Cerebrovascular Events in Patients With Significant Stenosis of the Carotid Artery Are Associated With Hyperhomocysteinemia and Platelet Antigen-1 (Leu33Pro) Polymorphism

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Background and Purpose—Although risk factors for carotid artery stenosis caused by atherosclerosis are known, it is unclear what triggers “activation” of the atherosclerotic plaques and the ensuing thromboembolic cerebral events. The aim of this study was to evaluate whether thrombophilic factors, platelet glycoprotein (GP) polymorphisms, and homocysteine are associated with a risk of ischemic events in patients with significant carotid stenosis.

Methods—Consecutive patients with ≥50% carotid stenosis, whether symptomatic (with ipsilateral ischemic events) or asymptomatic, who were evaluated and followed in a neurovascular clinic were tested for plasma levels of homocysteine, C677T mutation in methylenetetrahydrofolate reductase, G20210A mutation of factor II, factor V Leiden, antiphospholipid antibodies, and polymorphisms of platelet membrane GP: human platelet antigen (HPA)-1, GP Ia (C807T), and GP Ib (variable number of tandem repeats, Kozak, and HPA-2).

Results—Eighty-six asymptomatic and 67 symptomatic patients were evaluated. The former group was older (73.7 ± 6.9 versus 69.5 ± 9.1 years, \( P = 0.02 \)). Major risk factors for stroke were similar in both groups. In symptomatic patients versus asymptomatic patients, hyperhomocysteinemia was 3-fold more frequent (34.3% versus 12.8%, respectively; \( P = 0.002 \)) and HPA-1a/b was almost 2-fold more common (38.8% versus 20.9%, respectively; \( P = 0.01 \)). All other thrombophilic factors and platelet polymorphisms studied did not differ significantly between the 2 groups. Multivariate analysis revealed that hyperhomocysteinemia and the HPA-1a/b genotype conferred a significant risk of cerebral ischemic events, with odds ratios (95% CI) of 4.07 (1.7 to 9.7) and 3.4 (1.5 to 7.8), respectively.

Conclusions—Hyperhomocysteinemia and HPA-1a/b are independent risk factors for ischemic events in patients with significant carotid stenosis. (Stroke. 2001;32:2753-2758.)

Key Words: antigens ■ carotid stenosis ■ homocyst(e)ine ■ stroke, ischemic

Large atherosclerotic plaques at the bifurcation and proximal segments of the internal carotid arteries are common among elderly white individuals and cause significant (≥50%) stenosis in ~8% of subjects.1 A major complication of carotid artery stenosis is ipsilateral carotid territory stroke (ie, large-artery stroke),2,3 occurring at an annual incidence of 2% to 3.2%.3,4 Systemic factors such as hypertension, smoking, diabetes, hyperlipidemia, hyperhomocysteinemia, and certain infections have been found to promote and accelerate the atherosclerotic process,5–7 yet its complications by thrombotic or thromboembolic events appear to be induced by instability of the plaques.8,9 However, the appearance and composition of the carotid artery plaques as demonstrated by imaging or pathological examinations have not been consistent enough for prediction of thromboembolic episodes, such as transient ischemic attack (TIA) or stroke.8–10 Local factors that make the plaques thrombogenic or systemic prothrombotic factors could presumably be implicated in determining whether the patient will remain asymptomatic or be afflicted by a thromboembolic event.

Thrombophilic polymorphisms such as the factor II gene mutation G20210A (FII G20210A) and factor V gene mutation G1691A, also termed factor V Leiden (FVL), are established risk factors for venous thromboembolism,11–13 but their role in arterial thrombosis has been controversial.13–15 The role of the homozygous state for the C to T transition at nucleotide 677 of 5,10-methylenetetrahydrofolate reductase gene (MTHFR C677T) in arterial thrombosis is also debatable.16–18 The presence of antiphospholipid antibodies (APLAs) has been considered a well-known risk factor for...
venous and arterial thrombosis, yet this has also been recently disputed. However, an elevated plasma homocysteine (Hcy) level has been consistently shown to be an independent risk factor for ischemic stroke and atherosclerosis.

Platelets play a major role in thrombus formation, and enhanced platelet activation has been found in subjects with stroke. At sites of vascular injury, thrombus formation is initiated by binding of platelet glycoprotein (GP) Ib to von Willebrand factor and binding of platelet GP Ia/IIa to collagen, which give rise to platelet activation and aggregation by binding of the platelet GP Ib/IIa complex to fibrinogen and von Willebrand factor. Several polymorphisms in the genes encoding for platelet GPs have been associated with increased platelet adhesiveness and aggregation, and in some studies, they have been associated with an increased risk of arterial thrombosis. Thus, the homozygous form of the C807T polymorphism in the GP Ib gene was shown to be associated with an increased risk of stroke in young patients in one study but not in another study. The 3 polymorphisms in the GP Ib gene were all found to be associated with arterial thrombosis including stroke; they are as follows: Thr145Met (human platelet antigen [HPA]-2); variable number of tandem repeats (VNTRs) of 39 bp named A, B, C, and D; and a single dinucleotide polymorphism C/T at position −5 from the initiator ATG codon (Kozak sequence).

However, other studies have not confirmed these observations. A polymorphism in the GP Ibα gene (a single nucleotide change T/C, which causes Leu33Pro transition) has also been associated with coronary artery disease in some studies but not in patients with stroke.

The aim of the present study was to evaluate whether the development of stroke or TIA in patients with significant (≥50%) carotid artery stenosis is associated with inherited or acquired thrombophilic factors and/or platelet GP polymorphisms.

Subjects and Methods

Patients

Consecutive patients with significant (≥50%) carotid stenosis, whether symptomatic or asymptomatic, who attended a neurovascular clinic at Rabin Medical Center between September 1999 and May 2000 were included in the present study. This specific clinic serves as a referral center for evaluation of patients with carotid artery stenosis and identification of candidates for carotid endarterectomy. The study was approved by the Human Subject Ethics Committee of the Hospital, and informed consent was obtained from all the patients.

After a detailed medical history was obtained, all patients underwent cardiovascular and neurological examination, brain CT scan, carotid duplex, and transcranial Doppler (TCD). Echocardiography, Holter monitoring, and other cardiac or cerebrovascular imaging examinations were performed when indicated.

Evaluation of previous cerebrovascular events and events that occurred during the study were performed according to the World Health Organization definitions of stroke or TIA.

Patients with symptomatic carotid artery stenosis were defined as such if they had experienced an ipsilateral (carotid territory) TIA, ischemic stroke, or retinal ischemic event.

All other patients were defined as asymptomatic, and they were divided into 3 subgroups: (1) patients who were found to have a carotid artery bruit on routine physical examination or who had the stenosis detected by carotid artery duplex examination that was performed during an evaluation before major vascular surgery at other sites; (2) patients with nonspecific symptoms, such as vertigo, dizziness, syncope, headache, or blurred vision; and (3) patients with a history of cerebrovascular events unlikely to have been associated with the present carotid artery disease. These patients had either lacunar strokes mainly in the vertebrobasilar or contralateral territories or cardioembolic, postoperative, or undefined strokes; all had nonsignificant carotid artery disease at the time of their previous events.

Duplex scans were performed at least twice with the use of the same equipment (ATL, HDI 3000, Bothell), and the highest degree of stenosis observed at the proximal segment of the internal carotid artery was recorded. The severity of the stenosis was defined by means of peak systolic velocity and end-diastolic velocity (PSV and EDV, respectively) and divided accordingly into 4 "significant stenosis" groups: (1) moderate (50% to 69%), with PSV 200 to 300 cm/s and EDV <90 cm/s; (2) severe (70% to 89%), with PSV >300 cm/s and EDV ≥100 cm/s; (3) very severe (80% to 99%), with PSV >400 cm/s and EDV >200 cm/s; or (4) complete occlusion (100%). These duplex-based stenosis groups were compared with 60 carotid arteries measured blindly on conventional angiographies by North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria. Exact agreement was found in 80% of the cases, and there were no major (>1 stenosis group up or down) disagreements; all total occlusions were measured correctly.

Stenosis progression was defined as occurring when a follow-up duplex revealed a higher than a previous category of stenosis. TCD (Intraview, Rimed, Ltd) recordings were carried out according to established guidelines. In addition to the original interpretation of the CT scans, all scans were reviewed (as a standard procedure, unrelated to the present study) by one of the authors (J.Y.S.) to verify the presence and location of brain infarcts.

Laboratory Tests

Symptomatic patients were tested at least 3 months after the acute cerebral ischemic event. Asymptomatic patients were tested at one of their visits to the clinic. After at least 12 hours of fasting, blood was drawn for a complete blood count and Hcy, glucose, and cholesterol levels. Also, 9 parts of blood were drawn into 1 part of 3.8% sodium citrate. Citrated blood was centrifuged within 30 minutes at 2000g (20 minutes), and plasma aliquots were stored at −35°C.

Tests for APLAs

APLAs were evaluated by using 2 coagulation-based tests, the partial thromboplastin time–lupus anticoagulant test (Diagnostica, Stago) and diluted Russell’s viper venom test, and an immunological test (Systelisa Cardiolipin Antibodies Enzyme Immunoassay kit, Pharmacia and Upjohn Diagnostics), as published previously.

Plasma Hcy Determination

Total plasma Hcy was measured by the high-performance liquid chromatography–based method of Jacobson et al. The normal range of Hcy level was 5 to 15 μmol/L.

Determination of Thrombophilic Polymorphisms

DNA was extracted by a standard method. Factor V Leiden and factor II gene mutation G20210A were analyzed as previously described. The C677T substitution in the MTHFR gene was identified by the method of Frosst et al.

Determination of Platelet GP Polymorphisms

The 807 C/T in the GP Ia gene was detected by amplification of a DNA segment with use of a forward primer (5’-TATGTTGGGACCTCACGAAAC-3’), which contained a modified nucleotide (underlined) for XmnI recognition, and a reverse primer (5’-GATTTAACTTTCCCAGCGCCTTC-3’). XmnI digestion yielded 210- and 22-bp fragments on 3% agarose gel electrophoresis when the C allele was present, whereas the T allele yielded a 232-bp fragment.
The HPA-1 polymorphism, which is a substitution of Leu33Pro as a result of a C to T change in the GP IIIa gene, was detected by polymerase chain reaction (PCR) amplification of a 482-bp fragment and MspI digestion as described by Simsek et al.\(^4\,5\)

The HPA-2 polymorphism, a Thr145Met substitution in GP Ibα, was detected by PCR amplification of a 587-bp fragment with use of a forward primer described by Ishida et al.\(^4\,6\) and a reverse primer (5'-TATGCGTTTGGTGCGGGAATGTGC-3'), followed by BsuHI digestion and 2% agarose gel electrophoresis.

Identification of the VNTR polymorphism in the GP Ib gene was carried out by PCR amplification with use of a forward primer (5'-CCACTCTGGACCCAAGCAGGC-3') and a reverse primer (5'-GCTTGTCGACGACGCCAGGATGG-3') and separation on 3% agarose gel.

The Kozak C/T polymorphism at \(^5\) of the GP Ibα gene was detected by PCR amplification with use of a forward primer (5'-CCACTCTGGACCCAAGCAGGC-3') and a reverse primer (5'-GCTTGTCGACGACGCCAGGATGG-3'), followed by digestion with AvaII and 3% agarose gel electrophoresis.

**Statistical Analyses**

Analyses included the \(\chi^2\) test for the determination of significance for discrete variables or the Fisher exact test according to the size of the cells examined. Differences in age between the asymptomatic and symptomatic patients were assessed by the Student \(t\) test. Because the Hcy values were not normally distributed, the nonparametric Wilcoxon rank sum test for 2 samples was used to determine differences between the symptomatic and the asymptomatic groups, and the Kruskal-Wallis test was used for comparison of 2 subgroups of patients. The effects of hyperhomocysteinemia and HPA-1a/b on the risk of stroke or TIA adjusted for age, ethnic origin, and severity of carotid stenosis were estimated by using a multiple logistic regression model in a separate analysis. Another multiple logistic regression model was used to investigate the joint effect of hyperhomocysteinemia and HPA-1a/b on the risk of stroke. The adjusted odds ratios (ORs) and their 95% CIs were calculated.

**Results**

One hundred eighty-six patients with significant (≥50%) carotid artery stenosis were included in the study. Thirty-three patients (19 asymptomatic and 14 symptomatic) refused, for various reasons, to participate in the study. Of the remaining 153 patients, 86 (56.2%) were asymptomatic, and 67 (43.8%) were symptomatic. Forty-four (51.2%) asymptomatic patients complained of nonspecific symptoms (as specified in Subjects and Methods). Twenty patients (23.2%) were entirely asymptomatic, and 22 asymptomatic patients (25.6%) had a history of previous cerebrovascular ischemic events unrelated to the carotid stenosis. These events included 12 lacunar infarctions, 4 strokes or TIsAs that occurred during cardiovascular surgery, 1 cardioembolic stroke, and 6 undefined strokes. All these patients had an unremarkable duplex examinations of the appropriate ipsilateral (when applicable) carotid arteries at the time of the event. The risk factors for stroke in this subgroup were similar to the risk factors for the rest of the asymptomatic group except for a higher, but not statistically significant, prevalence of diabetes mellitus.

The demographic information and the prevalence of conventional risk factors for ischemic stroke in the symptomatic and asymptomatic patients are shown in Table 1. The symptomatic patients were older (\(P=0.02\)) and were different from the symptomatic group with respect to their ethnic origin. Whereas 36% of the symptomatic patients were of Asian African origin and 48% were of European American origin, the corresponding prevalences among the asymptomatic pa-

<table>
<thead>
<tr>
<th>TABLE 1. Demographic and Clinical Data</th>
<th>Asymptomatic Patients (n=86)</th>
<th>Symptomatic Patients (n=67)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD), y</td>
<td>73.7±6.9</td>
<td>69.5±9.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>58.1</td>
<td>71.6</td>
<td>NS</td>
</tr>
<tr>
<td>Origin, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>11.6</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Asia/Africa</td>
<td>18.6</td>
<td>35.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Europe/America</td>
<td>69.8</td>
<td>47.8</td>
<td></td>
</tr>
<tr>
<td>Risk factors, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>72.1</td>
<td>67.2</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>15.1</td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>44.2</td>
<td>53.7</td>
<td>NS</td>
</tr>
<tr>
<td>Never</td>
<td>40.7</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>55.8</td>
<td>56.7</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>34.9</td>
<td>29.9</td>
<td>NS</td>
</tr>
<tr>
<td>Other, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD, PVD</td>
<td>68.6</td>
<td>67.2</td>
<td>NS</td>
</tr>
<tr>
<td>s/p CABG</td>
<td>25.6</td>
<td>28.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; PVD, peripheral vascular disease; CABG, coronary artery bypass graft. Values other than those for age are percentage of group.

patients were 19% and 70%, respectively (\(P=0.02\)). No difference between the 2 groups was observed regarding the sex of the patient or conventional risk factors for stroke, ie, hypertension, smoking, hypercholesterolemia, diabetes mellitus, and ischemic coronary or peripheral vascular disease.

The results of imaging investigations are summarized in Table 2. Whereas the prevalence of a moderate degree of stenosis (50% to 69%) was similar in symptomatic and asymptomatic patients, there was a significantly higher prevalence of total occlusion among the symptomatic patients (overall correlation \(P=0.04\)). Symptomatic patients with total carotid occlusion (symptomatic occlusions) consisted of the following: 13 patients with CT-confirmed middle cerebral artery territory infarctions (3 of whom had their stroke preceded by a TIA); 11 patients who suffered from transient monocular blindness, 3 of whom, while awaiting a duplex scan, developed a middle cerebral artery stroke (2 patients) and a retinal stroke (1 patient); and 1 patient who had a hemispheric TIA. In all patients, an immediate (to the insult) duplex scan revealed the occlusion. In 6 patients, a prior carotid duplex had been performed for various reasons; of these, 2 patients had it performed within 4 months of their event, demonstrating 70% to 90% stenosis.

During the follow-up, no difference was observed with respect to progression of the atherosclerotic plaques or suspected intracranial stenosis (by TCD) between the 2 groups.

Carotid artery angiography was performed in 44.8% and 27.9% of the symptomatic and asymptomatic patients, respectively. This was followed by carotid endarterectomy in 37.3% and 26.7% of the symptomatic and asymptomatic patients, respectively.
TABLE 2. Imaging Evaluation

<table>
<thead>
<tr>
<th>Imaging Evaluation</th>
<th>Asymptomatic (n=86)</th>
<th>Symptomatic (n=67)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal carotid stenosis,* %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%–69%</td>
<td>24.4</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>70%–89%</td>
<td>34.9</td>
<td>14.9</td>
<td>0.04</td>
</tr>
<tr>
<td>90%–99%</td>
<td>25.6</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>15.1</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>Brain CT scan, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>65.1</td>
<td>26.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Appropriate infarct†</td>
<td>12.8</td>
<td>56.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Silent infarct‡</td>
<td>20.9</td>
<td>22.4</td>
<td>NS</td>
</tr>
<tr>
<td>Not performed</td>
<td>1.2</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Stenosis progression, %</td>
<td>47.1</td>
<td>51.6</td>
<td>NS</td>
</tr>
<tr>
<td>TCD, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected intracranial stenosis, %</td>
<td>31.4</td>
<td>34.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are percentage of group.

*Relates to the clinically affected side in the symptomatic patients.
†In asymptomatic patients, the infarct was unrelated to the carotid stenosis (see Subjects and Methods).
‡Four symptomatic patients had both appropriate and silent infarcts.

More than 34% of the symptomatic patients, compared with ≈15% of the asymptomatic patients, had elevated levels of plasma Hcy (P=0.002) (Table 3). Analysis of Hcy as a continuous variable revealed that mean Hcy concentrations were 15.8±10.4 μmol/L in symptomatic patients (median 13.5 μmol/L) and 12.5±7.6 μmol/L in asymptomatic patients (median 11.1 μmol/L) (P=0.016). Among the asymptomatic patients, the median values for Hcy in the 3 groups of never symptomatic patients, patients with nonspecific symptoms, and patients with unrelated symptoms were 12.7, 10.8, and 10.2 μmol/L, respectively (P=0.24).

The Hcy levels were also analyzed according to 4 age groups of the patients: <50 years, 50 to 59 years, 60 to 69 years, and ≥70 years. The mean±SD of Hcy in each group was 11.7±5.9, 11.8±5.2, 13.4±5.5, and 13.1±4.2 μmol/L, respectively. Although an increase in Hcy levels with increasing age was observed, the difference between the groups was not statistically significant (P=0.4). Similar findings were observed when the analysis was stratified according to asymptomatic or symptomatic status of the patient.

Analysis of Hcy levels according to ethnic origin of the patients revealed no association between Hcy levels and any ethnic origin (P=0.5).

Analysis of allele/genotype frequencies of platelet GP polymorphisms disclosed that the prevalence of HPA-1a/b polymorphism was almost 2-fold higher among symptomatic patients versus asymptomatic patients (OR 2.5, 95% CI 1.2 to 5.1; P=0.01) (Table 3). The prevalence of platelet HPA-1 was not significantly different among the 3 subgroups of asymptomatic patients: 30%, 19.5%, and 18.2% for never asymptomatic patients, patients with nonspecific symptoms, and patients with unrelated symptoms, respectively (P=0.6). After exclusion of the subgroup with unrelated symptoms from the main analysis, hyperhomocysteinemia and HPA-1a/b remained an independent risk factors for ischemic events (P=0.04 for each Hcy and HPA-1a/b).

Because there were significant differences between asymptomatic and symptomatic patients regarding ethnic origin and age, we included these variables in a multivariate stepwise logistic regression model. After adjustment for age and origin, hyperhomocysteinemia and the presence of HPA-1a/b increased the risk of stroke or TIA >4- and 2-fold, respectively (OR 4.21 and 2.33, respectively; 95% CI 1.80 to 9.86 and 1.1 to 4.94, respectively). No synergy was found between hyperhomocysteinemia and HPA-1a/b; the OR for the combination of hyperhomocysteinemia and HPA-1a/b was 4.83 (95% CI 1.33 to 17.58).

No statistically significant differences were observed between symptomatic and asymptomatic patients regarding the prevalences of all other platelet GP polymorphisms, ie, C807T in GP Ia and HPA-2b, VNTR-a/b, and Kozak C/T in GP Ib. Similarly, there were no differences in the prevalences of positive APLA test, FII G20210A, FVL, and C677T MTHFR polymorphisms.

The severity of carotid artery stenosis was also found to be associated with an increased risk of stroke (OR 2.09, 95% CI 1.02 to 4.26). To establish whether HPA-1a/b or hyperhomocysteinemia was an independent risk factor for stroke, regardless of the degree of carotid artery stenosis, we analyzed the prevalence of these variables after adjustment for age, origin, and extent of stenosis. After this adjustment, hyperhomocysteinemia and HPA-1a/b conferred a similar risk of stroke (for Hcy, OR 4.07 and 95% CI 1.7 to 9.7 [P=0.002]; for HPA-1a/b, OR 3.4 and 95% CI 1.5 to 7.8 [P=0.004]).

TABLE 3. Prevalence of Hyperhomocysteinemia and HPA-1a/b Polymorphism Among Patients With Significant Carotid Stenosis

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic (n=86)</th>
<th>Symptomatic (n=67)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated Hcy level</td>
<td>11 12.8</td>
<td>23 34.3</td>
<td>0.002</td>
<td>3.1 (1.4–7.1)</td>
</tr>
<tr>
<td>a/a</td>
<td>65 75.6</td>
<td>37 55.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a/b</td>
<td>18 20.9</td>
<td>26 38.8</td>
<td>0.01</td>
<td>2.5 (1.2–5.1)</td>
</tr>
<tr>
<td>NT</td>
<td>3 3.5</td>
<td>4 6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NT indicates not tested.
Discussion

The present study evaluated the role of elevated plasma Hcy, thrombophilic factors, and platelet membrane polymorphisms in the occurrence of cerebrovascular events in patients with significant (≥50%) carotid artery stenosis. By comparing symptomatic patients with a group of asymptomatic patients, all with ≥50% stenosis at the carotid bifurcation, we found that hyperhomocysteinemia and heterozygosity for HPA-1a/b were associated with a 4- and 3.4-fold increase in the risk of ipsilateral ischemic events, respectively, regardless of age, ethnic origin, and extent of carotid stenosis. After exclusion of asymptomatic patients who had previous ischemic cerebrovascular events that were unrelated to the stenosis, hyperhomocysteinemia and HPA-1a/b remained independent risk factors for the ischemic events.

Epidemiological studies have demonstrated that hyperhomocysteinemia is an independent risk factor for stroke,18–22 atherosclerosis in general,2,6 and carotid atherosclerosis in particular.7 The molecular mechanism by which Hcy promotes atherothrombosis is unknown. Experimental evidence suggests that the atherogenic propensity associated with hyperhomocysteinemia results from endothelial dysfunction and injury followed by platelet activation and thrombus formation. Although the exact mechanism of endothelial cell dysfunction is unknown, there is a growing evidence that Hcy exerts its effects by promoting oxidative damage.6 The association of hyperhomocysteinemia with ischemic events related to carotid artery stenosis observed in the present study is in agreement with other studies in which hyperhomocysteinemia was found to be associated specifically with ischemic strokes due to large-artery disease.18,47 A recent report48 showed that hyperhomocysteinemia impairs cerebrovascular reactivity in elderly persons, which in part explains the higher frequency of ischemic cerebrovascular events in this age group.

The common thrombophilic polymorphisms, FVL, FII G20210A, and C677T MTHFR, have been associated with venous thromboembolism11–13 but have not been consistently associated with arterial cerebrovascular events in adults, as observed in the present study. However, the power of our study for these markers was not strong enough to draw unequivocal conclusions.

Among the platelet GP polymorphisms studied, only HPA-1a/b was found to be associated with symptomatic carotid disease. In another study, in a subgroup of young white women, such an association was also found.38 However, other studies failed to demonstrate an association between HPA-1a/b and ischemic stroke.25,37 These conflicting results obtained from various studies can stem from differences in the studied populations and from different specific outcomes that were measured.

Our finding of a positive correlation between the HPA-1b allele and stroke in patients with significant carotid artery stenosis is not surprising because this allele was shown to decrease the threshold for ADP-induced binding of fibrinogen to platelets during activation.25 Moreover, a recent study suggested that the HPA-1b may be associated with thin-walled vulnerable plaques in coronary arteries that tend to rupture.34 By analogy with coronary arteries, we hypothesize that the HPA-1b allele in symptomatic patients with carotid artery stenosis increases their vulnerability to plaque rupture, resulting in thromboembolic events.

In some reports, the C807T polymorphism in GP Ia and 2 other polymorphisms in GP Ib (C/B of the VNTR and HPA-2b) were found to be associated with an increased risk of stroke.28,30,31 The lack of correlation between these platelet GP polymorphisms and ischemic events observed in the present study has been reported in other studies as well.29,32,33 However, the conclusions of lack of an association between these GP polymorphisms and ischemic events in the present study are not definitive because of our relatively small group of patients.

While awaiting confirmation by other studies, we suggest that all patients with significant carotid artery stenosis should be tested for plasma Hcy, because reduction of high Hcy levels may reduce the risk of cerebrovascular events.

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