Inherited Thrombophilic Disorders in Young Adults With Ischemic Stroke and Patent Foramen Ovale

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Background and Purpose—The pathogenic link between patent foramen ovale (PFO) and stroke remains unknown in most cases. We investigated the association between inherited thrombophilic disorders and PFO-related strokes in a series of young adults in the setting of a case-control study.

Methods—We investigated 125 consecutive subjects (age, 34.7±7.3 years) with ischemic stroke and 149 age- and sex-matched control subjects. PFO was assessed in all patients with transcranial Doppler sonography with intravenous injection of agitated saline according to a standardized protocol. Genetic analyses for the factor V $(FV)_{G1691A}$ mutation, the prothrombin $(PT)_{G20210A}$ variant, and the *TT*677 genotype of methylenetetrahydrofolate reductase (*MTHFR*) were performed in all subjects.

Results—A pathogenic role of PFO was presumed in 36 patients (PFO+). Interatrial right-to-left shunt either was not detected or was considered unrelated to stroke occurrence in the remaining 89 patients (PFO−). The $PT_{G20210A}$ variant was more frequent in the PFO+ group compared with control subjects and the PFO− group (PFO+ versus control subjects, 11% versus 2%; 95% CI, 0.04 to 0.94; PFO+ versus PFO−, 11% versus 1.1%; 95% CI, 1.09 to 109; $P=0.047$). A similar distribution was observed for subjects carrying either the $PT_{G20210A}$ variant or the FV_{G1691A} mutation (PFO+ versus control subjects, 19.4% versus 5.3%; 95% CI, 0.08 to 0.75; PFO+ versus PFO−, 19.4% versus 3.3%; 95% CI, 1.45 to 26.1; $P=0.021$). Combined thrombophilic defects were observed in 3 subjects of the PFO+ group, in 2 control subjects (8.3% versus 1.3%; 95% CI, 0.01 to 0.66; $P=0.015$), and in 0 subjects in the PFO− group. A trend toward a difference in the frequency of the FV_{G1691A} mutation between PFO+ and control subjects was found after bivariate analysis (11% versus 3.3%; $P=0.068$) but not after multinomial logistic regression analysis. No significant association was found in the distribution of the *TT MTHFR* genotype in the 3 groups.

Conclusions—In young adults, the $PT_{G20210A}$ variant and, to a lesser extent, the FV_{G1691A} mutation may represent risk factors for PFO-related cerebral infarcts. A role of systemic thrombophilic disorders in the pathogenesis of this specific subtype of stroke may be hypothesized. (*Stroke*. 2003;34:28-33.)

Key Words: amine oxidoreductase ■ factor V ■ heart septal defects, atrial ■ prothrombin ■ stroke, ischemic

Over the last decade, patent foramen ovale (PFO) has been identified as an independent risk factor for cerebral infarct, particularly in young adults with cryptogenic stroke.[1–4] However, the causal relationship is not established, and the pathophysiological process linking this abnormality to stroke remains elusive in most cases. Paradoxical embolism from the peripheral venous system,[5] embolization from thrombi formed within the atrial septum,[6,7] and the formation of thrombus as a result of transient atrial arrhythmias have been advocated.[8] Furthermore, the detection of PFO in a stroke patient with an otherwise unexplained infarct does not necessarily identify the cause of stroke. Owing to the uncertainty about the mechanism of brain ischemia, secondary

prevention for patients with PFO who have had a stroke is a subject of considerable debate.

Recently, sparse observations suggested that a disequilibrium of the hemostatic system toward a clotting diathesis may increase the propensity to form an unstable thrombus and affect the risk of cerebral embolism in the presence of interatrial septal abnormalities. The possibility that patients with PFO-related stroke have an underlying hypercoagulable state has potential implications for the understanding of the pathophysiological process and identification of the most appropriate therapeutic strategies. Most of the published data on this issue are either anecdotal[9,10] or documented in case series[11–14] and case-control studies not specifically designed

to test this hypothesis.[15–17] Thus, the association of PFO with inherited prothrombotic disorders in stroke patients remains to be determined.

The aim of the present study was to systematically explore such an association and to investigate the role of thrombophilic conditions in different pathogenic subtypes of ischemic stroke. To accomplish this purpose, we undertook a prospective case-control study including a series of young adults with first-ever ischemic stroke and a group of control subjects stratified by age and ethnic and geographic origin.

Subjects and Methods

Patients with first-ever ischemic stroke occurring at <45 years of age who were consecutively admitted to our department between January 1997 and December 2000 were invited to participate in a research program for the evaluation of gene-environment interactions in the development of ischemic cerebrovascular disease. From a series of 129 unrelated subjects, 125 were prospectively considered for participation. Unwillingness to undergo genetic analysis explains the exclusion of 4 subjects.

A detailed description of the diagnostic workup and the stroke subtype classification criteria have been presented previously.[18] Briefly, the standard protocol included neurological examination, brain CT and/or MRI scan, extracranial Doppler ultrasonography with frequency analysis and B-mode imaging, transcranial Doppler (TCD), 12-lead ECG, transthoracic and/or transesophageal echocardiography, and standard blood tests. Coagulation testing, including prothrombin and activated partial thromboplastin times, antiphospholipid antibodies, fibrinogen, protein C, protein S, activated protein C resistance, and antithrombin III, was also carried out. MR angiography and/or conventional angiography of the neck and cerebral vessels were performed in selected cases. Assessment of lower extremity venous Doppler was performed whenever the clinical history and examination justified the suspicion of deep-vein thrombosis.

One hundred forty-nine subjects from the staff of our hospital with no known history of vascular disease, matched to the cases by sex and age in 3-year bands, were invited to participate in the study as control subjects. Both cases and controls were white and were from the same geographic area and social status.

Demographic data (age, sex) and history of conventional vascular risk factors such as hypertension, diabetes mellitus, cigarette smoking, and hypercholesterolemia were assessed in each subject according to predefined criteria.[18]

Genetic analyses for inherited prothrombotic conditions including the G1691A mutation in the factor V (FV) gene, the G20210A mutation within the 3′-untranslated region of the prothrombin (PT) gene, and the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene were performed in both cases and controls.

The study was designed and carried out in observance of the ethical principles established by the local Institutional Guidelines on Clinical Investigation. Written, informed consent was provided by all study participants.

Assessment of PFO

PFO was assessed in all patients with TCD with intravenous injection of agitated saline. The technique is described in detail elsewhere.[19] Briefly, it consists of the injection of 20 mL of previously shaken saline as a contrast-enhancing agent into a peripheral vein while recording the flow velocity of the middle cerebral artery, insonated through the temporal window on the right side at a depth of 50 to 60 mm, with a handheld probe. The appearance of transient spikes on the velocity spectral curve within 10 seconds of the intravenous injection of contrast medium is considered positive for interatrial right-to-left shunt. The method has been previously validated in our institution and provides 90% sensitivity, 100% specificity, and an overall diagnostic accuracy of 95% compared with transesophageal echocardiography. All contrast

TCD studies were performed with a standard TC2020 EME (Nicolet Biomedical) device equipped with a 2-MHz transducer by 2 experienced examiners (M.M., A.C.) who were unaware of the results of laboratory investigations.

PFO-Related Infarcts

All patients had a temporal window suitable for TCD investigations. Interatrial right-to-left shunt was evident in 42 cases (33.6%) as an isolated abnormality (n=35) or in association with the echographic finding of mitral valve prolapse (n=1). The remaining 6 cases were detected in patients with angiographically proven spontaneous cervical artery dissection (n=4), probable atherosclerotic vasculopathy (n=1), and lacunar infarct (n=1). Right-to-left shunt was considered a coincidental finding in these cases, and in the etiologic classification, priority was given to the vascular mechanism of stroke. Thus, after the exclusion of these 6 cases, a total of 36 patients were entered into the group of PFO-related cerebral infarcts (PFO+). Three of them had deep-vein thrombosis at the time of stroke.

Genetic Analysis

Genomic DNA was isolated from −20°C frozen samples of EDTA-anticoagulated whole blood through standard DNA extraction. The G1691A mutation in the FV gene (factor V Leiden) and the G20210A mutation in the PT gene were determined according to a standardized multiplex polymerase chain reaction method.[20] The C677T MTHFR genotypes were determined according to the method of Frosst and coworkers[21] with polymerase chain reaction amplification and restriction digestion with HinfI to distinguish mutant from wild-type allele.

Statistical Analysis

Differences in the distribution of baseline categorical variables between cases and controls and among the PFO+ group, the PFO− group, and controls were compared with the use of Fisher's exact test and Pearson's χ^2 test computed by Monte Carlo procedure, respectively. A value of $P \leq 0.05$ on a 1-sided test (for Fisher's exact test) and on a 2-sided test (for Pearson's χ^2 test) was considered significant. A multinomial logistic regression model that included sex, age, hypertension, smoking status, hypercholesterolemia, and thrombophilic genotypes was used to examine the effect of these variables in the prediction of group status (PFO+, PFO−, control). Diabetes mellitus was not entered into the multiple regression equations because of the low frequency of this condition in the present series. Overall model fit for covariates and thrombophilic genotypes was tested by the likelihood ratio test between the model with the tested effect and the model without the effect. A value of $P \leq 0.05$ on the 2-sided likelihood ratio test was considered significant. Results are given as odds ratios (ORs) with 95% CIs. The analyses were undertaken with the SPSS (version 11.1) software package.

Results

Table 1 shows the prevalence of baseline demographics and vascular risk factors among patients and controls. As expected, patients were more often smokers and more often had hypertension compared with controls, whereas the prevalence of diabetes mellitus and hypercholesterolemia was not significantly different between the 2 groups.

Distribution of the Prothrombotic Genotypes in the Patient Group

The observed distribution of the genotypes closely resembled those previously reported in other series from Northern Italy.[22,23] The prevalence of subjects carrying at least 1 procoagulant genotype was significantly higher in the group of patients than in the group of controls (28% versus 18.7%; $P=0.049$). In contrast, no significant differences were ob-

TABLE 1.   Demographic and Clinical Characteristics of Stroke Patients and Controls

Characteristic	Stroke Patients (n=125)	Control Subjects (n=149)	Crude OR	95% CI	P Value*
Age	34.7±7.3	34.8±6.1			
Sex (male)	68 (54.4)	80 (53.7)	1.029	0.64–1.66	0.562
Smoking	59 (47.2)	40 (26.8)	0.411	0.25–0.68	<0.001
Hypertension	18 (14.4)	10 (6.7)	0.428	0.19–0.96	0.029
Diabetes mellitus	2 (1.6)	0 (0.0)			0.207
Hypercholesterolemia	31 (24.8)	30 (20.1)	0.764	0.43–1.35	0.218

Mean±SD and number (percentage) are presented for age and categorical variables, respectively.
*P values obtained using 1-sided Fisher's exact test.

served in the distribution of the FV_{G1691A} mutation, $PT_{G20210A}$ variant, and *TT MTHFR* genotype, as well as the frequency of subjects carrying either the FV_{G1691A} mutation or the $PT_{G20210A}$ variant, the FV_{G1691A} mutation or the *TT MTHFR* genotype, and the $PT_{G20210A}$ variant or the *TT MTHFR* genotype. Finally, the frequency of subjects with a combination of >1 prothrombotic defect did not differ significantly in the 2 groups. None of the study subjects was found to be homozygous for the FV_{G1691A} mutation or the $PT_{G20210A}$ variant. The overall distribution of the thrombophilic genotypes is shown in Table 2.

Distribution of the Prothrombotic Genotypes in the Group of PFO+ and PFO− Patients

A significant difference between the subgroup of PFO+ patients and the group of controls was observed in the distribution of the $PT_{G20210A}$ variant ($\chi^2=9.96$; $df=2$; $P=0.016$), the prevalence of subjects carrying either the FV_{G1691A} mutation or the $P_{G20210A}$ variant ($\chi^2=11.6$; $df=2$; $P=0.003$), and the frequency of subjects with a combination of >1 thrombophilic defect ($\chi^2=10.4$; $df=2$; $P=0.013$). A trend toward a difference ($\chi^2=5.59$; $df=2$; $P=0.068$) in the distribution of the FV_{G1691A} mutation was also found. Findings from the bivariate analysis were further investigated in the multinomial logistic regression model adjusted for demographic variables (age and sex) and vascular risk factors. The results were substantially unaltered. However, as opposed to

the $PT_{G20210A}$ variant, the FV_{G1691A} mutation turned out to play a marginal role in the occurrence of stroke in PFO+ patients.

Both the $PT_{G20210A}$ variant and the carriership of either the FV_{G1691A} mutation or the $PT_{G20210A}$ variant were also significantly associated in the subgroup of PFO+ patients compared with the subgroup of individuals whose cerebral infarct was unrelated to PFO (PFO−) (Table 3). In contrast, the distribution of the prothrombotic genotypes did not differ between PFO− patients and controls (data not shown).

Discussion

The literature on hypercoagulable states in stroke patients with PFO is scarce and mostly anecdotal. Ours is the first case-control study exploring the relationship between FV Leiden, the $PT_{G20210A}$ variant, and the *TT MTHFR* genotype and PFO-related cerebral infarcts. The present study provides evidence that the $PT_{G20210A}$ variant and, to a lesser extent, the FV_{G1691A} mutation, are associated in the subgroup of PFO+ patients, suggesting a role of such disorders in the pathogenesis of stroke related to interatrial septal abnormalities. In contrast, despite the reported association between the homozygous deficient *TT MTHFR* genotype and venous thrombosis,[24,25] the relationship of such a defect with cerebral ischemia in patients with PFO seems unlikely.

The role of prothrombotic states in patients with PFO-related stroke has seldom been satisfactorily investigated. Di

TABLE 2.   Distribution of Prothrombotic Genotypes in Stroke Patients and Controls

	Stroke Patients (n=125)	Control Subjects (n=149)	Crude OR	95% CI	P Value*
FV_{G1691A}	6 (4.8)	5 (3.3)	0.689	0.21–2.31	0.381
$PT_{G20210A}$	5 (4.0)	3 (2.0)	0.493	0.12–1.26	0.269
TT MTHFR	27 (21.6)	23 (15.4)	0.663	0.36–1.23	0.123
FV_{G1691A} or $PT_{G20210A}$	10 (8)	8 (5.3)	0.652	0.25–1.71	0.263
FV_{G1691A} or *TT MTHFR*	32 (25.6)	27 (18.1)	0.688	0.39–1.23	0.132
$PT_{G20210A}$ or *TT MTHFR*	31 (24.8)	26 (17.4)	0.674	0.37–1.21	0.121
FV_{G1691A} or $PT_{G20210A}$ or *TT MTHFR*	35 (28)	28 (18.7)	0.595	0.34–0.99	0.049
Combined thrombophilic defects	3 (2.4)†	2 (1.3)‡	0.553	0.09–3.36	0.418

*P values obtained using 1-sided Fisher's exact test.

†Heterozygosity for the FV_{G1691A} mutation+heterozygosity for the $PT_{G20210A}$ variant, heterozygosity for the FV_{G1691A} mutation+*TT MTHFR* genotype, and heterozygosity for the $PT_{G20210A}$ variant+*TT MTHFR* genotype, respectively.

‡Heterozygosity for the FV_{G1691A} mutation+*TT MTHFR* genotype, and heterozygosity for the $PT_{G20210A}$ variant+*TT MTHFR* genotype, respectively.

TABLE 3. Distribution and Adjusted Odds Ratios of Prothrombotic Genotypes in PFO+ and PFO− Patients

Genotype	PFO+ Patients (n=36)	PFO− Patients (n=89)	PFO+ vs Controls		PFO+ vs PFO−		P Value*
			Adjusted OR	95% CI	Adjusted OR	95% CI	
FV$_{G1691A}$	4 (11)	2 (2.2)	NS	NS	NS	NS	0.160
PT$_{G20210A}$	4 (11)	1 (1.1)	0.19	0.04–0.94	10.09	1.09–109	0.047
TT MTHFR	7 (19.4)	20 (22.4)	NS	NS	NS	NS	0.337
FV$_{G1691A}$ or PT$_{G20210A}$	7 (19.4)	3 (3.3)	0.24	0.08–0.75	6.16	1.45–26.1	0.021
FV$_{G1691A}$ or *TT MTHFR*	10 (27.8)	22 (24.7)	NS	NS	NS	NS	0.294
PT$_{G20210A}$ or *TT MTHFR*	10 (27.8)	21 (23.6)	NS	NS	NS	NS	0.339
FV$_{G1691A}$ or PT$_{G20210A}$ or *TT MTHFR*	12 (33.3)	23 (25.8)	NS	NS	NS	NS	0.115
Combined thrombophilic defects	3 (8.3)	0 (0.0)	0.09	0.01–0.66	...	...	0.015

*P values obtained using the Likelihood Ratio Test of Multinomial Logistic Regression Models for testing overall prothrombotic genotypes effect. NS indicates not significant.

Tullio et al[12] found a significantly higher prevalence of protein C deficiency among 25 stroke patients with PFO compared with 195 without PFO. However, the assessment of right-to-left shunt by transthoracic contrast echocardiography represents a substantial bias of that study. Barinagarrementeria et al[13] and Chaturvedi[11] obtained similar findings in small case series, whereas Schwarze et al[26] found an increased prevalence of right-to-left shunts in patients with cerebrovascular accidents and activated protein C resistance compared with patients without this thrombophilic disorder. It is notable that a large group of control subjects with no history of stroke was lacking in all these studies. Furthermore, coagulation tests did not include specific genetic analyses. Nongenetic laboratory assays for coagulopathies may be influenced by various physiological, pharmacological, and hematologic factors when performed during the acute phase of stroke.[27] In addition, a direct relationship between intracerebral concentrations of hemostatic proteins and circulating levels obtained from peripheral blood samples has never been proved.[28] Because none of these reports provided detailed information on such potential confounding, methodological limitations are critical for the interpretation of the results. In contrast, the use of genetic analyses, which are not likely to be affected by the acute-phase response, and the recruitment of a large cohort of control subjects represent the strengths of our study.

Given its character of association study, the present report can provide no data concerning the biological process linking coagulopathies and cerebral ischemia in patients with interatrial right-to-left shunt. Thus, any causal relationship can be only speculative.

Although paradoxical embolism is the favored hypothesis, deep-vein thrombosis in stroke patients with PFO is usually undetectable.[29] However, sparse reports suggest that this pathomechanism has probably been underestimated.[30] Recently, Cramer and coworkers[31] diagnosed paradoxical embolism in 29% of patients in a small series of subjects with cryptogenic stroke and suggested that deep-vein thrombosis may be missed in a significant proportion of cases if an extensive study of pelvic and calf vein is not performed, in addition to the routine popliteal and femoral veins investigation. The Paradoxical Embolism From Large Veins in Ischemic Stroke (PELVIS) study is ongoing to address these preliminary observations.[32]

Direct arterial embolism of thrombus from the atrial septum is another potential mechanism. In agreement with the concept of "vascular bed-specific hemostasis" proposed by Rosenberg and Aird,[33] Kistler and coworkers hypothesized that the rate of cardioembolic stroke might be the result of a combination of anatomic and hemostatic defects.[34] A systemic prothrombotic state affecting the coagulation process on the endocardial surface of the heart might be the first event in the pathophysiological mechanism leading to "local thrombus" formation.[33] The likelihood of this process and the rate of subsequent embolism are expected to increase in subjects carrying anatomic abnormalities within the heart such as a PFO[34,35] and might be even higher in the presence of additional predisposing conditions such as transient atrial arrhythmias.[8]

Our findings are apparently in disagreement with those of the recent Patent Foramen Ovale in Cryptogenic Stroke Study (PICSS) that showed no significant difference in the rate of recurrent stroke or death between patients with PFO and those with otherwise unexplained infarcts randomized to warfarin or aspirin.[36] If an association between PFO and prothrombotic disorders in stroke patients exists, one would theoretically expect a better response to oral anticoagulants than to platelet inhibitors. However, because PICSS was not specifically designed to explore the role of thrombophilic disorders in patients with PFO, no detailed information on the patients' coagulative status is available. A further explanation of this disagreement might be the age of the patients (59.0 and 34.7 years in PICSS and our series, respectively), which might exert an effect on the relation between cardiac abnormalities and thrombophilic disorders. Finally, contrary to PICSS, a recent meta-analysis found warfarin superior to antiplatelets in preventing recurrent ischemic events in patients with PFO.[37]

Study Limitations

Some limitations of our study should be noted. First, PFO is frequently associated with atrial septal aneurysm (ASA), and the presence of both abnormalities, as opposed to PFO alone, has been recently demonstrated to increase the risk of

recurrent stroke.[38] The TCD technique prevents the assessment of ASA, which represents the major drawback of the present study. Any further analyses comparing the prevalence of inherited thrombophilias in stroke patients with PFO, ASA, or both are hindered by the lack of precise data on the frequency of ASA in our series. Second, our protocol did not include any measurement, albeit semiquantitative, of PFO diameter, an anatomic marker that may allow the identification of patients at high risk of embolism.[39] However, because of the anatomic characteristics of the foramen ovale, it is not simple to obtain measures of the maximum size of the opening with contrast TCD, and it might be that the microbubble count does not reflect the exact amount of shunting.[40] Finally, the lack of a systematic search for right-to-left shunt in the group of controls hampers any comparisons between the PFO subjects with stroke and those without stroke. The clinical implications of these missing data are noteworthy, but it seems unlikely that they have significantly altered the results of our study.

Clinical Implications

Besides the potential impact on the understanding of the pathophysiology of PFO-related infarcts, an additional implication of our findings concerns the evaluation of the appropriateness of coagulation testing in patients with ischemic stroke and the selection of patients eligible for detailed coagulation studies.[41] Coagulation tests seem to be of little value in the diagnostic workup of young patients with ischemic stroke except in the small subgroup with PFO-related infarct. Accordingly, laboratory testing for coagulopathies in this specific subtype of stroke may be warranted and cost-effective.

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