implications in terms of nutritional recommendations for the prevention of atherosclerotic disease are discussed in other parts of this supplement[13]. Apart from genetic differences underlying geographical variation in folate requirements, it is generally recognized that a total folate intake from food and supplements of >400 μg/day usually ensures normal Hcy levels in the majority of the population. Higher doses may be necessary for individuals with decreased renal function or low folate status. A reason for concern is the evidence that folate supplementation may mask the symptoms of vitamin B12 deficiency or even precipitate the related neuropathy.

Patients with renal disease (end stage renal disease or ESRD, haemodialysis, peritoneal dialysis and transplant recipients) might be at particular risk. As a matter of fact cardiovascular disease is the major cause of death in these individuals. Even after stratification by age, gender, race, and presence of diabetes CVD mortality in dialysis patients is some 20 times higher than in the general population, while in transplant patients a fourfold increase can be observed. Apart from classical risk factors like diabetes, hypertension and hyperlipidemia frequently associated with renal disease, hyperhomocysteinemia, often of considerable degree, is a particular feature of these patients. Several explanations for this Hcy elevation have been proposed, probably the most likely being the reduction in glomerular filtration rate. Folate status represents the major determinant of fasting total plasma homocysteine levels in maintenance dialysis patients[14]. Subjects with chronic renal disease are somewhat

Table 1 MTHFR Val/Val genotype prevalences in different regions of Italy

1st Author	Journal	Year	Italian region	Frequency of Val/Val
Missiaglia	Thromb Haemost	1997	North East	13.7%
De Franchis	Am J Hum Genet	1996	South	15.1%
Motti	Atherosclerosis	1998	Central (Lazio)	16.1%
Legnani	Arterioscler Thromb	1997	North (Emilia)	17.5%
De Stefano	Thromb Haemost	1997	Central	18.0%
Girelli	Blood	1998	North East	18.2%
Grandone	Thromb Haemost	1997	South	18.2%
Sacchi	Thromb Haemost	1997	North	21.0%
Cattaneo	Thromb Res	1999	North	21.0%
Cortese	unpublished		South (Calabria)	20.5%

https://doi.org/10.1079/PHN2001159 Published online by Cambridge University Press

refractory to low-dose folic acid supplementation. From the published Hcy-lowering intervention studies conducted on these patients one can conclude that doses as high as 15 mg/d are necessary to lower Hcy in dialysis patients, while a high-dose combination B therapy (folic acid 5.0 mg/d, vitamin B6 50 mg/d and vitamin B12 0.4 mg/d) is able to normalize Hcy level in renal transplant patients[15].

The MTHFR Val/Val genotype has been reported to aggravate the increase of plasma Hcy in hemodialysis patients[16].There was no detectable effect of MTHFR polymorphism on plasma Hcy levels in ESRD patients undergoing haemodialysis receiving high-dose folate supplementation (2 g/die)[17]. On the other hand, MTHFR polymorphism is a significant predictor of plasma total Hcy and folate levels in kidney graft recipients[18]. In these individuals, homozygosity for Val allele significantly increases plasma total Hcy concentrations and lowers folate levels even in presence of good renal function. However, there are no data supporting a role of the MTHFR polymorphism as a determinant of renal transplant survival.

Apo E polymorphism as an example of diet-sensitive genetic factor for atherosclerosis

Based on the current evidence, the MTHFR polymorphism could add to the list of the gene variants potentially candidate for clinical use in the prevention of atherosclerosis. In this regard, the most intensively studied genetic polymorphism contributing to the development of atherosclerosis is represented by the apolipoprotein E polymorphism, which can be adopted as a standard model of gene/environment interaction in the context of a multifactorial disorder[19].

The apolipoprotein E polymorphism is another example of North-to-South gradient in the distribution of a genetic polymorphism relevant to the risk of atherosclerosis. It has been known for several years by now that three different alleles, namely $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$, determined by the combination of two distinct polymorphic sites at the apolipoprotein E gene locus, are responsible for the different affinity of the apoE-carrying lipoproteins with peripheral receptors. In the presence of the $\varepsilon 4$ allele, this modulation in affinity results in an increase in circulating cholesterol-rich lipoproteins. The effect of the $\varepsilon 4$ allele towards increasing cholesterol levels has been observed in random samples of normal people, normolipidemic individuals, those selected for health, and in diabetic and CHD patients. The atherogenic potential of the $\varepsilon 4$ allele is further supported by studies of its prevalence in various population samples and subsets with or without CHD. It has been consistently demonstrated that the frequency of the $\varepsilon 4$ allele tends to be higher in populations with increased CHD mortality rates, such as the Finnish population, and lower in those with reduced rates, such as the Chinese and Japanese populations. Furthermore, as shown recently, the $\varepsilon 4$ allele frequency varies widely across populations even within Caucasian and Asian samples. The frequency of the $\varepsilon 4$ allele appears to be higher in Northern Europe (eg: up to 0.30 in a Helsinki population), where CHD prevalence is high, than in southern regions (e.g.: as low as 0.04 in a population from Calabria, Southern Italy), where CHD prevalence is low. It is noteworthy that the relation of the $\varepsilon 4$ allele with an increase in CHD prevalence may be strengthened by the well-known interaction of this polymorphism with environmental factors, particularly with nutritional habits such as the amount of dietary saturated fatty acids[20].

As in the case of the thermolabile variant of MTHFR, a North-to-South gradient has been demonstrated for the $\varepsilon 4$ allele frequency, although in the opposite direction. Again, the reasons for this nearly eightfold difference is not easily explained. One can only speculate that different environmental factors and competing mortalities have exerted alternative selection pressures.

Conclusions

As pointed out elsewhere in this document, if a mutation in a candidate gene within a population is frequent enough to be defined a polymorphism, its penetrance is likely to be relatively low, even if the relation between the mutation and the altered phenotype is clear. Evidently, the interplay with other genetic and/or environmental factors significantly modulates the final effect, making genetic screening inconvenient and expensive. Undoubtedly, when dealing with the implementations of guidelines in the context of a population strategy aimed at achieving better nutritional habits and a healthier lifestyle, the easiest approach is to give recommendations concerning nutrients themselves. For instance, in relation to the two polymorphisms described above, an increased intake of folate and a decreased intake of saturated fatty acids would be advisable in general. Nevertheless, in the presence of a consistently demonstrated high prevalence of the risk-related allelic variants of candidate genes within a certain population, the same recommendations could be locally reinforced.

References

1 Welch GN, Lo Scalzo J. Homocysteine and atherothrombosis. *N. Engl. J. Med.* 1998; **338**: 1042–50.
2 Kang SS, Wong PWK, Susmano A, Sora H, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary heart disease. *Am. J. Hum. Genet.* 1991; **48**: 536–45.
3 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate gene risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* 1995; **10**: 111–3.
4 Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH,

https://doi.org/10.1079/PHN2001159 Published online by Cambridge University Press

Rosenberg IH. Selhub J. Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; **93**: 7–9.
5 Fletcher O, Kessling AM. MTHFR association with arteriosclerotic vascular disease? *Hum. Genet.* 1998; **103**: 11–21.
6 Pepe G, Camacho Vanegas O, Giusti B, Brunelli T, Marcucci R, Attanasio M, Rickards O, De Stefano GF, Prisco D, Gensini GF, Abbate R. Heterogeneity in World Distribution of the Thermolabile C677T mutation in 5,10-methylenetetrahydrofolate reductase. *Am. J. Hum. Genet.* 1998; **63**: 917–20.
7 Gudnasson V, Stansbie D, Scott J, Bowron A, Nicaud V, Humphries S. on behalf of the EARS group. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. *Atherosclerosis* 1998; **136**: 347–54.
8 Nygard O, Refsum H, Ueland PM, Stensvold I, Nordrehaug JE, Kvale G, Vollset SE. Coffee consumption and plasma total homocysteine: The Hordaland, Homocysteine Study. *Am. J. Clin. Nutr.* 1997; **65**: 136–43.
9 Stolzenberg-Solomon RZ, Miller ER 3rd, Maguire MG, Selhub J, Appel LJ. Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population. *Am. J. Clin. Nutr.* 1999; **69**: 467–75.
10 Food Standards. Amendment of standards of identity for enriched grain products to require addition of folic acid. *Federal Register* 1996; **61**: 8781–97.
11 Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N. Engl. J. Med.* 1999; **340**: 1449–54.
12 Motti C, Gnasso A, Bernardini S, Massoud R, Pastore A, Rampa P, Federici G, Cortese C. Common mutation in methylenetetrahydrofolate reductase. Correlation with homocysteine and other risk factors for vascular disease. *Atherosclerosis* 1998; **139**: 377–83.
13 Molloy AM, Scott JM. Folates and prevention of disease. (this issue)
14 Bostom AG, Shemin D, Lapane KL, Nadeau MR, Sutherland P, Chan J, Rozen R, Yoburn D, Jacques PF, Selhub J, Rosenberg IH. Folate status is the major determinant of fasting total plasma homocysteine levels in maintenance dialysis patients. *Atherosclerosis* 1996; **123**: 193–202.
15 Bostom AG, Culleton BF. Hyperhomocysteinemia in chronic renal disease. *J. Am. Soc. Nephrol.* 1999; **10**: 891–900.
16 Fodinger M, Mannhalter C, Wolfl G, Pabinger I, Muller E, Schmid R, Horl WH, Sunder-Plassmann G. Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. *Kidney Int.* 1997; **52**: 517–23.
17 Lee HA, Choi JS, Ha KS, Yang DH, Chang SK, Hong SY. Influence of 5,10-methylenetetrahydrofolate reductase gene polymorphism on plasma homocysteine concentration in patients with end-stage renal disease. *Am. J. Kidney Dis.* 1999; **34**: 259–63.
18 Fodinger M, Buchmayer H, Heinz G, Papagiannopoulos M, Kletzmayr J, Rasoul-Rockenschaub S, Horl WH, Sunder-Plassmann G. Effect of MTHFR 1298A → C and MTHFR 677C → T genotypes on total homocysteine, folate, and vitamin B(12) plasma concentrations in kidney graft recipients. *J. Am. Soc. Nephrol.* 2000; **11**: 1918–25.
19 Gerdes LU, Klausen IC, Sihm I, Faergeman O, Apolipoprotein E. polymorphism in a Danish population compared to findings in forty-five other study populations around the world. *Genet. Epidemiol.* 1992; **9**: 155–67.
20 Kesaniemi YA, Ehnholm C, Miettinen TA. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *J. Clin. Invest.* 1987; **80**: 578–81.