

Factor V Leiden and prothrombin gene mutation may predispose to paradoxical embolism in subjects with patent foramen ovale

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The role of paradoxical embolism through patent foramen ovale as a mechanism of cryptogenic stroke is controversial. If a venous source of emboli is relevant, prothrombotic states should be associated with patent foramen ovale and cryptogenic stroke. We assessed the occurrence of several prothrombotic states (factor V Leiden, prothrombin G20210A, deficiencies in protein S, protein C and antithrombin, lupus anticoagulant, anticardiolipin antibodies, elevated factor VIII, resistance to activated protein C) and classical risk factors for venous thrombosis in 57 adult patients with cryptogenic stroke and patent foramen ovale and in 104 matched controls. Prothrombotic states [odds ratio (OR) 2.8; 95% confidence interval (CI), 1.2–6.5; $P = 0.021$], migraine with aura (OR 4.4; 95% CI 1.8–10.8; $P = 0.001$) and classical risk factors for venous thrombosis (OR 2.5; 95% CI 1.1–5.7; $P = 0.037$) were independent risk factors for cryptogenic stroke. In particular factor V Leiden or prothrombin G20210A associated with cryptogenic stroke ($P = 0.022$) whereas other coagulation abnormalities did not ($P = 0.140$). Among

the patients with prothrombotic states, Valsalva manoeuvre was common at onset of stroke. Our results support the possibility of paradoxical embolism behind strokes in patients with patent foramen ovale. *Blood Coagul Fibrinolysis* 14:261–268 © 2003 Lippincott Williams & Wilkins.

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Introduction

In one-third of young adults, the aetiology of cerebral infarction remains cryptogenic despite extensive diagnostic work-up [1]. In patients 55 years of age or younger with cryptogenic brain infarction, patent foramen ovale (PFO) has been shown to be a more common finding (56%) than in controls (18%) and in patients with brain infarction of determined cause (17%), suggesting paradoxical embolism as the mechanism for stroke [2,3]. Assuming that strokes are due to paradoxical embolism in the presence of PFO, a high incidence of deep venous thrombosis (DVT) or a high frequency of either inherited or acquired conditions leading to an increased incidence of venous thromboembolism would be expected in this group of patients [3]. However, as a venous source of paradoxical emboli is seldom identified in patients with PFO and cerebral ischaemia [4], paradoxical embolism is often only assumed. Thus, the clinical significance of PFO as an important risk factor for cerebral ischaemia is still controversial [5].

Inherited prothrombotic states, such as factor V Leiden mutation, prothrombin gene G20210A mutation, ele-

vated levels of factor VIII and decreased activity of protein C, protein S and antithrombin, have been recently linked to an increased risk of venous thromboembolism [6,7], but there is still a lack of evidence supporting an association between these states and brain infarction in general, although an association between these disorders and some subgroups of ischaemic stroke has been suggested [6–11]. Interestingly, some studies suggest, though in the absence of convincing evidence, that prothrombotic states could be associated with cryptogenic stroke and PFO in particular [12,13]. Thus, prothrombotic states might serve as the 'missing link' between PFO and cerebral ischaemia of unknown origin.

We investigated whether prothrombotic states and PFO coexist in young patients with a cryptogenic brain infarction. Because venous thrombosis is widely considered a multi-causal disorder, we also assessed the role of other potential vascular risk factors and their interactions. Identification of an association between prothrombotic states and PFO would be important for a better understanding of the underlying mechanisms of

cryptogenic brain infarction and paradoxical embolism and for strengthening the status of PFO as a risk factor for stroke. This could also have implications for the targeting of diagnostic investigations in patients diagnosed with PFO and for management after stroke.

Materials and methods

General design and population

We conducted a case-control study of ischaemic brain infarction of undetermined cause and PFO among consecutive patients 15–60 years of age and treated at Oulu University Hospital, Finland between 1991 and 1998. All the patients seen in our clinic during this time period were considered potentially eligible and thus were screened for the study.

Exclusion criteria

We excluded those patients who could be classified as having one of the following stroke subtypes defined by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [14]: (1) large-artery atherosclerosis was defined as the presence of an occlusion or a stenosis on Doppler ultrasound or angiography with at least 50% diameter reduction of a brain-supplying artery corresponding to clinical symptoms. The percentage stenosis on angiography was calculated according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [15]. (2) Small-vessel disease was defined as the presence of one of the traditional lacunar syndromes (e.g. pure motor stroke, pure sensory stroke, sensorimotor stroke, ataxic hemiparesis, and dysarthria-clumsy hand syndrome), infarction less than 1.5 cm of diameter or normal computed tomography/magnetic resonance imaging examination, and the absence of acute cerebral cortical dysfunction. (3) Cardioembolism was defined as the presence of high or medium risk sources of cardiac embolism [14]. (4) Stroke of other determined aetiology included patients with myeloproliferative disorders, arterial dissection, vasculitis, and other non-atherosclerotic vasculopathies. Furthermore, we excluded those patients in whom two or more potential causes were identified and those in whom the cause of stroke had remained undetermined due to incomplete evaluation. The following definition for ischaemic stroke was applied: rapidly developing clinical signs of focal or global disturbance of cerebral function lasting for more than 24 h with no apparent cause other than vascular origin. Patients with transient ischaemic attacks were excluded.

The patients were considered to have cryptogenic brain infarction if they met the published TOAST criteria for 'stroke of undetermined aetiology, negative evaluation' [14], except that we accepted the presence of PFO or atrial septal aneurysm (ASA). To exclude other brain diseases a head computed tomography scan was per-

formed on all and magnetic resonance imaging on 45% of the patients. Conventional angiography was performed on 67% and magnetic resonance angiography on 4% of the patients, and the rest underwent colour duplex ultrasonography of the carotid and vertebral arteries. The majority of the cases (90%) were identified prospectively during their treatment at the Department of Neurology, the rest were identified by screening the hospital discharge records. We identified 105 patients with a cryptogenic ischaemic brain infarction, of whom 58 had PFO.

We recruited 104 community-based controls. The patients were asked if their spouse or a friend of the same age could participate as a control, but the relatives of the patients were not eligible. If the spouse or a friend was not available, we used randomly selected age- and sex-matched controls from the population register of the hospital catchment area. Controls with a history of ischaemic stroke were excluded.

Informed consent was obtained from all the subjects. The Research Ethics Committees of Oulu University Hospital and Finnish Red Cross Blood Transfusion Service approved the study.

Echocardiography and definition of patent foramen ovale

As a part of the clinical investigation, all patients had undergone a contrast transoesophageal echocardiographic examination, which was performed by trained echocardiographers using 5-MHz omniplane transducers to detect cardiac abnormalities. The patients were examined in a fasting state, and they received topical anaesthesia of the oropharynx. A four-chamber view of the atrial septum was obtained before the injection of contrast agent. The contrast agent of the study was a mixture of 0.9% sodium chloride (9 ml) and air (1 ml) agitated vigorously in a syringe. To obtain a good bolus of air microbubbles, the contrast solution was injected immediately after preparation rapidly through a 17-gauge catheter placed into a right antecubital vein. This procedure was performed once during normal breathing and once or more during the end phase of a Valsalva manoeuvre. PFO was considered to be present if one or more bright microbubbles were seen to cross the atrial septum and to appear in the left atrium within three heart cycles after contrast opacification of the right atrium. Positive contrast studies were classified into three grades by counting the maximum number of microbubbles in the left atrium within three heart cycles after the contrast media filling of the right atrium. A small shunt was defined as a count of one to five microbubbles, a moderate shunt was defined as a count between six and 25 microbubbles and a large shunt was considered to be present if the count was over 25 microbubbles. ASA was diagnosed when the

atrial septum exhibited at least a 10 mm excursion into the left or right atrium or both.

Data collection

The data were collected by personal interviews conducted by the same interviewer using a structured questionnaire, a review of the medical records and analysis of blood samples. The interviewer was not aware of the patient's status with regard to PFO when filling in the questionnaire. Information was obtained from family members if the patient was unable to communicate. The subjects were considered to be hypertensive if they were on antihypertensive medication. The current smoking category included subjects who had been smoking regularly within the past year. Migraine with aura and migraine without aura were defined based on the International Headache Society (IHS) criteria [16]. None of the patients fulfilled the IHS criteria for migrainous infarction. Persons were considered to have diabetes if they were on an antidiabetic diet or medication. The subjects were asked if they had ever been diagnosed for pulmonary embolism or DVT. For women, current use of oral contraceptives or oestrogen replacement therapy was recorded. Any history of DVT and ischaemic stroke in first-degree relatives was recorded. Predisposing events for venous thrombosis were taken to include minor or major surgery, hospitalization for any reason, traumatic injury, or long-distance travel by car, bus, train or plane (more than 3 h), all of which were to have occurred either within 1 or within 3 months before the stroke, or before the interview date in the controls. Valsalva manoeuvre at stroke onset implies sexual intercourse, lifting of heavy objects, defecation, coughing, or heavy physical exertion.

Specimen collection and analysis

Blood samples for coagulation tests were taken more than 2 months after the stroke, in order to avoid acute phase reactions. Blood was drawn into Vacuette tubes (Greiner Labor Technik, Kremsmünster, Austria) containing 0.109 mol/l trisodium citrate. Plasma was prepared by centrifugation for 20 min at 20°C (1600 *g*) and stored at -70°C until used. For lupus anticoagulant tests, plasma was filtered before freezing by using a Acrodisc PF Syringe Filter with 0.8/0.2 µm Supor Membrane (Pall Gelman Laboratory, Ann Arbor, Michigan, USA). For the identification of factor V Leiden and prothrombin G20210A, the polymerase chain reaction amplification and restriction fragment length polymorphism techniques were used [17,18]. Antithrombin, protein C and protein S activities were measured with STA Stachrom AT III, STA Stachrom Protein C and STA Staclot Protein S (Diagnostica Stago), respectively. Factor VIII clotting activity was measured with the one-stage method using Automated APTT (Organon Teknika, Durham, USA). Activated protein C (APC)

resistance was tested with Coatest APC Resistance (Chromogenix AB, Mölndal, Sweden). Lupus anticoagulant was tested with DVVtest (American Diagnostica Inc, Greenwich, Connecticut, USA) and PTT-LA (Diagnostica Stago). Anticardiolipin antibodies were determined as described earlier, except that the reagent for residual coating of the microplates and for the serum and enzyme-conjugate dilutions was phosphate-buffered saline, pH 7.2, containing 10% bovine serum [19]. The cut-off values were 38 GPL (IgG phospholipid unit) for immunoglobulin (Ig)G and 20 MPL (IgM phospholipid unit) for IgM. The laboratory staff were blinded to the case or control status. In case of abnormal test results in the assays for APC resistance (in the absence of factor V Leiden mutation), antithrombin, protein C, protein S, anticardiolipin antibodies, or lupus anticoagulant, a new sample was requested. Only repeatedly abnormal results were considered as proof of a coagulation abnormality. In one of the 58 patients with stroke and PFO blood analysis was not performed due to a missing sample.

Statistical analysis

The data were analysed with the SPSS (release 10.0.5; SPSS Inc., Chicago, Illinois, USA). Categorical variables were compared using Fisher's exact test or the Pearson χ^2 test. Continuous variables were compared between the groups using Student's *t*-test or the Mann-Whitney U test. Multivariate odds ratios (ORs) for risk factors, with 95% confidence intervals (CIs), were calculated by unconditional multiple logistic regression (maximum likelihood method). Stepwise logistic regression was used to test the differences in the prevalence of variables between cases and controls. All *P* values presented are two-tailed.

Results

The baseline characteristics of the patients and control subjects are shown in Table 1. There was no significant difference in the ages between patients and controls (*P* = 0.551). Significant risk factors for cryptogenic brain infarction in univariate analysis were migraine with aura, presence of a predisposing event within 1 month and presence of a predisposing event within 3 months. Of the 58 patients with PFO 22% had a small to moderate shunt and 78% had a large shunt. Eight (13.8%) of the 58 patients with PFO had an associated ASA. The prevalence of ASA increased with the degree of shunt: 0, 14, and 19% in patients with small, moderate, and large shunts, respectively.

The results of the coagulation tests and the numbers of subjects tested for each parameter are shown in Table 2. The presence of any coagulation abnormality was more common in the patients with a cryptogenic brain infarction and PFO than in the controls (*P* = 0.012). Of the individual prothrombotic factors, the specific pre-

Table 1 Characteristics of patients with cryptogenic brain infarction having patent foramen ovale and controls

Characteristic	Cases (n = 58)	Controls (n = 104)	Unadjusted OR (95% CI)
Age, mean (SD), years	44.3 (9.9)	45.3 (10.0)	
Men	32 (55%)	59 (57%)	
Women	26 (45%)	45 (43%)	
Body mass index, mean (SD), kg/m ²	26.0 (5.1)	26.0 (4.5)	
Current smoking	17 (29%)	25 (24%)	1.3 (0.6–2.7)
Hypertension	9 (16%)	13 (13%)	1.3 (0.5–3.2)
Diabetes	1 (2%)	4 (4%)	0.4 (0.0–4.0)
History of deep venous thrombosis	2 (3%)	5 (5%)	0.7 (0.1–3.8)
Family history of deep venous thrombosis	9 (16%)	12 (12%)	1.4 (0.6–3.6)
Family history of ischemic stroke	8 (14%)	18 (17%)	0.8 (0.3–1.9)
Migraine without aura	6 (10%)	9 (9%)	1.2 (0.4–3.6)
Migraine with aura	19 (33%)	12 (12%)	3.7 (1.7–8.4)*
Use of oestrogens in women	7 (27%)	12 (27%)	1.0 (0.3–3.0)
Acquired risk factor for deep venous thrombosis			
Predisposing event within 1 month	14 (24%)	12 (12%)	2.4 (1.0–5.7)**
Predisposing event within 3 months	17 (29%)	15 (14%)	2.5 (1.1–5.4)***

OR, odds ratio; CI, confidence interval. * $P = 0.001$; ** $P = 0.036$; *** $P = 0.023$.

Table 2 Coagulation abnormalities found in patients with cryptogenic brain infarction having patent foramen ovale and controls

Variable	n [†]	Cases (n = 57)	Controls (n = 104)	Unadjusted OR (95% CI)
Factor V Leiden mutation	161	4 (7%)	1 (1%)	7.8 (0.8–71.3)
Prothrombin G20210A mutation	160	2 (4%)	0 (0%)	1.0 (1.0–1.1)
Antithrombin deficiency [‡]	160	1 (2%) [‡]	2 (2%)	0.9 (0.1–10.5)
Protein C deficiency [‡]	155	0 (0%)	0 (0%)	ND
Protein S deficiency [‡]	155	1 (2%) [‡]	0 (0%)	1.0 (1.0–1.1)
Factor VIII activity > 150%	159	7 (13%)	9 (9%)	1.5 (0.5–4.2)
IgG anticardiolipin antibodies	159	0 (0%)	0 (0%)	ND
IgM anticardiolipin antibodies	159	1 (2%)	1 (1%)	1.9 (0.1–30.2)
Lupus anticoagulant	160	1 (2%)	1 (1%)	1.9 (0.1–30.5)
APC resistance [§]	148	1 (2%)	0 (0%)	1.0 (1.0–1.1)
Combined results				
Any coagulation abnormality		17 (30%)	14 (13%)	2.7 (1.2–6.1)*
Factor V Leiden or prothrombin mutation		6 (11%)	1 (1%)	12.4 (1.4–105.5)**
Coagulation abnormality, mutations excluded		11 (19%)	13 (13%)	1.7 (0.7–4.0)

OR, Odds ratio; CI, confidence interval; ND, no data; Ig, immunoglobulin; APC, activated protein C. [†]The numbers differ because of anticoagulant treatment or missing sample. [‡]Activity repeatedly below normal range. [§]One patient had a combination of antithrombin and protein S deficiency. [§]In the absence of factor V Leiden mutation. * $P = 0.012$; ** $P = 0.008$.

sence of either the factor V Leiden or the prothrombin gene mutation was more common in the patients than in the controls ($P = 0.008$). Factor V Leiden alone was found more often in the cases than in the controls, but this difference did not quite reach statistical significance ($P = 0.053$). All the subjects with the factor V Leiden or prothrombin mutation were heterozygous for the mutation, and none of them had both mutations simultaneously. One patient had APC resistance in repeated testing in the absence of any known physiological or pharmacological factor that might have affected the coagulation test result. One patient had a combination of repeatedly low levels of both antithrombin and protein S activity, and two controls had repeatedly low levels of antithrombin activity. As family members were not tested, it could not be determined whether the abnormal test results were due to acquired or inherited causes.

In multivariate analysis, we found that migraine with aura, the presence of any coagulation abnormality and the presence of a predisposing event for DVT within 3 months were significantly and independently associated with the occurrence of brain infarction in the patients with PFO (Table 3), model 1. However, migraine with aura was not associated with coagulation abnormalities. The prevalence of coagulation abnormalities was 35 versus 33% in patients with and without migraine with aura, respectively. To investigate the role of the mutations separately, another model – model 2 – where the coagulation abnormalities were divided into two groups according to the presence or absence of the factor V Leiden and prothrombin G20210A mutation was created. It appeared that the presence of either of the mutations and migraine with aura were significantly associated with cryptogenic brain infarction, whereas the presence of other coagulation abnormal-

Table 3 Major risk factors for cryptogenic brain infarction in subjects having patent foramen ovale in unconditional multivariate logistic regression models

	Model 1		Model 2	
	OR	95% CI	OR	95%CI
Migraine with aura	4.36	1.76–10.83*	4.72	1.87–11.94 [†]
Any coagulation abnormality	2.75	1.17–6.49**		
Factor V Leiden or prothrombin mutation			13.99	1.47–133.6 [‡]
Other coagulation abnormality			2.05	0.79–5.31
Predisposing event within 3 months	2.45	1.06–5.69***	2.13	0.89–5.08

OR, odds ratio; CI, confidence interval. In Model 2, the coagulation abnormalities are divided into mutations and other abnormalities. Odds ratios are adjusted for age and sex, and for other variables included in the model. * $P = 0.001$; ** $P = 0.021$; *** $P = 0.037$; [†] $P = 0.001$; [‡] $P = 0.022$.

ities was not statistically significant as a risk factor. Because long-distance travelling may not represent a similar thrombogenic predisposition as major surgery or traumatic injury, we also tested the models after omitting long-distance travelling. When that was done, the predisposing event became a significant risk factor in both model 1 (OR 12.8; 95% CI 2.5–64.4; $P = 0.002$) and model 2 (OR 10.5; 95% CI 2.1–53.9; $P = 0.005$).

Table 4 shows that the association of coagulation abnormalities with cryptogenic ischemic brain infarction was more marked in the patients who had also had a predisposing event whereas in the group without a predisposing event the association was non-significant. In fact, a predisposing event associated with a coagulation abnormality in 12% of the cases, whereas the corresponding figure for the controls was 1%. This suggests some interaction between coagulation abnormalities and predisposing events. However, when we tried to fit an interaction term into the multivariate model, it failed to show statistical significance. We did not find any evidence of interaction for the other variables in the models.

The occurrence of previous DVT, a family history of DVT or a family history of ischaemic stroke were not associated with either the presence of a mutation or a

Table 4 Presence of a coagulation abnormality in patients with cryptogenic brain infarction having patent foramen ovale and controls in relation to the presence of a predisposing event within 3 months

	Predisposing event present		Predisposing event absent	
	Cases	Controls	Cases	Controls
Coagulation abnormality				
Not present	10	14	30	76
Present	7	1*	10	13**

* $P = 0.041$, ** $P = 0.154$.

positive finding in any coagulation test. However, we found that Valsalva manoeuvre-like activities at stroke onset were significantly associated with the presence of a coagulation abnormality. Five of the six patients with either the factor V Leiden or prothrombin G20210A mutation (83%) had had a Valsalva manoeuvre at stroke onset, compared with only eight (16%) in the group of 49 patients without those mutations ($P = 0.002$). Furthermore, three of the four patients with the factor V Leiden mutation (75%) reported a Valsalva manoeuvre ($P = 0.035$). A Valsalva manoeuvre had been present in 41% of the patients with any coagulation abnormality, as compared with 15% of the patients without coagulation abnormalities ($P = 0.046$).

Coagulation abnormality was present in 38% of patients with small to moderate shunt and in 31% of patients with large shunts. Either of the mutations was present in 13% of patients with small to moderate shunt and in 10% of patients with large shunts. There was a non-significant trend for an association between the presence of ASA with the presence of a coagulation abnormality; 43% of the patients with both PFO and ASA also had a coagulation abnormality compared with 29% in those with isolated PFO. The prevalence of either of the prothrombotic mutations was 14% in the patients with both PFO and ASA, and 10% in patients with isolated PFO.

In Table 5 we present a comparison of patients with cryptogenic brain infarction with and without PFO, and controls. More complete data concerning patients without PFO are published elsewhere [20]. The presence of any coagulation abnormality, or either of the mutations between patients with and without PFO was not statistically different ($P = 0.354$ and $P = 0.293$, respectively). The presence of any coagulation abnormality, or either of the mutations between patients without PFO compared with controls was not statistically different ($P = 0.20$ and $P = 0.22$, respectively). A Valsalva-like activity was more common at stroke onset in patients with PFO than in those without PFO ($P = 0.019$).

Discussion

The main findings here were that migraine with aura and venous thrombosis-promoting hypercoagulable states, factor V Leiden and prothrombin G20210A mutations in particular, were found more frequently in patients with cryptogenic brain infarction and PFO than in age- and sex-matched controls from the general population. The frequencies of the conventional risk factors for arterial atheromatous disease were not different between the patients and controls. The higher frequency of underlying risk factors for venous thrombosis in our patients supports the possibility of paradoxical embolism through PFO as an important mechanism

Table 5 Comparison of patients with cryptogenic brain infarction with and without patent foramen ovale, and controls

Characteristic	Cryptogenic stroke with PFO (n = 58)	Cryptogenic stroke without PFO (n = 46)	Controls (n = 104)
Any coagulation abnormality	17 (30%)*	10 (22%)	14 (13%)
Factor V Leiden or prothrombin mutation	6 (11%)**	2 (4%)	1 (1%)
Acquired risk factor for deep venous thrombosis			
Predisposing event within 1 month	14 (24%)*	5 (11%)	12 (12%)
Predisposing event within 3 months	17 (29%) [†]	8 (17%)	15 (14%)
Valsalva-like activity at stroke onset	13 (24%) [‡]	3 (7%)	ND

PFO, patent foramen ovale; ND, no data. * $P = 0.012$ between patients with PFO and controls; ** $P = 0.008$ between patients with PFO and controls; *** $P = 0.036$ between patients with PFO and controls, [†] $P = 0.023$ between patients with PFO and controls, [‡] $P = 0.019$ between patients with PFO and patients without PFO.

behind ischaemic cryptogenic stroke. This hypothesis is further supported by three findings: (1) our patients had had a higher number of events predisposing them to venous thrombosis during the 3 months before the stroke than the controls. (2) There seemed to be some interaction between the presence of a coagulation abnormality and the presence of a predisposing event. (3) We found a strong association between the presence of coagulation abnormalities, especially the factor V Leiden and prothrombin G20210A mutations, and the history of Valsalva manoeuvre-like activity at stroke onset. Furthermore, a Valsalva-like activity was more common at stroke onset in patients with PFO than in those without PFO.

Potential biases of this hospital-based case-control study merit consideration: (1) referral bias is unlikely because our hospital is the only centre for acute neurological patients in the area of Northern Ostrobothnia, taking care of most cerebral infarcts between the ages 15–60 years. (2) All the subjects lived in the same geographical area and were of similar ethnic background. (3) The controls were chosen from the general population, which allowed us to investigate the role of several common risk factors present in the population. (4) Interpretation bias was avoided because the laboratory staff were unaware of the characteristics of the participants. Furthermore, acute phase reactions were avoided by testing the patients in the convalescent phase and by repeating the coagulation testing if the result was abnormal. (5) Ascertainment bias was avoided by using a single interviewer and a structured questionnaire and by addressing the questions without knowledge of the presence or absence of PFO.

However, we cannot exclude selection bias towards patients in which paradoxical embolism was suspected because the physicians in charge judged whether transoesophageal echocardiography was needed or not.

We found no statistical interaction between coagulation abnormality and predisposing events, probably because of the small numbers. It is probably due to these small

numbers that there was no association between any coagulation abnormality and previous DVT, a family history of DVT or a family history of ischaemic stroke. A trend for a difference was observed in the presence of coagulation abnormalities among cryptogenic stroke patients with and without PFO. This difference was non-significant, however, and may be explained by the small number of cases in both groups.

Our finding of the factor V Leiden and prothrombin G20210A mutations being associated with ischaemic stroke in adults is contradictory to many previous studies [6–11]. Prospective cohort studies in particular have yielded negative results. Ischaemic stroke consists of a number of different subtypes, however, and these may have different genetic profiles. Studies lacking stroke subtyping may fail to detect important associations.

We found the factor V Leiden in 7% of the patients and 1% of the controls, while the carrier frequencies previously reported in Finland have varied from 1 to 6% [21–23]. In the largest population-based survey to be conducted in Finland the prevalence of factor V Leiden mutation was 2.4% (95% CI 1.4–4.0%) and for prothrombin G20210A mutation it was 0.5% (95% CI 0.1–1.4%) (L. Hiltunen, personal communication). We used community-based age- and sex-matched controls. Thus bias is unlikely and the prevalence reported here can be considered a reliable estimate for the population of the area.

Migraine with aura was found to associate with PFO in patients with cryptogenic stroke. The association between migraine with aura and PFO is well known, as is also the association between migraine and brain infarction [24–26]. However, the underlying mechanisms of how migraine predisposes to ischaemic stroke are still unclear [27,28]. It has been suggested that coagulation abnormalities could play a role in predisposing young adults with migraine to stroke, but the hitherto published data are contradictory [21,29–32]. In our study, coagulation disorders were not associated with migraine.

We consider verification of PFO justified in young adults with cryptogenic brain infarction. If PFO is found, screening for hypercoagulable states is recommended. If the coagulation testing is positive, paradoxical embolism could be presumed as the mechanism of stroke. If the patient has recently suffered a trauma or undergone surgery, or if an underlying subclinical venous thrombosis is found, the diagnosis of paradoxical embolism is further strengthened. A positive history of a Valsalva manoeuvre at stroke onset also increases the possibility of finding a coagulation abnormality and could also be considered indirect proof of paradoxical embolism.

This study does not answer the question of whether all prothrombotic states are of equal importance. Demonstration of statistical associations will require a larger number of subjects, which is beyond the resources of a single study site. Also, we do not know what is the optimal strategy for secondary stroke prevention in patients with cryptogenic stroke and PFO, and whether those with a coagulation abnormality should be treated differently. The current therapeutic options for patients with PFO vary from antiplatelet or anticoagulant therapy to surgical or transcatheter closure of the PFO [3,33]. One recent study suggested that those patients with both PFO and ASA who have had a cryptogenic stroke constitute a subgroup with a higher risk of recurrent stroke [34], but another recent study could not confirm this finding [35]. Thus far the only published controlled trial, PFO in Cryptogenic Stroke Study (PICSS), examined stroke recurrence and death in stroke patients with and without PFO, who were randomly assigned to warfarin or aspirin [35]. Among those with a cryptogenic stroke, there was a non-significant trend toward primary event reduction in warfarin-treated patients. Long-term anticoagulant therapy is apparently effective in reducing the incidence of thrombosis, but also carries an increased risk of haemorrhage. Although inherited thrombophilias, including the prothrombin and factor V gene mutations, have been associated with an increased risk for recurrent venous thromboembolic complications in patients with venous thrombosis, prospective studies that would have evaluated the benefit-to-risk ratios of anticoagulant therapies of different durations are lacking, and therapy should be tailored to suit the individual patient [36–38]. In some patients, being off anticoagulant treatment affects the quality of life negatively because of serious fear of a recurrent episode of thrombosis, whereas others find the anticoagulant therapy and its monitoring a burden [39]. Regardless of whether a coagulation abnormality is present or not, patients with PFO need counselling about their diagnosis, the high-risk situations for DVT and the need to initiate prophylaxis in this setting, and about symptoms of DVT that require medical attention. Closure of the PFO will prevent paradoxical

embolism, but will not lower the risk of DVT or pulmonary embolism. Two randomized trials have been set up with the intention of comparing medical treatment with percutaneous PFO closure in patients with suspected paradoxical embolism [40].

In conclusion, the current evidence suggests that when PFO is diagnosed in a young adult with cryptogenic brain infarction, paradoxical embolism is a possible or even probable causative mechanism. Our study is the first case-control study to support the assumption that paradoxical embolism could be a significant mechanism, particularly in subjects with an inherited thrombophilia, and that stroke may occur, provoked by predisposing and triggering factors. As coagulation abnormalities were found in 30% of the patients with cryptogenic brain infarction who also had PFO, screening for thrombophilia in cases of such strokes is indicated. Prothrombotic states were not found in the rest of the patients, however, which means that alternative and still unknown risk factors and mechanisms for cerebral ischaemia must be present.

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