Original Contributions

Genetic Analysis of the Thermolabile Variant of 5,10-Methylenetetrahydrofolate Reductase as a Risk Factor for Ischemic Stroke

Dawn L. Harmon, Rachael M. Doyle, Raymond Meleady, Marguerite Doyle, Denis C. Shields, Rosemary Barry, Davis Coakley, Ian M. Graham, Alexander S. Whitehead

Abstract—Mild hyperhomocysteinemia is a risk factor for atherosclerotic vascular disease. Homozygosity for the C677T mutation in the gene for 5,10-methylenetetrahydrofolate reductase (MTHFR) is frequently associated with hyperhomocysteinemia, particularly in individuals with low levels of serum folate, and has been directly associated with cardiovascular disease in certain populations. The purpose of this study was to establish whether the C677T mutation, which causes thermolabile MTHFR, is a risk factor for ischemic stroke in the Irish population. The homozygous C677T genotype has previously been associated with coronary heart disease in Ireland. We collected blood from 174 individuals (minimum age 60 years) who had suffered an ischemic stroke that was confirmed by computed tomography brain scan. Control subjects (n=183) aged ≥60 years, who had never suffered a stroke or transient ischemic attack, were recruited from hospitals and active retirement groups in the same geographical area. MTHFR genotypes were determined and other known risk factors for stroke were documented. In the control group, the frequency of subjects with the homozygous C677T genotype was 10.4%. In patients who had suffered ischemic stroke, the frequency was 15.5%. This difference was not statistically significant. The odds ratio of stroke for C677T homozygotes, with other genotypes as a reference group, was 1.59, 95% CI=0.85, 2.97. The data indicate that the homozygous C677T MTHFR genotype is at most a moderate risk factor for ischemic stroke. (Arterioscler Thromb Vasc Biol. 1999;99:208-211.)

Key Words: homocysteine ■ genetics ■ MTHFR ■ ischemic stroke

Moderate elevation of homocysteine in plasma (moderate hyperhomocysteinemia) is an established risk factor for atherosclerotic vascular disease including cerebrovascular disease. In addition it is associated with thickening of the carotid arterial wall. The magnitude of the risk of vascular disease conferred by hyperhomocysteinemia varies between populations. A meta-analysis of the data indicates that hyperhomocysteinemia confers at least a 2.5-fold increased risk of stroke.

Homocysteine is a sulphur amino acid generated by the many transmethylation reactions that consume S-adenosyl methionine (SAM) (Figure). Homocysteine is remethylated to methionine (from which SAM is then regenerated) by the vitamin B12-dependent enzyme methionine synthase. This remethylation uses 5-methyl tetrahydrofolate as the methyl donor. 5-methyl tetrahydrofolate is itself generated from 5,10-methylene tetrahydrofolate by the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR). Under normal circumstances, homocysteine can be irreversibly degraded by the transsulfuration pathway, the first step of which is catalyzed by the vitamin B6-dependent enzyme cystathionine β-synthase (CBS).

Plasma homocysteine levels are therefore influenced by both nutritional (folic acid and vitamins B12 and B6) and genetic factors, including mutations in the genes encoding methionine synthase, CBS, and MTHFR. In particular, a biochemically defined “thermolabile” variant of MTHFR is associated with moderate hyperhomocysteinemia. The genetic change underlying thermolabile MTHFR is a C to T base transition at nucleotide 677 in the MTHFR cDNA, which results in the substitution of valine for alanine. The frequency of homozygotes for the thermolabile MTHFR mutation is between 5% and 15% in different European populations.

Recently, homocysteine for the thermolabile genotype has been associated with a nearly 10-fold increased risk of an individual being in the top 5% of the population for homocysteine distribution. This is a homocysteine level known to confer a 2- to 3-fold risk of vascular disease. However, the association between elevated plasma homocysteine and the thermolabile genotype is dependent on serum folate status. Significant gene-nutritional interactions are therefore likely, and twin studies indicate that approximately half of homocysteine variation may be a result of genetic factors.
Several studies of the association between the homozygous thermolabile genotype and coronary artery disease (CAD) have been reported. A recent UK-based study has established that, as in CAD, hyperhomocysteinemia is a significant risk factor for stroke. However, a recent study by Markus et al., the homozygous thermolabile genotype was not associated with ischemic cerebrovascular disease (CVD), despite the significant association of the genotype with elevated homocysteine in the patient group. In the present study, we investigated the possibility that this genotype may be a risk factor for ischemic stroke in the Irish population, as the genotype confers a particularly high (2.9-fold) increase in risk of CAD in this population.

Methods
Stroke was defined as an episode of sudden-onset lateralized neurological deficit and was confirmed by objective evidence of ischemia on computed tomography brain scan. After ethical approval and informed consent had been obtained, we recruited 174 patients from St James’s Hospital who had survived an ischemic stroke at 60 years. (St James’s Hospital is an urban teaching hospital in Dublin that serves a population of ~400,000.) Patients were drawn from both the acute medical and rehabilitation wards and the day hospital. Information regarding known risk factors for stroke was recorded for each patient (ie, hypertension, current smoking, history of stroke or transient ischemic attack, carotid endarterectomy, ischemic heart disease, diabetes mellitus, and peripheral vascular disease).

Control subjects (n = 183) were recruited from the same age group and geographical area. The sole exclusion criterion for control subjects was a past history of stroke. Of the control subjects, 143 were recruited from active retirement groups, 38 were attending a day hospital for other reasons, 1 was a relative of a staff member, and 1 was a hospital employee. Recruitment of patients and control subjects occurred between May 1996 and June 1997.

DNA was prepared from 30 μL of whole blood collected in EDTA. MTHFR genotypes were determined by the method of Frosst et al. by the use of PCR amplification and restriction digestion with HinfI to distinguish C677T and wild type alleles.

Results
The frequency of the homozygous thermolabile MTHFR genotype was higher in ischemic stroke cases (15.5%) than in the age matched control subjects (10.4%). This trend (odds ratio = 1.59, 95% CI = 0.85, 2.97) was not significant (P = 0.16). The distribution of MTHFR genotypes in the case and control groups is shown in Table 1.

Control subjects had lower frequencies of known risk factors for stroke, including atrial fibrillation, ischemic heart disease (IHD), diabetes, hypertension, and current smoking (Table 2). MTHFR genotype was not significantly associated with any of the documented risk factors that were present at a high enough frequency to allow analysis (ie, hypertension, current smoking, atrial fibrillation, or IHD). Among the 174 stroke cases, 49 (28.2%) suffered from IHD. The frequency of the genotype in these individuals was 16.5%, similar to the overall frequency in the stroke cases. A number of stroke patients had a history of stroke (51.1%), transient ischemic attack (10.3%), carotid stenosis or carotid endarterectomy (6.3%), previous to the index event leading to their enrollment. The frequency of the homozygous thermolabile genotype in the 105 individuals with a history of one of these conditions was 14.3%. In addition, 10.3% of stroke patients had been diagnosed as having multi-infarct dementia.

Discussion
We have shown that the homozygous C677T MTHFR genotype may be a moderate risk factor for ischemic stroke in Ireland; however, the association is not strong enough to reach statistical significance in the number of individuals recruited for this study.

A meta-analysis of 9 case-control studies that support hyperhomocysteinemia as a risk factor and 2 prospective studies, in which no significant association was found, indicates that hyperhomocysteinemia confers at least a 2.5-fold increased risk of stroke. A third prospective study has
since found a significantly increased incidence of stroke in middle-aged British men with elevated homocysteine levels. However, the first study of the homozygous thermolabile genotype in UK patients with ischemic CVD, which included both stroke and CT-negative TIA, found no association of the genotype with CVD. The proportional contribution of the homozygous C677T genotype to mild hyperhomocysteinemia has also been studied. In 1 study, nearly half of the individuals whose homocysteine levels were above the 95th percentile of the normal distribution were C677T homozygotes. However, the association between the genotype and homocysteine is dependent, at least in part, on serum folate levels; Harmon et al found that elevated homocysteine was only associated with the genotype in individuals with folate levels below the population median. In the present study, the folate and homocysteine status of the population has not been determined. There is good evidence that vitamin deficiency states are common in the elderly. It may be that the relative contribution of the C677T MTHFR genotype to raised plasma homocysteine levels (and therefore to ischemic stroke) is lower than might be expected simply because such genetic factors are heavily outweighed by nutritional deficiencies (for example, of folate or B12) in this elderly group, leading to high homocysteine levels. If the present population is not folate deficient, then we cannot discount the possibility that the interaction of the C677T MTHFR genotype with low folate levels is a strong risk factor for stroke in those who are nutritionally compromised. Overall, our results indicate that the homozygous C677T genotype is, at most, a modest risk factor for stroke in a population with a high prevalence of other stroke risk factors. Other estimates of the frequency of the C677T MTHFR genotype in healthy Irish subjects, based on control groups in a study of Parkinson’s disease (n=184, C677T homoygote frequency 7.1%) and CAD (n=61, C677T homoygote frequency 6.6%), indicate that the genotype frequency in Ireland is lower (≈7.0%) than the 10.4% observed in the elderly control subjects reported above. When our control population is combined with these additional control groups, then the frequency of the homozygous C677T genotype is significantly higher in stroke cases (P=0.01, odds ratio = 2.0, 95% CI = 1.2-3.4). This observation endorses the need to undertake large prospective studies to establish more accurately the level of risk conferred by the genotype. If confirmed, an odds ratio of between 1.5 and 2 would have significant public health implications.

The association of the homozygous C677T MTHFR genotype with CAD has been the subject of many recent studies. In Ireland,14 the Netherlands,15 and Japan16,17 the genotype has been associated with a significantly increased risk of CAD, and in French Canada with a nonsignificant increase in risk. However, no association between the genotype and CAD or myocardial infarction has been found in Australia,19,20 England,21,25 the Physicians Health Study (United States),22 the Boston Area Health Study,23 or additional studies in the Netherlands26 and the United States.27,28 Investigators have also failed to find any association between the genotype and angiographically significant obstructive coronary artery disease,20 atherosclerotic peripheral vascular disease,29 and (in a recent study) ischemic CVD.29

The different findings and conclusions reached in the above studies may be partially explained by differences in study size and design. However, if the risk conferred by the genotype is largely dependent on an additional factor such as serum folate level, which may vary between populations, then the impact of the homozygous C677T MTHFR genotype might differ widely. Indeed, homocysteine levels are known to vary significantly between populations,44 and it has yet to be determined what proportion of this variation is attributable to diet and/or to genetic factors, including the C677T MTHFR genotype. However, in most of the above studies in which homocysteine was measured, the genotype was associated with elevated homocysteine levels, and it is therefore surprising that in many of these studies it does not appear to be a significant risk factor for vascular disease.

To conclude, we have shown that the MTHFR genotype may be a modest risk factor for stroke in the Irish population. However, other factors such as nutritional deficiency, acting through additional genetic and nongenetic mechanisms and leading to raised plasma homocysteine levels, may be relatively more important in an elderly population. In view of the conflicting results generated by the many retrospective studies of the association between the genotype and vascular disease, a prospective study to accurately quantify the risk conferred in stroke is needed. Such a study should include the measurement of homocysteine and of serum and red cell folate levels and should also include dietary information.

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References


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