Prothrombotic Mutations as Risk Factors for Cryptogenic Ischemic Cerebrovascular Events in Young Subjects With Patent Foramen Ovale

Nicoletta Botto, PhD; Isabella Spadoni, MD; Sandra Giusti, MD; Lamia Ait-Ali, MD; Rosa Sicari, MD, PhD, FESC; Maria Grazia Andreassi, PhD

Background and Purpose—Patent foramen ovale (PFO) has been identified as a potential risk factor for cerebrovascular ischemia. Procoagulant mutations may increase the risk and impact the choice of appropriate therapy for secondary prevention. We evaluated the prevalence of the 2 most common genetic risk factors for thromboembolism, factor V Leiden (G1691A) and prothrombin G20210A, in young PFO patients who were referred for percutaneous transcatheter closure of their PFO.

Methods—Ninety-seven patients (50 men; mean±SD age, 40.9±10.0 years) with first-ever cerebrovascular events before the age of 55 years and 160 age-matched control subjects (69 men; mean±SD age, 40.4±10.5 years) were recruited into the study. Factor V Leiden and prothrombin G20210A mutations were detected by using a multiplex allele-specific polymerase chain reaction assay.

Results—The prevalence of subjects carrying at least 1 prothrombotic genotype was significantly higher in the group of PFO patients than in the group of controls (10.3% vs 2.5%; χ²=72.7, P=0.008). Two patients (2.1%) versus 1 control subject (0.6%) and 8 cases (8.2%) versus 3 controls (1.9%) were carriers for factor V Leiden and prothrombin G20210A mutations, respectively. After adjustment for other vascular risk factors, the combination of either factor V Leiden or prothrombin G20210A and PFO was associated with a 4.7-fold (95% CI=1.4 to 16.1; P=0.008) increased risk of cerebral ischemia in young patients.

Conclusions—Our results indicate that prothrombotic mutations are important risk factors for cerebral ischemia in young patients with PFO. Screening for thrombotic mutations should be considered in young patients with PFO-related ischemic events. (Stroke. 2007;38:2070-2073.)

Key Words: genetics ■ patent foramen ovale ■ stroke care ■ vein thrombosis

Patency of the foramen ovale (PFO) is present in ~25% of the general population and in most cases never causes a health disturbance.1 However, the presence of a PFO is considered a possible cause of unexplained, presumably embolic, ischemic neurological events in young patients.2–4 The causal relation between PFO and cryptogenic thromboembolic cerebral events has not been established, and the suggested mechanisms are likely complex, including paradoxical embolism from the peripheral venous system,5 embolization from thrombi formed within the atrial septum,6 and the formation of a thrombus as a result of transient atrial arrhythmias.7 To date, no randomized, clinical trial has been conducted to identify those high-risk patients who may benefit from mechanical treatment, so no optimal management strategy has been designed so far.2–4

Procoagulant conditions may have an impact on the strategy for prevention of recurrent events in patients with cryptogenic stroke and PFO. In particular, the risk of paradoxical embolization may be increased by the presence of the 2 most common genetic risk factors for venous thrombosis: factor V Leiden (G1691A) and prothrombin (PT) G20210A. Indeed, both FV Leiden and PT G20210A polymorphisms are relatively frequent in white general population, with the prevalence of heterozygous carriers of 1% to 5% and of 1% to 3%, respectively.8

Recently, prothrombotic mutations have been suggested as genetic risk factors for “cryptogenic” ischemic cerebrovascular disease in young adults,9,10 and a relation between the prothrombotic mutation and cerebral ischemia risk in younger PFO patients has also been reported.11–13 However, the prevalence of coagulation mutations in patients with a PFO has not been consistently studied. Furthermore, the question of whether it is beneficial to recommend laboratory testing for inherited coagulopathies in patients with PFO-related
strokes remains an unresolved issue. The present study was designed to determine the prevalence of FV Leiden and PT G20210A mutations in young patients with ischemic cerebral events who were referred to our institution for percutaneous transcatheter closure of their PFO and in a group of healthy subjects.

Subjects and Methods

Study Population

From January 2004 to June 2006, 185 patients were referred to the G. Pasquini Hospital for PFO transcatheter occlusion; of these, 97 (50 men; mean±SD age, 40.9±10.0 years) were selected according to the following criteria: (1) a history of unequivocal ischemic stroke, documented clinically by a neurologist and radiographically by either cranial computed tomography or magnetic resonance imaging, or an episode of a transient ischemic attack, defined as a transient neurological deficit with full recovery within 24 hours and confirmed clinically by a neurologist; (2) the presence of a PFO with or without atrial septal aneurysm, diagnosed by contrast transesophageal echocardiography or transcranial Doppler examinations; and (3) age younger than 55 years. Seventy-four patients were not included, either because they were >55 years or because they did not consent to participate in the study (n=14).

At the time of blood sampling, a complete history, including vascular risk factors such as hypertension, dyslipidemia, and cigarette smoking, was collected from all patients. The following definitions were used for vascular risk factors: hypertension, as blood pressure ≥140/90 mm Hg (confirmed by measurements on several occasions) or antihypertensive therapy; and dyslipidemia, as an LDL value ≥130 mg/dL and HDL <35 mg/dL, triglycerides ≥200 mg/dL, or use of lipid-lowering medications. The diagnosis of diabetes mellitus was established according to World Health Organization criteria: fasting plasma glucose ≥7.0 mmol/L, or antidiabetic medication use. Smokers were classified as individuals who smoked at least 3 cigarettes per day at the time of analysis, past smokers had quit smoking for at least 6 months, and nonsmokers were individuals who had never smoked. Smoking patients were the combined groups of past and current smokers. Accordingly, each risk factor was coded as either present or absent. None of the patients eligible for the study had undergone genetic testing before admission to our institution. Moreover, physicians in charge of patient care were unaware of the genetic test results.

For the control group, we selected 160 healthy subjects in the same age group (69 men; mean±SD age, 40.4±10.5 years) from our staff with no previous history of cardiovascular diseases and no history of cancer. Written, informed consent was obtained from all study participants. The institutional ethics committee approved the study protocol.

Clinical and Genetic Characteristics and Associated Risk

<table>
<thead>
<tr>
<th></th>
<th>PFO Patients (n=97)</th>
<th>Controls Subjects (n=160)</th>
<th>OR (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD age, y</td>
<td>40.9±10.0</td>
<td>40.4±10.5</td>
<td>1.0 (0.9–1.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Sex (male), n (%)</td>
<td>50 (51.5)</td>
<td>69 (43.1)</td>
<td>1.4 (0.8–2.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Smoking habit, n (%)</td>
<td>17 (17.5)</td>
<td>27 (16.9)</td>
<td>1.0 (0.5–2.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>2 (2.1)</td>
<td>4 (2.5)</td>
<td>0.8 (0.1–4.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>12 (12.4)</td>
<td>7 (4.4)</td>
<td>3.1 (1.2–8.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>14 (14.4)</td>
<td>14 (8.8)</td>
<td>1.7 (0.8–3.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>PT G20210A, n (%)</td>
<td>8 (8.2)</td>
<td>3 (1.9)</td>
<td>4.7 (1.2–18.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>FV Leiden, n (%)</td>
<td>2 (2.1)</td>
<td>1 (0.6)</td>
<td>3.3 (0.3–37.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Either FV or PT mutation, n (%)</td>
<td>10 (10.3)</td>
<td>4 (2.5)</td>
<td>4.5 (1.4–14.7)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Genotyping

Genetic analyses for inherited prothrombotic conditions including the G1691A mutation in the FV gene and the G20210A mutation within the 3′-untranslated region of the PT gene were performed in all subjects. Genotyping analysis was performed by using a multiplex allele-specific polymerase chain reaction assay (Nuclear Laser Medicine), as previously described.

Statistical Analysis

All statistical analyses were conducted with the Statview statistical package, version 5.0.1 (SAS Institute, Abacus Concepts, Inc, Berkeley, Calif). Data were expressed as the mean±SD. Differences between means of the 2 continuous variables were evaluated by Student’s t test. Differences in noncontinuous variables and genotype distribution were tested by χ² analysis. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% CIs for the association between prothrombotic mutations and cerebral ischemia risk. The ORs were also adjusted for other vascular risk factors. A probability value of 0.05 was considered statistically significant.

Results

The clinical and genetic characteristics for cases and controls are provided in the Table. Mean age, smoking habit, and diabetes mellitus were statistically comparable between cases and controls. Cases were more often male (51.5% versus 43.1%) and hypertensive (14.4% versus 8.8%), although the difference compared with controls did not reach statistical significance. The prevalence of dyslipidemia was significantly higher in patients with a PFO than in control subjects (12.4% versus 4.4%; P=0.02). The frequency of subjects carrying at least 1 prothrombotic genotype was significantly higher in the group of patients than in the group of controls (10.3% versus 2.5%; χ²=7.2, P=0.008). Specifically, heterozygosity for the FV Leiden mutation was found only in 2 patients (2.1%) and in 1 control subject (0.6%). The PT 20210A mutant variant was detected in 8 cases (8.2%) and 3 controls (1.9%). None of the study subjects was found to be a carrier of both mutations. Logistic-regression analysis showed that the combination of either FV Leiden or PT G20210A and PFO was associated with a 4.5-fold increased risk of cerebral ischemia in young patients (95% CI=1.4 to 14.7; P=0.008). No significant alterations were observed with regard to other coagulation defects, including protein C, protein S, and antithrombin III. In multivariate analyses, an independent effect for cerebral ischemia was observed for the presence of either prothrombotic mutation (OR=4.7, 95%
CI = 1.4 to 16.1; \( P = 0.008 \) and dyslipidemia (OR = 3.1, 95% CI 1.1 to 8.6; \( P = 0.03 \)).

**Discussion**

PFO has been implicated in the etiology of cryptogenic stroke or transient ischemic attack,\(^2\)\(^6\)\(^-\)\(^8\) \(^-\)\(^1\)\(^8\) and its closure has been suggested as an effective treatment for recurrences. However, the optimal strategy (medical or invasive) remains undefined because no randomized trial has compared PFO closure with medical therapy. In addition, catheter closure of PFO has inherent potential risks like any other interventional procedure. Evidence of a genetic coagulation defect may have practical implications for patient management. In fact, genetic thrombophilic defects may affect the potential risks and reduce the expected benefits of percutaneous PFO closure.\(^3\)

In this study, we found that the presence of either the FV Leiden or the PT G20210A mutation was associated with an \( \sim \) 4-fold increased risk of cerebral ischemia in PFO patients. Currently, the role of prothrombotic variants for arterial vascular disease is unclear.\(^15\) A large body of evidence showed a significantly higher prevalence of the PT G20210A mutation in young patients with stroke of unknown origin\(^9\)\(^,\)\(^10\)\(^,\)\(^1\)\(^0\); however, only a few studies have investigated the prevalence of procoagulant mutations in patients with PFO and cerebral ischemia.

Pezzini et al\(^1\)\(^1\)\(^1\) have demonstrated that in young adults, the PT G20210A and, to a lesser extent, the FV Leiden mutation, may represent risk factors for PFO-related cerebral infarct, suggesting a role for these thrombophilic disorders in the pathogenesis of ischemic stroke. Accordingly, the G20210A variant in the PT gene was confirmed to be significantly associated with cerebral ischemia in patients with PFO.\(^1\)\(^1\)\(^1\)\(^-\)\(^1\)\(^3\)

Recently, a case-control study demonstrated that only “major” venous-to-arterial circulation shunts, usually due to PFO, were associated with stroke in young adults, but no relation was found between thrombophilia and ischemic stroke.\(^20\) However, major venous-to-arterial circulation shunts were identified by transcranial Doppler after intravenous microbubble ultrasound contrast in only 24 (25%) patients compared with 12 (12%) control subjects.\(^20\) Nonetheless, it may be argued that this study had a limited sample size to detect the implications of prothrombotic genetic variants in PFO-related stroke.

Our data indicate that coexistence of a PFO and inherited hypercoagulable states may identify subjects at higher risk for paradoxical embolism. The present data are consistent with previous reports\(^1\)\(^1\)\(^1\)\(^-\)\(^1\)\(^3\) and have important clinical implications. From a clinical viewpoint, it is critical to define in which cases PFO per se constitutes a risk high enough to warrant percutaneous closure and then define the best treatment.

PFO closure appears to be at least as effective as medical treatment for prevention of recurrent cerebrovascular events in cryptogenic stroke patients with PFO.\(^21\) In any case, patients with such mutations who have had an ischemic event (particularly at a young age) are probably at higher risk for recurrent events after successful PFO closure without an aggressive anticoagulant regimen.\(^22\)

However, our study has several potential limitations. First, although cases were recruited consecutively and control subjects were randomly selected from the community, potential confounding factors can never be completely excluded in an observational study. Second, although the present study seems to be sufficiently powered in instances where the OR exceeds 4, the large CIs may make the results of our interaction analysis statistically unstable. Third, there was the potential for selection bias because we were able to evaluate only those patients who were referred to our institution for PFO treatment. It is possible that patients with a prothrombotic mutation may have been diagnosed and counseled elsewhere and may not have been evaluated at our institution. Nonetheless, these parameters have not entered the clinical arena as standard risk factors, so it is unlikely that patients were excluded because of the presence of a prothrombotic profile.

On the basis of the present results, a more thorough risk assessment of this set of patients is warranted. The presence of prothrombotic mutations may be a marker for identification of individuals in whom the incidence of stroke is higher and in turn, may provide critical information for the most appropriate treatment. Nonetheless, randomized trials are needed to assess the real weight of genetic profiling in patients with PFO with and without stroke.

**Disclosures**

None.

**References**


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*Stroke*. 2007;38:2070-2073; originally published online May 24, 2007; doi: 10.1161/STROKEAHA.106.480863

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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