Hyperhomocysteinemia, MTHFR 677C→T Polymorphism, and Stroke

To the Editor:
We welcome the report from Madonna and co-workers examining the role of prothrombotic and homocysteine (Hcy) pathway polymorphisms in risk of ischemic stroke in young adults.1 We wish to comment on several issues raised by their article relating to the design of studies of genetic risk factors for complex phenotypes such as ischemic stroke, which we believe to be important when interpreting their findings. As the report points out, genetic predisposition to a complex human phenotype such as stroke is unlikely to be mediated by a large influence of 1 or 2 genes. Many observers agree that it is likely to result from a combination of relatively small individual effects of several genes, each predisposing to stroke via their influence on intermediate phenotypic traits, such as hypertension or hyperlipidemia.2–4 This assumption has several implications for the design of epidemiological studies examining candidate genetic risk factors for stroke.

First, given the small anticipated effect size associated with any single candidate polymorphism, the group sample sizes required to robustly demonstrate an association will be very large. This is important to avoid a potentially erroneous conclusion that no association exists (type 2 error). This point is particularly relevant in the case of the MTHFR 677C→T polymorphism. Most prospective and retrospective studies to date have indicated that mildly elevated plasma Hcy is independently associated with ischemic stroke, and that the MTHFR TT genotype is associated with elevated Hcy. Paradoxically, most studies have not demonstrated an association between ischemic stroke and TT genotype.5 One potential explanation for this apparent inconsistency is that the majority of studies lacked sufficient power to detect an association. Given a background rate of 17% in the general population, as reported in the article by Madonna and colleagues, and an anticipated relative risk of 20% associated with the TT genotype, we estimate that at least 10,181 subjects per group are required to detect an effect with 80% power (α = 0.05). The practical difficulties that this creates may be overcome by pooling data from multiple centers, or by employing alternative family-based designs, such as those currently underway by the NIH-funded Siblings With Ischemic Stroke Study (SWISS).

Also, given the heterogeneity of underlying biological mechanisms predisposing to ischemic stroke, careful definition of stroke subtype and intermediate traits (eg, hypertension, atrial fibrillation) is important when studying candidate genetic polymorphisms.2,3 This is important because a susceptibility gene that increases stroke risk by influencing 1 trait (eg, hypertension) is unlikely to be overrepresented in a study sample that contains a large proportion of patients with stroke due to other mechanisms (eg, cardiogenic embolism). Stratification by subtype and intermediate traits may increase the ability to detect the influence of prespecified candidate genes, by decreasing “noise” and reducing variability in the sample. This is relevant when interpreting studies examining the role of Hcy pathway polymorphisms. Although greatly elevated Hcy is associated with arterial thrombosis in vivo and activation of coagulation pathways in vitro, mildly elevated Hcy levels in the range described in the report by Madonna et al have been reported to cause cerebrovascular disease primarily by predisposing to large- and small-vessel atherosclerosis.6–7 In studies of this nature, underrepresentation of subjects with large- and small-vessel stroke may further dilute the ability to detect an association of stroke with Hcy pathway polymorphisms, such as MTHFR TT genotype.

Finally, it is important to control for potential modification of genetic influences by environmental factors, such as vitamin status in studies of Hcy pathway genes.2–4 Unfortunately, this information was not provided in the report. It is particularly relevant to studies of the MTHFR 677C→T substitution, as data consistently indicate that the adverse effect of the substitution on plasma Hcy are abolished by adequate folate intake, suggesting that the TT genotype may be relatively more important in populations with low folate intake. This is relevant when interpreting their results in the US context, as recent data indicate that folate levels have increased in the US population since folate fortification of cereals in 1998, with accompanying reductions in plasma Hcy.8

P.J. Kelly, MB, MSc, MRCPI
M. Barron, BS
K.L. Furie, MD, MPH
Stroke Service
Department of Neurology
Massachusetts General Hospital and Harvard University
Boston, Massachusetts


Response:
We thank Dr Kelly et al for their letter that emphasizes major aspects concerning the design of our study as well as the role of some genetic predisposing factors. As acknowledged by Kelly et al, we interpret our data to support the concept that, rather than single genes, the combination of several genes, each causing a small effect per se, is important to lead to complex human phenotypes such as stroke. Nevertheless, our data emphasize that, similar to factors such as cigarette smoking, hypertension, diabetes, and hyperlipidemia, hyperhomocysteinemia is more common in cases than in controls and is important to help identify subjects with a history of early-onset ischemic stroke.
The point of stratification of the sample(s) according to the type of event (atherothrombotic, cardioembolic) is well taken. Several groups believe that rather than in atherosclerosis, homocysteine is involved in platelet activation and thrombosis. Nor we have found conclusive clinical data that at low concentrations, homocysteine is atherogenic while it is thrombogenic at higher concentrations. Thus, it is not worth looking for the association of this polymorphism with the event in larger sample sizes; it only suggests that the impact that these findings may have for the identification of subjects at risk is likely to be rather limited.

The type of event will help clarify the role of the MTHFR TT mutation. This does not imply that the impact that these findings may have for the identification of subjects at risk is likely to be rather limited.

Helicobacter pylori, CagA-Positive Strains, and Ischemic Stroke

To the Editor:

In their interesting study, Heuschmann et al1 showed the chronic Helicobacter pylori infection to be associated with a higher risk of stroke caused by small-artery occlusion, a subtype of ischemic stroke also sharing pathomechanisms with atherosclerotic disease. A similar trend was demonstrated for stroke caused by large-artery atherosclerosis, although this trend was not statistically significant. We would like to make some comments on it. Several studies have shown that the clinical outcome of H pylori infection is strictly related to the genetic polymorphism of both the bacterium2 and the host.3 In fact, recent findings have demonstrated H pylori infection to be only weakly associated with ischemic heart disease in multivariate analysis. On the other hand, the more cytotoxic H pylori strains, bearing the cytotoxin-associated gene A (CagA), were highly prevalent in patients with ischemic heart disease.4 On the basis of these observations, it could be interesting to investigate the prevalence of CagA-positive strains of the bacterium in patients with ischemic stroke. Various pathogenetic mechanisms have been postulated to explain the association between infection by the cytotoxic H pylori strains and the atherosclerotic process. Because atherosclerosis is a chronic inflammatory disease, the stronger persistent low-grade immunoinflammatory burden evoked by the infection by CagA-positive strains could play a role in this association. Moreover, a cross-mimicry between CagA and antigens of the endothelial wall of cerebral arteries has been demonstrated5 and has been postulated to promote the atherosclerotic process by inducing endothelial damage. More recently, authors hypothesized that CagA-positive strains of H pylori, predisposing to gastric atrophy, may induce vitamin malabsorption and therefore hyperhomocysteinemia, a well-documented risk factor for ischemic heart disease. However, not all patients infected by H pylori and its CagA-positive strains are affected by atherosclerosis-related diseases. The most convincing explanation for this observation could be found in the complex interaction between bacterium and host. In conclusion, the presence of particular factors, such as infection by CagA-positive strains, in subjects with a genetic susceptibility to develop ischemic stroke may explain the different clinical outcome of the infection in different patients.

M. Gabrielli, MD
P. Pola, MD
A. Gasbarrini, MD

Department of Internal Medicine
Catholic University of Sacred Heart
Rome, Italy


Response

We appreciate the comments of Dr Gabrielli and colleagues and their interest in our article on chronic Helicobacter pylori and Chlamydia pneumoniae infection in acute ischemic stroke.1 We agree that chronic H pylori infection in general seems only to have a weakly positive association with coronary artery disease, but this association could be caused by confounding and/or biases.2 Gabrielli and colleagues hypothesized that infection with more virulent H pylori strains, eg, strains bearing cytotoxin-associated gene A (CagA), might increase the relative risk of coronary artery disease, based on the findings of a retrospective case-control study on 88 subjects.3 However, a recently published prospective study failed in reproducing these results.4 Although there have been dozens of studies seroepidemiologically linking coronary artery disease and H pylori infection, until now 3 studies have investigated the potential association between ischemic stroke and chronic H pylori infection.5,6 All of these studies found similar results, as follows: Grau et al.6 reported elevated antibodies to H pylori in ischemic stroke by athero-

### Influenza Virus Infection, Infectious Burden, and Atherosclerosis

**To the Editor:**

We read with great interest the study by Philippa Lavallée et al. in a recent issue of *Stroke*. The authors found in a case-control study of 450 subjects that the risk of stroke was significantly reduced in the subjects vaccinated against influenza during the last 5 years. The authors conclude that influenza vaccination may protect against brain infarction by reducing infections.

A possible relationship between influenza and atherosclerosis, in particular myocardial infarction, was first suggested after epidemics of influenza struck Europe and the United States in the early 1900s. Increasing evidence exists indicating that inflammation and possible infections play an important role in atherogenesis. The hypothesis of infectious agents that may play an important role in the atherogenesis is supported by the results of several epidemiologic studies, suggesting a possible atherogenic potential from particular pathogens, like *Helicobacter pylori*, cytomegalovirus (CMV), herpes simplex virus (HSV), and Chlamydia pneumoniae. However, existing epidemiologic data about the association of some of these pathogens and atherosclerosis are conflicting, and although attractive, the microbial pathogenesis theory for atherosclerosis remains unproven. Clinical data about the association of influenza virus seropositivity (in unvaccinated subjects) and atherosclerosis are lacking. We tested the possible association between influenza type A and B infection and presence of atherosclerosis of coronary arteries (defined as >50% diameter stenosis of at least 1 coronary artery assessed angiographically) in a study of 218 consecutive patients (not previously vaccinated against influenza) undergoing coronary angiography.

Blood of all subjects was tested for serum IgG antibodies to influenza A and B and for seromarkers of 4 other pathogens (C pneumoniae, hepatitis A virus [HAV], H pylori, and CMV). Of the 218 patients (54.6% men, mean age 64.6 years), 95 (43.6%) had anti-influenza A IgG antibodies and 141 (64.7%) had anti-influenza B IgG antibodies. Coronary artery disease (CAD) prevalence was 69.5% in anti-influenza A seropositive patients and 61% in anti-influenza A seronegative patients (P=0.25). CAD was found in 66% of anti-influenza B seropositive subjects and 62.3% of anti-influenza B seronegative patients (P=0.69). This analysis demonstrates that influenza (A and B) seropositivity represents no predictor of risk for CAD. Moreover, seropositivity for each other pathogen (tested in our study) was not associated with CAD. In contrast, the number of infectious pathogens to which an individual has been exposed ("infectious burden") correlated with prevalence of CAD. Four or more of the 6 seromarkers tested for particular infections were positive in 48.8% of patients with CAD and in 31.2% of patients in patients without CAD (P=0.02). Five or 6 seromarkers were positive in 21.3% of patients with CAD and in 9% of patients without CAD (P=0.03).

Therefore, we support the interpretation of the results by Philippa Lavallée et al. that infections subsequent to influenza may play a role in promoting the complications of atherosclerotic disease (in particular, brain and myocardial infarction) and may also induce hypercoagulation, rather than influenza virus infection playing a causal role in atherogenesis. However, some triggers like additional exposure to other pathogens or nonspecific stimulation of the immune system could influence the susceptibility to the atherogenic effects of infection with a particular pathogen agent.
Letters to the Editor

Johann Auer, MD
Robert Berent, MD
Thomas Weber, MD
Bernd Eber, MD

Department of Cardiology and Intensive Care
General Hospital Wels
Wels, Austria

To the Editor:

mortality were Glasgow Coma Score, age
orrhage (ICH). Factors independently associated with 30-day
mortality in patients following a spontaneous intracerebral hem-
to determine a reliable grading score for the prediction of 30-day


Testing the ICH Score

Hemphill et al1 present an analysis of 161 patients carried out to determine a reliable grading score for the prediction of 30-day mortality in patients following a spontaneous intracerebral hemorrhage (ICH). Factors independently associated with 30-day mortality were Glasgow Coma Score, age >80 years, ICH volume, ICH of infratentorial position, and presence of intraventricular hemorrhage. A score based on these variables was assigned to each patient. All patients within their dataset with an ICH score of 0 survived, and all patients with a score of 5 (highest score assigned) died.

Hemphill et al restricted the testing of the scoring system to the data that produced it. We were interested in whether this scoring system could be of similar predictive value in patients treated in our unit. From 1994 to date, all patients admitted following a spontaneous supratentorial ICH have been recorded on a prospective database and followed up to 6 months after ictus. Although we do not have specific mortality at 30 days, we have recorded outcome at neurological discharge, which was on average 2 to 4 weeks after ictus. Up to August 1999, 440 patients had been entered. Data were missing on 47 patients, not allowing a score to be calculated, but the remaining 393 patients were scored as shown in Figure 1.

We had only 1 patient scoring either of the highest 2 scores, as only 5% of patients in our database had an infratentorial ICH. A single patient scored 6 (<1%), 17 scored 4 (4%), 74 scored 3 (19%), 116 scored 2 (30%), 97 scored 1 (25%), and 88 patients scored 0 (22%). We also found 100% mortality in patients scoring 5 or 6 (although this is only 1 patient) but a 5% mortality with a score of 0 or 1, as shown in Figure 2.

We were also interested to see whether this scoring system could predict unfavorable outcome (severe disability, death, or vegetative state) at neurological discharge. From Figure 3 it is apparent that for all patients scoring above 2, the rate of unfavorable outcome approaches 100%. In fact, 38% of patients with a score of 0 and 70% with a score of 1 are not independent at neurological discharge.

It therefore appears that this ICH scoring system is generally applicable inasmuch as the mortality is low in patients with an ICH score of 0 or 1. Thirty-day mortality rises steeply with a score of 2 or above. Unfavorable outcomes, however, are common in patients with a low ICH score, and this rises to almost 100% with scores of 2 and above. We feel that some sort of system to predict those capable of making an independent recovery from their ICH would be more helpful and is not provided by this simple ICH grading score.

Helen Fernandes, FRCS
Barbara A. Gregson, PhD
M. Shahid Siddique, FRCS
A. David Mendelow, PhD, FRCEd
STICH
Department of Neurosurgery
University of Newcastle upon Tyne
United Kingdom

Response:

The ICH Score is a clinical grading scale designed for risk stratification of patients after acute nontraumatic intracerebral hemorrhage (ICH).1 The outcome measure chosen for scale development was mortality at 30 days, which is a commonly used measure in other ICH prediction models. The ICH Score was demonstrated accurate in the population from which it was developed. However, in order for a scale such as the ICH Score to be useful, it must demonstrate validity in other patient populations. Additionally, in studies of acute neurologic catastrophes such as stroke or traumatic brain injury, functional outcome, and not just mortality, may be an important outcome measure as well.
When Is a Stroke Actually “Stable”?  


to the Editor:
Lazar et al.1 recently published an interesting study on the possibility of midazolam to transiently unmask focal deficits in patients with a good recovery after a stroke. The authors pointed to a direct γ-aminobutyric acid (GABA)-mediated inhibition of neural activity, thus suggesting a possible role for this neurochemical mechanism in poststroke recovery.

We would like to stress an additional effect of benzodiazepine (BDZ) on cerebral blood flow (CBF). It is known that BDZ causes a global reduction of CBF in normal subjects.2–4 A state of chronic hypoperfusion, which in turn could affect particularly the peri-infarct areas, has been demonstrated in stroke patients in the acute as well as in the chronic phase.5–7 These findings suggest the role of transient cerebral hypoperfusion in unmasking an otherwise clinically silent tissue with a reduced “perfusion reserve.”

Therefore, in addition to a direct GABA-mediated action, we suggest that a global reduction of CBF, linked to BDZ administration, should also be considered. This raises the possibility that a drug-induced critical hypoperfusion of peri-infarct tissue might contribute to the reemergence of previous symptoms.

Lazar et al.1 brilliantly demonstrated that a stroke lesion is never “stable,” even in the later stages. If a contribution to the reappearance of the old symptoms might be also ascribed to CBF reduction, the latter factor should shed a new light on the complex interaction between drugs and poststroke recovery.

Vittorio Di Piero, MD, PhD
Silvia Di Legge, MD
Marta Altieri, MD
Gian Luigi Lenzì, MD

V Chair of Neurology
Department of Neurological Sciences
University “La Sapienza”
Rome, Italy


Response
We thank Dr Di Piero and his colleagues for bringing to our attention the relevance of recent findings that benzodiazepines produce changes in cerebral blood flow. It is certainly possible that, in addition to direct GABA-mediated inhibition of compensatory mechanisms, midazolam-induced global hypoperfusion could be reducing the ability of perilesional brain regions to carry out new functions. Although there could be a lowered perfusional threshold in a chronically hypoperfused area adjacent to the infarct, an alternative explanation might be that there are fewer neurons in the compensatory zone that participate in the target function, making them vulnerable to lowered cerebral blood flow. Experimental observations support the notion that compensatory networks after stroke consist of a markedly smaller neural pool than the original system.1 Research using transcranial magnetic stimulation in patients after stroke reveals smaller motor evoked potentials in the affected limbs after stroke as compared with normal, suggesting fewer descending neurons responsible for the generation of movement in these limbs.2 More important, however, is that Di Piero and his colleagues assume that the perilesional area is the region solely responsible for the restitution of function. Investigations with positron-
Microbleeding on MRI as a Marker for Hemorrhage After Stroke Thrombolysis

To the Editor:

We noted with interest the article by Dr Kidwell et al,1 who describe an MRI application that detects microbleeds. Kidwell et al propose that this technique can identify patients who might be at high risk for hemorrhagic complications, if intravenous or intra-arterial thrombolysis is given. We agree that this application merits further study and have recently seen an example of asymptomatic hemorrhage after thrombolyis, in an intravenous tissue plasminogen activator (IV tPA)–treated patient. This 76-year-old female had no evidence of hemorrhage on pretreatment noncontrast CT. IV tPA was initiated 149 minutes after onset of symptoms, because of persisting right hemiplegia and global aphasia. MRI was performed 25 minutes after tPA infusion was initiated. Six microbleeds were seen on T2*-susceptibility weighted image using a 3-Tesla MRI scanner (Signa Medical Systems) as illustrated in Figure 1. The following day, CT brain scan was repeated, identifying an asymptomatic hemorrhage after tPA. This was in the general area where a microbleed was visualized initially (Figure 2). This case example supports the claim by Kidwell et al of an association between microbleeding and tPA-related hemorrhage. Imaging at 3 Tesla has greater sensitivity to magnetic susceptibility and, therefore, may increase the sensitivity in identifying these lesions. We also note that the identification of microbleeds could predict long-term complications of anticoagulation. Our patient with multiple microbleeds received long-term anticoagulation for chronic atrial fibrillation, which was presumed to be the cause of her stroke. This decision is a problematic one, especially when considering that warfarin-related intracerebral hemorrhage occurs in approximately 0.2% to 0.6% of treated patients.2 If an elderly population has a rate of microbleeding in the range of 5%,3 as previous studies have suggested, this MRI finding could also represent an important predictor of this serious long-term complication.

Larger-scale studies are needed to evaluate if the presence of microbleeds is useful in predicting patients who are at a higher risk for intracerebral hemorrhage from thrombolyis or long-term anticoagulation therapy.

Shelagh Coutts, MB, MRCP(UK)
Richard Frayne, PhD
Robert Sevick, MD, FRCP
Andrew Demchuk, MD, FRCP
Seaman Family MR Centre
Departments of Clinical Neurosciences and Radiology
University of Calgary
Calgary, Alberta, Canada

Outcomes in Carotid Endarterectomy Performed by Vascular Surgeons or Neurosurgeons

To the Editor:

The article on New York State carotid endarterectomy (CE) outcomes by Hannan et al.1 reports an admirably low adverse outcome rate across all cases studied but also shows, as their title suggests, a surprising difference in outcome favoring the patients of vascular surgeons versus neurosurgeons (adjusted odds ratio for adverse events in neurosurgeons relative to vascular surgeons, 3.17 [95% CI, 1.26 to 7.97]). The authors themselves point out several reasons why this result may be erroneous, such as a possibly higher rate of detecting postoperative strokes by neurosurgeons. Another important difference that might have influenced the result was that the proportion of asymptomatic patients was 67% for vascular surgeons and 53.3% for neurosurgeons; additionally, the adverse outcome rate for asymptomatic patients is lower in this and other series.2 Perhaps more importantly, the study sample was clearly not representative of all CE procedures done in New York State. The authors studied a voluntary registry, including only 7% of the surgeons in the state doing CE, among whom the adverse event rate was substantially lower than for the rest of the state.

Most importantly, the authors acknowledge that this difference between surgeons was not found in four other studies of CE outcomes. Given the increasing recognition among research methodologists that Bayesian reasoning is required to interpret statistically significant findings from new research in the context of preexisting research,3,4 we would propose that the finding of outcome differences across surgical specialties needs to be interpreted with great caution.

The study by Hannan et al.1 uses elegant analytic methods and has shown that very low adverse outcome rates can be achieved with CE. However, we believe that the finding of a significant difference in outcomes between the two surgical specialties studied is not externally valid.

Thomas E. Feasby, MD
James Kennedy, MB, MSc
Department of Clinical Neurosciences

Edward L. Hannan, PhD
A. John Popp, MD
Mark R. Chassin, MD, MPP, MPH
Ethan Halm, MD, MPH

Department of Health Policy, Management and Behavior
School of Public Health
University at Albany
Albany, New York
Outcomes in Carotid Endarterectomy Performed by Vascular Surgeons or Neurosurgeons
Thomas E. Feasby, James Kennedy, Hude Quan and William A. Ghali

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