Inherited Prothrombotic Conditions and Premature Ischemic Stroke

Sex Difference in the Association With Factor V Leiden

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Abstract—At a young age, ischemic stroke is an uncommon event in which prothrombotic factors are likely to play an important role. In 202 referred cases, 105 men and 97 women, median age 39 years (range, 3 to 50), with a history of ischemic stroke and in 1036 age frequency-matched apparently healthy individuals from the same ethnic background, we have investigated whether inherited prothrombotic conditions increase the risk of ischemic stroke. Neither abnormal plasma levels of natural anticoagulants and fibrinogen nor significant increase of the prothrombin A^{20210} allele was found in stroke cases compared with controls. Hypertension (odds ratio [OR], 22.61), male sex (OR, 2.30), smoking (OR, 2.78) and alcohol habits (OR, 0.14), a personal history of venous thromboembolism (OR, 4.53), a family history of stroke (OR, 1.93), high circulating levels of fibrinogen ($P=0.0190$), and total cholesterol ($P=0.101$) were all independently associated with ischemic stroke. Compared with noncarriers, carriers of the factor V (FV) Leiden mutation (OR, 2.56), and to a lesser extent, of the methylenetetrahydrofolate reductase (MTHFR) TT genotype (OR, 1.60), had an independent higher estimated risk of having a history of ischemic stroke. The relationship with the FV Leiden mutation was greater in women (OR, 3.95). Thus, in addition to established determinants, FV Leiden mutation is independently associated with the occurrence of ischemic stroke in this setting. The greater association in women suggests the possibility of an interaction of this genotype with female hormones. **(*Arterioscler Thromb Vasc Biol*. 1999;19: 1751-1756.)**

Key Words: stroke ■ genes ■ thrombosis ■ polymorphisms

Ischemic stroke is uncommon at a young age and, despite extensive investigations, a large proportion of ischemic strokes are of undetermined etiology.[1–3] A series of studies have been carried out to elucidate the mechanisms of this ischemic event. The results of the studies are not entirely consistent, but the majority of the data support the concept that thrombosis, rather than atherosclerosis, is important for juvenile ischemic stroke. Abnormalities within the gene loci encoding for natural anticoagulants (antithrombin, protein C, and protein S)[4–6] and for fibrinogen[7,8] have been shown to be rather uncommonly associated with ischemic stroke. Other acquired (antiphospholipid antibodies, hyperhomocysteinemia, high plasma levels of fibrinogen, hypofibrinolysis) and inherited (resistance to activated protein C, prothrombin A^{20210} allele) prothrombotic conditions have also been studied. Hyperhomocysteinemia has been documented to be an independent risk factor for stroke.[9,10] Hyperhomocysteinemia is often related to a thermolabile variant of the enzyme methylenetetrahydrofolate reductase (MTHFR),[11] a C→T transition[12] at nucleotide position 677. Mutations of the coagulation factor V (FV) Leiden and of the prothrombin (a G→A transition at nucleotide position 20210) genes account for a large number of cases of venous thromboembolism.[13–15] Some evidence suggests a role for these gene variants in the risk of arterial thrombosis leading to stroke.[16–21] However, these claims have been challenged.[22–29]

We have investigated a relatively young (≤50 years of age) population with a history of ischemic stroke, in whom a genetic and prothrombotic influence is conceivable to be most clearly evident, to assess the relationship with carriership of hereditary prothrombotic risk factors.

Methods

Patients

Between May 1996 and June 1998, 202 individuals aged ≤50 years, 105 men and 97 women, with a history of ischemic stroke were referred to one of the participating centers for a thrombophilic workup. All of the subjects had survived an ischemic stroke from 3 to 12 months before being enlisted. In each case, a nuclear magnetic resonance and/or CT scan confirmed the clinical diagnosis and served to define the type of stroke.[30] The median age at the time of

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the first thrombotic episode was 39.0 years (range, 3 to 50). Three patients suffered from autoimmune disease and 1 had a T-cell lymphoma.

Controls

While patients were being recruited, we interviewed 1272 apparently healthy employees of the Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Southern Italy. The 1084 subjects, who were aged 50 years or less, were invited to participate in the study. Of them, 33 refused and untypability was observed the blood specimens of another 12 subjects; thus, 1039 subjects were enrolled. Three male subjects had documented evidence of coronary heart disease after their enrollment and were excluded from the analysis. All subjects were white, and all of their parents and grandparents had been born in the same region. The male to female ratio was 0.74 (males=440, 42.5%; females=596, 57.5%).

A complete clinical summary, with emphasis on personal and family history for stroke, angina pectoris, myocardial infarction, peripheral arterial disease, venous thromboembolism, and vascular risk factors (high blood pressure, hyperlipidemia, diabetes mellitus, cigarette smoking, alcohol consumption), was obtained from all subjects by a specially trained staff according to a previously described questionnaire.[31] In addition to detailed and specific questions about symptoms of ischemic heart disease, peripheral vascular disease, and previous vascular surgery (as defined according to the World Health Organization questionnaire for cardiovascular disease), the questionnaire contained specific questions concerning stroke and the habitual use of drugs. Hypertension was defined as a longstanding use of antihypertensive drugs or as a systolic blood pressure >140 mm Hg and/or a diastolic blood pressure >90 mm Hg in the sitting position on at least 3 different occasions at the time of admission. Subjects with fasting blood glucose levels >7.8 mmol/L or on treatment with diets and drugs lowering plasma glucose levels were classified as diabetics. Alcohol consumers were divided into current drinkers and past consumers or subjects who never drank. Smokers were divided into subjects who currently smoke and those who never did. A family history for arterial and venous thrombosis was defined as the occurrence of stroke, myocardial infarction, or venous thromboembolism in parents and first-degree siblings. After approval of the local Ethics Committees, the study was carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all the subjects.

Materials

Deoxynucleotide triphosphatase (dNTP), KCl, $MgCl_2$, gelatin, and mineral oil were from Perkin Elmer-Cetus; proteinase K was from USB, Corp; and HEPES, Tris-HCl, EDTA, ethidium bromide, and SDS, were from Sigma Chemical Co. Restriction enzymes, *Hin*fI, *Mnl*I, and *Hin*dIII were from New England Biolabs Inc. The concentrations of total cholesterol were detected enzymatically with commercially available reagents (Roche). Both the reagent and the apparatus (CoA Data 2000) for the measurement of the fibrinogen were from Boehringer Mannheim.

Blood Collection and Coagulation Studies

Blood samples were collected into vacuum plastic tubes containing 0.129 mol/L trisodium citrate and centrifuged at 2000*g* for 15 minutes to obtain platelet-poor plasma. The latter was frozen and stored in small aliquots at −70°C until assayed. Fibrinogen, antithrombin, protein C, amidolytic and immunological (Behring), and total and free protein S antigen (ELISA, Diagnostica Stago) were determined in all patients at the Center of recruitment, as reported elsewhere.[32,33] Pooled normal plasma from 65 normal donors served as control plasma. Clotting assays were performed on a KC4 Amelung coagulometer. Normal ranges were 80% to 120% (antithrombin), 70% to 140% (protein C), 70% to 140% (protein S, total), 60% to 130% (protein S, free), 3.3 to 6.8 mmol/L (total cholesterol), and 2.0 to 4.5 g/L (fibrinogen). Interassay and intraassay coefficients of all the variables never exceed 8.0% and 5.0%, respectively.

DNA Extraction and Analysis

DNA was extracted from peripheral blood leukocytes according to standard protocols.[31] Amplification was carried out on 50-μL volume samples in a Perkin Elmer-Cetus thermal cycler. A 220-bp DNA fragment of the FV gene that included the nucleotide 1691 was amplified and digested with *Mnl*I as previously described,[34] with some modifications.[35] To identify the G→A mutation of the prothrombin gene, a 345-bp fragment was obtained and digested using the *Hin*dIII endonuclease.[15] Screening for the MTHFR C→T677 substitution was performed by amplification of a 198-bp DNA fragment followed by *Hin*fI digestion as described,[12] with modifications.[36]

Statistical Analysis

All of the analyses were performed according to the Statistical Package for Social Science (SPSS 6.1 for Macintosh). The significance of differences in means was evaluated by nonparametric tests, whereas the χ^2 statistic or the Fisher's exact test, as appropriate, tested the significance of differences in proportions. The allele frequencies were estimated by gene counting, and genotypes were scored. The observed numbers of each FV Leiden, prothrombin, or MTHFR genotypes were compared with those expected for a population in Hardy-Weinberg equilibrium using the χ^2 test. The significance of the difference of observed alleles and genotypes between the groups were tested using the χ^2 analysis after grouping homozygous and heterozygous carriers of the FV Leiden mutation, homozygous and heterozygous carriers of the A^{20210} prothrombin allele, and homozygous and heterozygous carriers of the C677 MTHFR allele. Prevalence odds ratios [ORs], considered as prevalence of existing disease, and 95% confidence intervals [CIs] were calculated employing the Normal approximation. Logistic-regression models calculated adjusted ORs and 95% CIs. Statistical significance was taken as $P<0.05$.

Results

Clinical characteristics of the study sample as a whole, and after stratification according to sex, are shown in Table 1. Cases were older ($P<0.0001$), more often current smokers (OR, 2.75; 95% CI, 2.01 to 3.74), and less often alcohol consumers (OR, 0.23; 95% CI, 0.16 to 0.33) than controls. Among subjects with ischemic stroke, there was a higher proportion of males (OR, 1.47; 95% CI, 1.08 to 1.98), hypertensives (OR, 27.58; 95% CI, 15.47 to 49.16), diabetics (OR, 8.12; 95% CI, 3.27 to 20.12), a history of myocardial infarction ($P<0.0001$, Fisher's exact test) and venous thromboembolism (OR, 5.86; 95% CI, 2.78 to 12.33). In addition, cases more frequently had a family history of stroke (OR, 2.77; 95% CI, 1.92 to 4.00) and venous thromboembolism (OR, 1.93; 95% CI, 1.07 to 3.51) than controls. The 2 groups also differed with respect to total cholesterol ($P<0.0001$) and plasma fibrinogen levels ($P<0.0001$). Similar findings were observed when the entire sample was stratified according to sex (Table 1).

No case or control carried inherited abnormalities of antithrombin, protein C, and protein S, or abnormally low fibrinogen plasma levels. In patients, mean±SD levels of antithrombin were 105.3±8.2% and those of protein C 112.0±11.6%. Total and free plasma levels of protein S were 103.2±6.5% and 99.7±5.8%, respectively.

Among cases, 50 individuals (24.8%; 95% CI, 18.8 to 30.8) were homozygous for the T allele of the MTHFR gene, the frequency of the T allele being 49.3% (95% CI, 44.4 to 54.2). One hundred ninety-six healthy subjects (18.9%; 95% CI, 16.5 to 21.3; $P=0.05733$) were T677 MTHFR homozygotes. Their T allele frequency was 43.7% (95% CI, 41.6 to 45.8; P=not significant [n.s.]). The median age at the time of

TABLE 1. Demographic Characteristics and Genotypes of Patients and Controls

	All		Men		Women	
	Controls (n=1036)	Cases (n=202)	Controls (n=440)	Cases (n=105)	Controls (n=596)	Cases (n=97)
Median age, y (range)	35 (22 to 50)	39 (3 to 50)*	36 (22 to 50)	41 (4 to 50)*	34 (22 to 50)	39 (3 to 50)§
Male sex, % (n)	42.5 (440)	52.0 (105)§	...	...	...	...
Smokers, % (n)	24.4 (253)	47.0 (95)*	29.3 (129)	61.0 (64)*	20.8 (124)	32.0 (31)°
Alcohol consumers, % (n)	54.6 (566)	21.8 (44)*	75.5 (332)	35.2 (37)*	39.3 (234)	7.2 (7)*
Fibrinogen, g/L (SD)	2.91 (0.64)	3.20 (0.90)*	2.81 (0.66)	3.21 (0.98)*	2.99 (0.61)	3.19 (0.81)‡
Cholesterol, mmol/L (SD)	4.85 (0.92)	5.54 (1.08)*	4.94 (0.95)	5.30 (1.2)‡	4.79 (0.89)	5.19 (0.93)*
Hypertension, % (n)	1.5 (16)	30.2 (61)*	1.4 (6)	36.2 (38)*	1.7 (10)	24.2 (23)*
Diabetes mellitus, % (n)	0.8 (8)	5.9 (12)*	0.0 (0)	6.7 (7)*	1.3 (8)	5.2 (5)‖
AMI, % (n)	0.0 (0)	2.5 (5)*	0.0 (0)	3.8 (4)‡	0.0 (0)	1.0 (1)
VT, % (n)	1.4 (14)	7.4 (15)*	0.5 (2)	5.7 (6)†	2.0 (12)	9.3 (9)†
Family history of AMI, % (n)	15.6 (162)	16.8 (34)	15.9 (70)	19.1 (20)	15.4 (92)	14.4 (14)
Family history of stroke, % (n)	11.4 (118)	26.2 (53)*	23.9 (25)‡	10.9 (65)	28.9 (28)*	
Family history of DVT, % (n)	4.2 (44)	7.9 (16)§	4.1 (18)	8.6 (9)	4.4 (26)	7.2 (7)
FV Leiden, % (n)	4.2 (43)	14.9 (30)*	5.0 (22)	14.3 (15)†	3.5 (21)	15.5 (15)*
PT A^{20210} allele, % (n)	4.2 (43)	5.0 (10)	4.1 (18)	3.8 (4)	4.2 (25)	6.2 (6)
MTHFR TT 677, % (n)	18.9 (196)	24.8 (50)	18.0 (79)	25.7 (27)	19.6 (117)	23.7 (23)

Mean (SD), except for age (median and range), and percentage (number) are presented for continuous and categorical variables, respectively. AMI indicates acute myocardial infarction; FV, factor V; PT, prothrombin; MTHFR, methylene tetrahydrofolate reductase; VT, venous thromboembolism.

*$P<0.0001$; †$P<0.001$; ‡$P<0.005$; §$P<0.05$.

the ischemic stroke was 36.1 years (range, 3 to 50) in T677 MTHFR homozygotes and 40.0 years (range, 11 to 50) in noncarriers (Mann-Whitney *U* test, $P=0.05$). In this setting, carriers of the FV Leiden mutation were 30 (14.9%; 95% CI, 10.0 to 19.8), 29 heterozygotes and 1 homozygote, among cases, and 43 heterozygotes (4.2%; 95% CI, 3.0 to 5.4; χ^2, 34.884; $P<0.0001$) among controls in this setting. The OR associated with FV Leiden was 4.03 (95% CI, 2.46 to 6.60). The median age at the time of the ischemic stroke was 44.5 years (range, 17 to 49) in carriers of the FV Leiden mutation and 39.0 years (range, 3 to 50) in noncarriers (Mann-Whitney *U* test, $P=0.08$). The prothrombin A^{20210} mutation was detected in 10 cases (5.0%; 95% CI, 2.0 to 8.0), 9 heterozygotes and 1 homozygote, and in 43 controls, all heterozygotes (4.2%; 95% CI, 3.0 to 5.4; $P=$n.s.). The median age at the time of the ischemic stroke was 46.0 years (range, 32 to 49) in carriers of the prothrombin A^{20210} mutation and 39.0 years (range, 3 to 50) in noncarriers (Mann-Whitney *U* test, $P=0.08$). Frequencies of all the mutations were similar in men and in women (Table 1). The observed distribution of genotypes showed no significant difference compared with that predicted from the Hardy-Weinberg equilibrium (χ^2 test).

When stratified according to types of stroke, 82 cases (40.6%) were atherothrombotic, 14 (6.9%) cardioembolic, 46 (22.8%) occlusions of small artery, 4 (2.0%) vasculitis, and 56 (27.7%) were of undetermined etiology or a result of more than one cause. No significant differences were observed when cases were analyzed according to such stratification (*P* always >0.05). In the whole sample, a personal history of venous thromboembolism was associated with the FV Leiden mutation (Fisher exact test, 0.00549), the prothrombin $G\rightarrow A^{20210}$ mutation (Fisher exact test, 0.03219), and the MTHFR TT genotype (χ^2, 3.98204; $P=0.04599$). A family history of venous thromboembolism was associated with the presence of FV Leiden mutation (Fisher exact test, 0.00405), but not the prothrombin $G\rightarrow A^{20210}$ mutation (Fisher exact test, 0. 12620) and MTHFR TT genotype (χ^2, 0.26143; $P=$n.s.).

The data relative to FV Leiden were further analyzed. Stratification according to smoking habit or a family history of stroke showed an additive effect of the gene variant (Table 2). In contrast, the combined presence of FV Leiden mutation and 1 or more vascular risk factors, ie, hypertension, diabetes mellitus, myocardial infarction, and hypercholesterolemia (Table 2), led to risk estimates (OR, 10.72; 95% CI, 5.46 to 21.04) that exceeded the separate effects of FV Leiden and the vascular risk factors, respectively (OR, 1.93; 95% CI, 0.82 to 4.57; and OR, 1.94; 95% CI, 1.40 to 2.70).

The findings from the univariate analysis were further investigated in a logistic model after adjustment for age (in years), sex, alcohol and smoking habits, hypertension, diabetes mellitus, a history of myocardial infarction and of thromboembolic episodes, a family history of stroke and of venous thromboembolism, and plasma levels of fibrinogen and total cholesterol. (Table 3). Under these circumstances, FV Leiden mutation was still independently and significantly associated with the occurrence of ischemic stroke, whereas the MTHFR TT genotype played a marginal, although significant, role. A separate analysis for sex was carried out. An independent and significant association between the occurrence of the cerebrovascular event and the FV Leiden mutation (OR, 3.95; 95% CI, 1.55 to 10.05), a personal history of venous thromboembolism (OR, 4.58; 95% CI, 1.55 to 13.50), and a family history of stroke (OR, 2.34; 95% CI, 1.23 to 4.49) was found in women. In men, significant associations were found with smoking consumption (OR, 4.48; 95% CI, 2.38 to 8.40)

TABLE 2. Current Smoking Habit, Family History of Stroke, Vascular Risk Factors§ and Factor V Leiden: Separate and Combined Effect on Ischemic Stroke

Variable	Factor V Genotype	Controls	Cases	OR	95% CI
Current smoking					
No	Wildtype	751	92	1*	...
No	Leiden	31	15	3.95	2.06 to 7.59
Yes	Wildtype	241	80	2.71	1.94 to 3.78
Yes	Leiden	12	15	10.20	6.82 to 15.26
Family history					
No	Wildtype	883	131	1*	...
No	Leiden	35	18	3.47	1.91 to 3.30
Yes	Wildtype	110	41	2.51	1.68 to 3.76
Yes	Leiden	8	12	10.11	4.06 to 25.20
Vascular risk factors					
No	Wildtype	686	92	1*	...
No	Leiden	27	7	1.93	0.82 to 4.57
Yes	Wildtype	307	80	1.94	1.40 to 2.70
Yes	Leiden	16	23	10.72	5.46 to 21.04

*Reference category.

§Hypertension, diabetes mellitus, myocardial infarction, and hypercholesterolemia.

and plasma fibrinogen levels (OR, 1.65; 95% CI, 1.15 to 2.35, for an increase of 1 g/L). Hypertension (women, OR, 16.86; 95% CI, 6.50 to 43.86; men, OR, 25.15; 95% CI, 8.77 to 72.11), total cholesterol (women, OR, 1.36; 95% CI, 1.04 to 1.79; men, OR, 1.32; 95% CI, 1.01 to 1.73, for an increase of 1 mmol/L), and alcohol consumption (women, OR, 0.12; 95% CI, 0.05 to 0.29; men, OR, 0.12; 95% CI, 0.06 to 0.23) were significantly related with the event in both sexes. In a multiple logistic regression model, evidence for an interaction was found for smoking habit and sex ($P=0.0249$), but not for FV Leiden mutation and smoking habit, sex, family history of stroke, and vascular risk factors (P always >0.1).

Discussion

Atherosclerosis is a lifelong degenerative event whose progression leads to major ischemic complications such as myocardial infarction and ischemic stroke. In the young, because of the limited lag of time for atherosclerosis to progress, ischemic stroke is likely to be a well-suited clinical setting to investigate the role of prothrombotic conditions, in particular inherited ones, in the risk of ischemic stroke.

Anecdotal observations have suggested a link between congenital deficiencies of natural anticoagulants (antithrombin, protein C, and protein S) or fibrinogen and cerebral infarction.[4–8] No inherited abnormalities of natural anticoagulants nor of fibrinogen were found in this setting. No association between the mutant A^{20210} allele of the prothrombin gene and a history of cerebral ischemia was observed in an Italian case-control study.[29] In the present report, in which allele and genotype frequencies closely resembled those reported in other Italian series,[28,29,35–38] the data are consistent with such a formulation, and imply that abnormalities of fibrinogen, natural anticoagulants, and the mutant A^{20210} allele of the prothrombin gene are a rather uncommon cause of ischemic stroke in young adults. In addition to established determinants (eg, hypertension and smoking habit), FV Leiden mutation exhibited a significant and independent relationship with the occurrence of ischemic stroke in this setting. The association was still significant when all the variables in which cases and controls differed were taken into account in a multivariate logistic regression model. In carriers of the FV Leiden mutation, the estimated risk of ischemic stroke was at least additive to that observed in current smokers, in subjects with a family history of stroke, and in those with vascular risk factors. A borderline significant association between the MTHFR TT genotype and the occurrence of ischemic stroke was found. The C→T substitution at nucleotide 677 within the MTHFR gene is a relatively frequent missense mutation.[12,36,37,39–41] Especially in settings carrying low plasma folate levels, the presence of MTHFR TT homozygosity has been associated with moderate hyperhomocysteinemia.[37,39–41] The latter is a risk factor for stroke.[9,10] Our

TABLE 3. Factors That Independently Identify Subjects With a History of Ischemic Stroke

Variable	b	SE	df	*P*	OR	95% CI
Hypertension	3.0732	0.3574	1	0.0000	22.61	10.73 to 43.54
Alcohol consuming	−1.9539	0.2548	1	0.0000	0.14	0.09 to 0.23
Smoking habit	1.0220	0.2109	1	0.0000	2.78	1.13 to 3.21
Male sex	0.8341	0.2231	1	0.0002	2.30	1.49 to 3.57
History of VT	1.5101	0.5015	1	0.0026	4.53	1.69 to 12.10
FV Leiden	0.9403	0.3550	1	0.0081	2.56	1.28 to 5.14
Total cholesterol (mmol/L)	0.2549	0.0991	1	0.0101	1.29	1.06 to 1.57
Family history of stroke	0.6574	0.2567	1	0.0104	1.93	1.17 to 3.19
Fibrinogen (g/L)	0.3224	0.1375	1	0.0190	1.38	1.05 to 1.81
MTHFR TT 677 genotype	0.4684	0.2375	1	0.0486	1.60	1.00 to 2.54

The multivariate logistic regression contained the following nonsignificant covariables: age, history of myocardial infarction, diabetes mellitus, family history of myocardial infarction, family history of DVT, Prothrombin A^{20210}/allele. b, indicates estimated coefficient; df, default; VT, venous thromboembolism.

findings somewhat differ from those of Markus et al,[41] in which no association between MTHFR TT homozygosity and cerebrovascular disease was found. The interaction with plasma folate and the low informativeness of the marker used may also account for inconsistencies. Plasma samples collected at the time of the ischemic event do not allow for measurements of the levels of homocysteine and folate in the present setting. Thus the present data cannot rule out the possibility that differences in the intake of folate account, at least in part, for the association of the MTHFR TT genotype with the occurrence of ischemic stroke.

Inconclusive results are present in the literature on the association between FV Leiden mutation and stroke.[16–19,21–27] Although the inconsistency may reflect the play of chance, alternative explanations have to be considered. Whereas a relationship has been documented in younger adults or specific settings,[16–19,21] the presence of the FV Leiden mutation was not associated with stroke in studies that enlisted older adults or elderly patients.[22–27] For example, Catto et al[24] studied 386 randomly selected elderly patients (median age, 74 years) with acute stroke. In the present study, median age was 39 years, 25.8% of women and 20% of men aged 30 years or less when the ischemic event took place. The US Physicians' Health Study[23] included only male physicians. In the study by Press et al[25] the percentage of men in the stroke group was 91%. In the present study, women were 48% of cases.

After stratification for sex, FV Leiden mutation was independently associated with an increased risk estimate of ischemic stroke in women (OR, 3.95). Recently, an increased risk of myocardial infarction has been reported in young women with FV Leiden mutation.[42] Endogenous estrogens and oral contraceptives increase the resistance to activated protein C regardless of the presence of the FV Leiden mutation.[43] In oral contraceptive users, FV Leiden mutation has been described to further increase the risk of venous thrombosis.[44] In the present sample, the small number of oral contraceptive users (n=9) did not allow for a reliable analysis of the role of oral contraceptives alone and in combination with the FV Leiden mutation on the occurrence of ischemic stroke.

Epidemiological evidence suggests a U-shaped association between alcohol consumption and cardiovascular disease, with moderate intake protecting against stroke.[45,46] In our study population, alcohol intake was associated with an independent lower risk of ischemic stroke (OR, 0.14). The beneficial effect of the alcohol consumption has been attributed to inhibition of the atherogenic potential of LDL cholesterol.[46] We did not address this issue. However, the higher percentage of alcohol drinkers and the lower mean levels of total plasma cholesterol in the control group further suggest an important role for blood lipids in the pathogenesis of stroke.

Because we only enlisted subjects that survived their stroke event, one could argue that this could have led to biased results. Raised circulating levels of some parameters measured, such as fibrinogen, may have been caused by the disease process because fibrinogen is an acute-phase protein. However, established determinants of ischemic stroke (eg, hypertension, male sex, smoking and alcohol habits, family history of stroke, and high circulating levels of fibrinogen and total cholesterol) are all strongly and independently associated with the occurrence of ischemic stroke in the present setting. Case fatality in stroke largely depends on age, with 88% of all deaths from stroke occurring in people older than 65 years.[2] Furthermore, it has not been proven whether subjects who died would have had an over- or under-representation of these gene variants. Thus, the data tend to exclude the possibility that the relationships that we have found are significantly affected by the selection of stroke survivors, and support the possibility that our case group is representative of a stroke population.

We conclude that FV Leiden mutation and, to a lesser extent MTHFR TT genotype, are independently associated with the occurrence of ischemic stroke in young adults aged 50 years or less. Similar to myocardial infarction, the relevance of FV Leiden mutation appears to be restricted to women in this setting, suggesting a role for endogenous and exogenous female hormones in such an association. The clinical implications of these data need to be addressed in prospective ad hoc studies.

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