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Methylenetetrahydrofolate Reductase and Methionine Synthase Gene Association with Homocysteine Metabolism and Coronary Artery Disease

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INTRODUCTION

Coronary artery disease (CAD), a major cause of death in developed countries, is a multifactorial disease. Various factors such as hypertension, diabetes, hyperlipidemia, and smoking, interact to increase the risk of developing CAD.[1] Recently, even a moderate elevation of plasma homocysteine (hcy) was found to be an independent risk factor for the development of CAD and was shown to have a close relation to several conventional risk factors.[1),2),3)] In fact, meta-analysis of studies published in recent years indicate that a 5 mol/l rise in plasma total hcy concentrations is equivalent to a 0.5 mmol/l increase in total cholesterol levels in terms of risk of CAD.[4)]

Hcy as an independent risk factor for CAD

Despite the strong evidence that total hcy is an independent risk factor for CAD, the exact mechanism for hyperhomocysteinemia leading to the development of CAD remains unclear.[5)] Experimental evidence suggests that the mechanism may be related to the redox properties of the sulfhydryl group of hcy.[3)] Other proposed mechanisms include platelet activation, hypercoagulability, cytotoxicity, and stimulation of LDL oxidation.[2)] Recently, it was reported that hcy has an inhibitory effect on endothelial cell growth and a stimulatory effect on vascular smooth muscle cell proliferation.[1),6),7)] Furthermore, there are some reports that hcy enhances endothelial cell-associated factor V activity, and inhibits thrombomodulin activity, protein C activation, and tissue plasminogen activator binding.[6)] Most of these studies, however, were *in vitro* studies, where supraphysiological concentrations of hcy were used.

Hcy metabolism

Hcy, a non-dietary sulphur-containing amino acid, is formed from methionine as a product of numerous S-adenosylmethionine-dependent transmethylation reactions.[3)] Once formed, the hcy can be trans-sulfurated to form cysteine or remethylated to form methionine.[3),5),8)] Hcy is converted to cysteine via the trans-sulfuration pathway in 2 sequential reactions, catalyzed by cystathionine -synthase (CBS), and -cystathionase, using pyridoxal phosphate (PLP), the active form of vitamin B_6, as a coenzyme.[3),5),9)] However, not all hcy is degraded to cysteine, the excess being remethylated to form methionine.

In remethylation, there are two pathways: the liver-based vitamin-independent betaine pathway and the folate-vitamin B_{12} pathway.[5)] The latter reaction in most tissues is catalyzed by methionine synthase (MS), which requires vitamin B_{12} as a cofactor and 5-methyl tetrahydrofolate (THF) as a cosubstrate.[3)] 5-Methyl THF, the principal circulating form of folate, is synthesized from 5,10-methylene THF (MTHF) by the enzyme 5,10-methylene THF reductase (MTHFR).[9)] Thus, hcy metabolism can be regulated by 3 key enzymes (CBS, MS and MTHFR) as well as by the cofactors (folate, vitamin B_{12}, and vitamin B_6).[9)]

Hyperhomocysteinemia, MTHFR genotypes and folate

Plasma normally contains an average of 10 mol/L of hcy due to the existence of a cellular hcy export mechanism.[9)] This export mechanism complements the catabolism of hcy to maintain its low intracellular level.[9)] Inhibition of hcy metabolism as a result of enzyme defects or vitamin deficiencies causes hcy export into extracellular compartments and thereby hyperhomocysteinemia. Genetically depressed levels of the enzyme CBS can cause hyperhomocysteinemia. Heterozygosity for deficiency of CBS had been postulated as a risk factor for mild hyperhomocysteinemia. A recent report, however, suggests that CBS deficiency is not common in patients with CAD.[8)]

A mutation (677CT) in the MTHFR gene, an alanine-to-valine substitution (the T allele), is the only common genetic change (with a prevalence of about 10% in Caucasians) that is associated with mild hyperhomo-

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cysteinemia[2,8] MTHFR reduces 5,10-MTHF to 5-methyl THF, the major methyl donor for remethylation of hcy to methionine.[10] The MTHFR TT genotype shows increased thermolability and about 30% of the normal activity.[1] Individuals with the MTHFR TT genotype may have a higher folate requirement for regulation of plasma hcy concentrations than those with the other genotypes.[9,10]

A polymorphism in the MTHFR gene, particularly among individuals with low plasma folate level, is a major determinant of the total hcy concentration, which is about 25% higher in the TT than the CC genotype.[2] There is, however, uncertainty as to the association between MTHFR mutation and CAD. Although some investigators reported the presence of a homozygous mutation to be a risk factor for CAD, Verhoef *et al.*[11] summarized the results of 12 studies and reported that 8 of them did not find an increased risk of CAD for this genotype. This suggests that an association between MTHFR mutation and the risk of CAD might be swamped by other risk factors.[10]

Hyperhomocysteinemia, vitamin B_6 and vitamin B_{12}

Although the homozygous mutant genotype, in combination with low folate status, predisposes individuals to hyperhomocysteinemia,[8] other variables that could contribute to hyperhomocysteinemia are the blood levels of PLP and vitamin B_{12}. In hcy metabolism, the transsulfuration pathway may be particularly sensitive to levels of PLP, the cofactor for CBS.[8] However, the interruption of trans-sulphuration by the lack of PLP is the least critical in hyperhomocysteinemia.[5]

Vitamin B_{12} deficiency, which is common in vegetarians and the elderly, leads to failure of the transfer of the methyl group from 5-methyl THF to hcy during remethylation. This also leads to the methyl-folate trap situation in which folate is trapped in the 5-methyl THF form, making it unavailable to other metabolic processes.[5] In fact, Mann *et al*[5] reported higher plasma hcy levels in vegetarians, and particularly in vegans, than in omnivores.[5] In addition, Pancharuniti *et al*[12] found an inverse relationship between vitamin B_{12} and plasma hcy, but when plasma hcy and other CAD risk factors were controlled, vitamin B_{12} status correlated positively with CAD. Especially in CAD men, there is an interaction between the MTHFR homozygous mutant genotype, and folate and vitamin B_{12} status in the elevation of plasma hcy.[15]

It has been estimated that two thirds of hyperhomocysteinemia cases can be attributed to inadequate plasma concentrations of one or more of the B-vitamins: folate, vitamin B_{12} or vitamin B_6.[13] Many studies have shown inverse associations between plasma folate and hcy, and to a lesser extent between vitamin B_{12} and hcy, but not between vitamin B_6. Plasma hcy levels are also influenced by the kind of B-vitamins supplementation. Selhub *et al.*[13] found that vitamin B_6 supplementation alone did not reduce plasma hcy level, whereas Mann *et al.*[5] found vitamin B_{12} and folate supplementations could reduce about 15%, 42 % of hcy level, respectively.[5]

Hyperhomocysteinemia and MS genotypes

MS catalyzes the remethylation of hcy to methionine in a methylcobalamin-dependent reaction, and a deficiency of MS activity results in hyperhomocysteinemia.[14] In the MS D919G mutation, the capacity to maintain a reduced form of cobalamin on the MS apoenzyme is impaired. It has been suggested that the MS D919G mutation, common in the general population, might lead to mild hyperhomocysteinemia with a consequent impact on vascular disease.[14] However, several recent studies showed that the MS D919G mutation is unlikely to have a major effect on hcy metabolism, as no association was shown between this MS mutation and elevated plasma hcy.[15]

CONCLUSION

A summary of results from many studies showed that the MTHFR TT genotype was associated with increased plasma hcy levels. Although elevated plasma total hcy is an independent risk factor for CAD, there is a lack of association between the MTHFR gene and CAD. Additionally, in contrast to the variation in MTHFR genotype, the MS D/G polymorphism was not found to have had a major effect on hcy metabolism. The results from intervention trials with hcy reducing therapy are generally effective on moderate hyperhomocysteinemia. Further studies are needed to clarify how changes in hcy metabolism and their effects on CAD are genetically and environmentally determined.

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