Thrombophilia screening in young patients with cryptogenic stroke

Prevalence of gene polymorphisms compared to healthy blood donors and impact on secondary stroke prevention

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Summary

The clinical relevance of thrombophilia screening in stroke patients is still a matter of debate, and descriptions of larger patterns of genetic variability are rare. We assessed the frequency of hereditary hypercoagulability in young patients with cryptogenic stroke (n = 44) and in healthy blood donors (n = 282) without prior cardiovascular event. Furthermore, we focused on the impact of thrombophilia screening on secondary stroke prevention. Results: Compared to the control group (19–67 years; median 38.5 years; 64% women), there was a lower prevalence of the FVII-R353Q mutation (p = 0.033) in stroke patients (17–52 years; median 36 years; 59.1% women). Of note, the FVII-R353Q mutation lowers FVII plasma levels, probably reducing the risk of cardiovascular events. The prevalence of the remaining 13 gene polymorphisms did not differ significantly. However, the prevalence of FV Leiden mutation tended to be higher among stroke patients. Conclusion: Overall, extended screening for inherited thrombophilia had an impact on medical stroke prevention in every sixth patient with cryptogenic stroke.

Keywords

Ischaemic stroke, secondary stroke prevention, FVII-R353Q mutation, genetics

Zusammenfassung

Die Wertigkeit einer Thrombophiliediagnostik bei Schlaganfallpatienten wird kontrovers gesehen. Ziel dieser retrospektiven Studie war es, die Häufigkeit genetisch determinierter thrombophiler Diathesen bei jungen Patienten mit kryptogenem Schlaganfall (n = 44) im Vergleich zu Blutspendern ohne bisheriges vaskuläres Ereignis (n = 282) zu erfassen und die Relevanz der Thrombophiliediagnostik für die Schlaganfallprävention zu beurteilen. Ergebnisse: Im Vergleich zu gesunden Probanden (19–67 Jahre; Median 38.5 Jahre; 64,3% Frauen) zeigte sich in der Schlaganfallkohorte (17–52 Jahre; Median 36 Jahre; 59,1% Frauen) eine niedrigere Prävalenz der FVII-R353Q-Mutation (p = 0,033), die protektiv für das Auftreten von kardiovaskulären Ereignissen sein könnte. Die Verteilung von weiteren 13 Genpolymorphismen unterschied sich hingegen nicht signifikant. Es fand sich lediglich ein Trend für eine höhere Prävalenz der FV-Leiden Mutation in der Schlaganfallkohorte. Schlußfolgerung: Anhand der Befunde der genetischen Thrombophiliediagnostik erfolgte bei einem Sechsten der jungen Patienten mit kryptogenem Schlaganfall eine Umstellung der bisherigen medikamentösen Sekundärprävention.

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Genetische Thrombophiliediagnostik nach kryptogenem Schlaganfall bei jungen Patienten – Prävalenz von Mutationen im Vergleich zu einer gesunden Vergleichspopulation und Relevanz für die medikamentöse Sekundärprävention

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Despite comprehensive clinical diagnostics, the cause of approximately every fourth ischaemic stroke remains undetermined (so-called cryptogenic stroke) (10, 19). This is commonly the case in young adults. Nevertheless, establishing stroke aetiology is crucial for secondary stroke prevention and for determining prospective stroke risk in those patients.

The impact of inherited or acquired coagulation disorders (thrombophilia) on the risk for ischaemic stroke is still uncertain and remains a matter of discussion (4, 10, 16). However, the majority of case-control studies demonstrated no association between predictors of venous thromboembolism and increased stroke risk (4, 22). Notably, even in stroke patients with cardiac right-to-left shunt, there was no increased prevalence of thrombophilic diathesis (22, 31). So far, thrombophilia work-up regarding the arterial system has been predominantly focused on the diagnosis of antiphospholipid syndrome (9). However, the detection of antiphospholipid antibodies has no implications for secondary stroke prevention, as oral anticoagulants have been proven to be non-superior to antiplatelet drugs in such patients (20). Current guidelines do not recommend extended thrombophilia screening in patients with ischaemic stroke from a neurologic right-to-left shunt (12), particularly with regard to the high costs of thrombophilia screening (30).

In recent years, new methods for detection of hereditary gene polymorphisms have been developed. These thrombophilic disorders might cause ischaemic stroke by affecting the arterial system. Various mechanisms have been associated with arterial thrombosis – namely, an
increased concentration of coagulation factors (e.g. β-fibrinogen mutation),
decreased activation of coagulation inhibitors (thrombomodulin mutation),
impaired inhibition of coagulation activators [tissue factor pathway inhibitor (TFPI) mutation],
decreased activity of fibrinolytic enzymes [tissue-type plasminogen activator (t-PA) mutation], and
increased activity of fibrinolysis inhibitors [plasminogen activator inhibitor type 1 (PAI-1) mutation].

Both a decreased concentration of coagulation factors [factor (F) VII mutation] and decreased fibrin cross-linking (FXIII mutation) are thought to prevent arterial thrombus formation and consequently lower ischaemic stroke risk (24).

Thrombophilia screening might help to establish stroke aetiology especially in those patients with cryptogenic stroke. Therefore, we retrospectively assessed the frequency of hereditary hypercoagulability in young adults with cryptogenic stroke in comparison with healthy participants who had no prior vascular events. Moreover, we assessed how often thrombophilia screening influenced secondary stroke prevention treatment paradigms.

Patients, material, methods

The study protocol was approved by the Charité’s ethics committee. We retrospectively analyzed data from 44 cryptogenic stroke patients who underwent treatment in our stroke outpatient clinic. Cryptogenic stroke was defined according to TOAST criteria (1) in the absence of relevant pathological diagnostic findings. In accordance with the German Society of Neurology guidelines (7), we included results from the following diagnostic tests: electrocardiography, echocardiography, laboratory screening, and imaging of the brain and brain-supplying arteries. A major inclusion criterion was (in- or outpatient, partial stepwise) thrombophilia screening.

We excluded stroke patients who were 55 years old or older at the time of stroke. Moreover, those patients with a “non-cryptogenic” stroke according to TOAST criteria (e.g. those with a history of atrial fibrillation, advanced heart failure, proven cardiac thrombus, dissection or relevant stenosis of a brain-supplying artery) or those stroke patients with “concurrent” risk factors were excluded.

Overall, 86% of the enrolled patients were admitted to a stroke unit of which 82% were treated at one of the Charité’s stroke units. At this time, about 25% of all stroke patients at the Charité were classified as having “cryptogenic” stroke. However, not all patients who received initial treatment at the Charité underwent follow-up treatment in a stroke outpatient clinic associated with the Charité.

We recorded the following findings: patient’s age at the time of stroke, gender, type of stroke, affected vascular territory, medication prior to stroke, medication at the time of hospital discharge, and medication after completion of thrombophilia screening. Additionally, we recorded a history of arterial hypertension, coronary heart disease, lipid metabolic disorders, diabetes mellitus, peripheral arterial disease, atrial fibrillation, and smoking.

The control group consisted of 282 healthy blood donors (14) in which pre-existing vascular disease (e.g. deep venous thrombosis, pulmonary embolism, myocardial infarction, coronary heart disease, stroke or peripheral arterial disease) was ruled out using a standardized questionnaire.

The prevalence of the following haemostatic gene polymorphisms were detected:
- plasminogen activator inhibitor type I (PAI-I) [-675 insertion/deletion (5G/4G)],
- tissue plasminogen activator (t-PA) [intron h deletion/insertion],
- factor V APC [1691G>A] (Leiden),
- factor V HR2 [4070A>G],
- prothrombin [20210G>A],
- β-fibrinogen [455G>A],
- tissue-factor-pathway-inhibitor (TFPI) [536C>T],
- thrombomodulin (TM) [127G>A],
- factor VII [R353Q],
- factor VII-activating protease (FSAP Marburg I) [1601G>A],
- factor XIII [Val34Leu],
- methylenetetrahydrofolate reductase (MTHFR) [677C>T],
- angiotensin converting enzyme (ACE) [intron 16 insertion/deletion],
- glycoprotein Ia (gpIa) [807C>T].

Heterozygous and homozygous mutations were distinguished from wild type (no mutation).

Genomic DNA was extracted from whole blood using GenoPrepTM Cartridges B and the GenoMTM-6 system (GenoVision, Vienna, Austria) (14). DNA samples were analyzed by an amplification refractory mutation system using allele-specific primer pairs. All primers were synthesized by TIB Molbiol (Berlin, Germany). The polymerase chain reaction was carried out in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Darmstadt, Germany) using the following temperature profiles: denaturation (96°C for 2 min), followed by 10 cycles (96°C for 15 s, 65°C for 60 s) and 20 cycles (96°C for 10 s, 61°C for 50 s, 72°C for 30 s). Amplification products were separated using electrophoresis and made visible using ethidium bromide and UV transillumination.

Statistical analysis

The results were reported as percentages or mean and standard deviation. The Fisher’s exact test was used to test differences in proportions for dichotomous characteristics between independent groups. Data were analyzed using SPSS Statistics 19. Due to the limited number of stroke patients, all statistical tests have to be regarded as exploratory and p-values < 0.05 indicate possible associations. P-values were not adjusted for multiple testing.

Results

Study cohort

Baseline data of patients with cryptogenic ischemic stroke are depicted in Table 1. Median patient age at time of stroke was 36 years (range 17–52), and 59.1% were female. No patient had a history of prior stroke and none were taking antithrom-
Thrombotic medication at the time of stroke onset. One (2.3%) patient had a past medical history of a deep vein thrombosis or pulmonary embolism. Two (4.5%) patients reported a history of repeated abortions. Echocardiography revealed a cardiac right-to-left shunt in five (16.7%) patients. Twelve (27.3%) stroke patients had no cardiovascular risk factors.

On hospital discharge, 19 (45.2%) stroke patients received (temporary) oral anticoagulation (INR 2–3), which was warranted in five (11.4%) patients by diagnosis of thrombophilia. Additionally, five (11.4%) patients were suspected of having a dissection in a brain supplying vessel and two (4.5%) patients were suspected of having antiphospholipid antibody syndrome. However, these diagnoses could not be confirmed. Three (6.8%) patients likely suffered in-hospital stroke recurrence due to clinical deterioration. A cardiac right-to-left shunt was given as reason in two (4.5%) patients. Of these, one (2.3%) patient had a thrombus in the cranial artery basilaris and one a right cardiac thrombus without a right-to-left shunt. Thirteen (29.5%) patients were still anticoagulated and 30 (68.2%) patients were receiving antithrombotic medication when they first arrived at our outpatient stroke clinic. One patient had stopped taking prescribed antithrombotic medication on her own account. The control cohort was 38.5 years old (median; IQR 28.7–49.8; range 19–67 years), and 64.1% were women.

**Laboratory diagnostics**

The prevalence of gene polymorphisms is depicted in Table 2. While the rate of homozygous FVII-R353Q mutation was comparable, a heterozygous mutation was found in 3.4% stroke patients and 20.2% controls (p = 0.033). Prevalence of all further investigated gene polymorphisms did not differ significantly. Furthermore, heterozygous FV-Leiden mutations tended (p = 0.073) to be more prevalent in stroke patients. Prevalence of the FV-HR2 mutation was comparable in stroke patients and controls and nearly matched prevalence of the FV-Leiden mutation in stroke patients. In contrast to the more seldom mutations (TFPI, thrombomodulin, FSAP, and prothrombin), the frequency of β-fibrinogen, FXIII, PAI-1, t-PA, ACE, MTHFR, and gpla were equally frequent in both cohorts.

There was no evidence for (venous) thrombosis in medical history in stroke patients with confirmed cardiac right-to-left shunt. The following gene polymorphisms were found in patients with a right-to-left shunt:

- patient 1: homozygous mutation of FVII and ACE,
- patient 2: heterozygous mutation of gpl,a,
- patient 3: homozygous mutation of MTHFR and PAI-1,
- patient 4: homozygous mutation of gpl,a, heterozygous mutation of MTHFR and ACE,
- patient 5: no mutation.

**Impact of thrombophilia screening on medical stroke prevention**

In eight (18.2%) stroke patients, a relevant change in antithrombotic medication was founded on results of genetic diagnostics. In four (9.1%) stroke patients, oral anticoagulation was terminated in favour of antithrombotic medication, as no relevant thrombophilia had been found. In four (9.1%) patients, oral anticoagulation was maintained indefinitely due to proven thrombophilia:

- patient 1: homozygous mutation of PAI-1, heterozygous mutation of FV-Leiden, gpl,a, t-PA,
- patient 2: homozygous mutation of β-fibrinogen and ACE, heterozygous mutation of FV-Leiden, MTHFR, FV-HR2 and prothrombin,
- patient 3: homozygous mutation of PAI-1 and t-PA, homozygous mutation of β-fibrinogen, FXIII and FV-HR2,
- patient 4 (proven permanent foramen ovale): homozygous mutation of PAI-1, homozygous mutation of t-PA.

**Discussion**

Within the present retrospective analysis, prevalences of hereditary thrombophilic disorders in young patients with cryptogenic stroke were compared to healthy controls without cardiovascular events. In addition, this study intended to evaluate the therapeutic value of an extended thrombophilia screening in young stroke patients.

In comparison to healthy controls, we found a lower prevalence of (heterozygous)
FVII-R353Q mutation (Tab. 2) in stroke patients. Because FVII-R353Q mutation lowers the activity and concentration of factor VII (21), a preventive effect on ischemic strokes is possible. The comparatively low prevalence of a FVII-R353Q mutation in stroke patients could therefore be equivalent to a heightened individual stroke risk. A recent study reported that stroke patients (≤45 years) have significantly lower prevalences of FVII-R353Q mutations when compared to healthy controls (22). This study did not report, however, the distribution of hetero- and homozygous FVII-R353Q mutations. Additionally, the reported prevalence of 12.8% (22) was considerably higher than in our cohort (Tab. 3). According to multivariate analysis, FVII-R353Q mutation was not an independent risk factor for ischemic stroke (20). However, cardiovascular risk profiles were more pronounced compared to our cohort (20). In an unsselected cohort of stroke patients, no significant difference in prevalence of FVII-R353Q mutations was found compared to controls (11). Interestingly, FVII-R353Q mutations were associated with a lower risk of myocardial infarction (15). Future large prospective studies are needed to determine if FVII-R353Q polymorphism identification is suited for evaluating an individual cardiovascular risk profile.

In accordance with our results, several studies (17, 18, 29) have shown that mutations of TFPI and FISAP were seldom (to never) found in stroke patients or healthy controls (Tab. 2). This is also the case for the thrombomodulin polymorphism. To the knowledge of the authors of this study, no data has been published on thrombomodulin polymorphisms in stroke patients at this time. In contrast, mutations of β-fibrinogen, FXIII, PAI-1 und t-PA were found frequently in both cohorts, rendering their clinical significance questionable. Comparable data on the prevalence of these polymorphisms were found in patients with (cryptogenic) stroke (Tab. 3).

The prevalence of hereditary thrombophilic diatheses predominantly concerning the venous system did not differ in stroke patients and controls (Tab. 2). Heterozygous FV-Leiden mutations did tend to be more prevalent in stroke patients. The significance of this polymorphism for thrombosis in the arterial system therefore cannot be ruled out, particularly regarding limited number of cases. We found a lower prevalence of the heterozygous FV-Leiden mutation than previous studies (Tab. 3), though there was no significant difference between controls (3, 5, 32). On the other hand, a significantly higher prevalence of this polymorphism has been reported in female patients (≤44 years) with acute myocardial infarction (26). A metaanalysis of published case-control-studies on unselected stroke patients up until 2003 showed a higher prevalence of the FV-Leiden mutation in stroke patients (4). The prevalence of the FV-HR2 mutation was comparable to the prevalence of FV-Leiden mutation in our cohort, while two patients had both polymorphisms. We are unaware of other studies that have published data regarding the prevalence of the FV-HR2 mutation in stroke patients (4).

Finally, extended thrombophilia screening had an impact on individual medical
stroke prevention in every sixth young stroke patient. However, due to limited data in available literature a high autonomy in physician’s choice has to be taken into account. The limited therapeutic value of an extended thrombophilia screening has to measured against the estimated cost of about 30 Euro per analysed gene polymorphism.

At the Department of Neurology of the Charité, a standardised thrombophilia screening (prothrombin mutation, FV-Leiden mutation, antithrombin III-, protein C- and protein S-concentration, antiphospholipid antibodies) is only performed in stroke patients with a cardiac right-to-left shunt or evidence of venous thrombosis. Assaying further hereditary thrombophilic markers is no clinical routine.

**Limitations**

This study has several limitations. Both the retrospective design and comparably low case number have to be taken into account. This renders our data analysis exploratory. Therefore, no valid conclusion can be drawn on the prevalence of thrombophilic disorders in stroke patients with proven right-to-left shunt. Furthermore, not all described gene polymorphisms have been assayed in all stroke patients. Moreover, follow-up durations varied. Thus, the frequency of stroke recurrence could not be correlated to thrombophilic polymorphisms. As with other complex disorders (33), we have to expect that the cerebral embolic risk is higher when multiple thrombophilic polymorphism exist (4). The strength of the present study is the large number of analysed gene polymorphisms and the size of the control group.

**Conclusion**

The existence of a hereditary thrombophilia should be considered when stroke aetiology remains cryptogenic after cardiovascular diagnostics. Because data on the efficacy of secondary stroke prevention are missing for nearly all gene polymorphisms, the therapeutic value of thrombophilia screening in stroke patients is estimated to be small. In relation to resulting costs, thrombophilia screening even in young stroke patients should not be regarded as a standard diagnostic tool. In the present study, FVII-R353Q mutation was found only occasionally in young patients with cryptogenic stroke if compared to healthy
blood donors. Larger prospective cohort studies are needed to determine the extent to which FVII-R353Q mutation allows refined stratification of stroke risk.

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Conflict of interest

KGH reports lecture fees and a study grant by Sanofi-Aventis and Bayer Healthcare. ME has received honoraria from AstraZeneca and Sanofi-Aventis, has participated in advisory board meetings of Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, MSD, Pfizer and has received honoraria from Astra Zeneca, Bayer, Berlin-Chemie, Bristol-Myers Squibb, Boehringer-Ingelheim, Desitin, Eisei, Ever, Glaxo Smith Kline, MSD, Novartis, Pfizer, Sanofi-Aventis, Takeda, Trommsdorff. GJJ received consulting and lecture fees from Bayer Health Care, Boehringer Ingelheim, Genzyme, Novartis, Pfizer, Sanofi-Aventis and Takeda. JH, BH, RK, UM and JK report no conflict of interest.

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