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FACTOR V LEIDEN AND ISCHEMIC STROKE RISK: THE GENETICS OF EARLY ONSET STROKE (GEOS) STUDY

Ali G. Hamedani, B.S.¹, John W. Cole, M.D. M.S.^{1,2}, Yuching Cheng, Ph.D.³, Mary J. Sparks, R.N. B.S.N.¹, Jeffrey R. O'Connell, D.Phil.³, Oscar C. Stine, Ph.D.³, Marcella A. Wozniak, M.D. Ph.D.^{1,2}, Barney J. Stern, M.D.^{1,2}, Braxton D. Mitchell, Ph.D. M.P.H.³, and Steven J. Kittner, M.D. M.P.H.^{1,2}

¹Department of Neurology, University of Maryland School of Medicine, Baltimore, MD

²Baltimore Veterans Affairs Medical Center, Baltimore, MD

³Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

Abstract

Background and Purpose—Factor V Leiden (FVL) has been associated with ischemic stroke in children, but not in adults. Although the FVL mutation is associated with increased risk for venous thrombosis, its association with ischemic stroke in young adults remains uncertain. Therefore, we examined the association between FVL and ischemic stroke in participants of the Genetics of Early Onset Stroke (GEOS) Study.

Methods—A population-based case-control study identified 354 women and 476 men aged 15–49 years with first-ever ischemic stroke, and 907 controls. Participant-specific data included vascular risk factors, FVL genotype and, for cases, the ischemic stroke subtype by modified TOAST criteria. Logistic regression was used to calculate odds ratios for the entire population and for subgroups stratified by risk factors and ischemic stroke subtype.

Results—The frequency of the FVL mutation was similar between ischemic stroke patients (3.6%, 95% CI: 2.5%–5.1%) and non-stroke controls (3.8%, 95% CI: 2.7%–5.2%). This frequency did not change significantly when cases were restricted to patients with stroke of undetermined etiology (4.1%, 95% CI: 2.6%–6.4%).

Conclusions—Among young adults, we found no evidence for an association between Factor V Leiden and either all ischemic stroke or the subgroup with stroke of undetermined etiology.

INTRODUCTION

The Factor V Leiden (FVL) mutation causes activated protein C resistance and is the most prevalent prothrombotic genetic mutation. It is associated with venous thromboembolism in patients of all ages [1] and ischemic stroke in children [2], but not with ischemic stroke in the general adult population [3]. However, studies of FVL and ischemic stroke in young adults have yielded conflicting reports, due in large part to limitations in sample size and method of patient selection [5–21]. The clinical importance of FVL in young adults with

Corresponding author: John W. Cole, M.D. M.S. University of Maryland School of Medicine Department of Neurology Bressler Res. Bldg. room 12-006 655 W. Baltimore St. Baltimore, MD 21201-1509 phone: (410) 706-0414 fax: (410) 706-0816 jcole@som.umaryland.edu.

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ischemic stroke of undetermined etiology is uncertain. To address this issue, we examined the association between FVL and first-ever ischemic stroke in a large biracial case-control study, the Genetics of Early Onset Stroke (GEOS) Study.

METHODS

The Genetics of Early Onset Stroke (GEOS) Study is a population-based case-control study designed to identify the genetic determinants of early-onset ischemic stroke and to characterize interactions of identified stroke genes and/or SNPs with environmental risk factors such as smoking and oral contraceptive use. This analysis is based on all 830 cases from the GEOS study and compares cases with first ischemic stroke at 15–49 years of age to a similar number of non-stroke controls, identified from the Baltimore-Washington area.

Cases with a first ischemic stroke were identified by discharge surveillance from one of 59 hospitals in the greater Baltimore-Washington area and by direct referral from regional neurologists. Cases and controls were recruited in 3 different time periods: Stroke Prevention in Young Women-1 (SPYW-1) conducted from 1992–1996, Stroke Prevention in Young Women-2 (SPYW-2) conducted from 2001–2003, and Stroke Prevention in Young Men (SPYM) conducted from 2003–2007. SPYW-1 included cases aged 15–44 years recruited within one year of stroke and was designed with a 1:2 case-to-control ratio. SPYW-2 and SPYM included cases aged 15–49 recruited within three years of stroke and was designed with a 1:1 case-to-control ratio. Control participants without a history of stroke were identified by random-digit dialing. Controls were balanced to cases by age and region of residence in each study and were additionally balanced for ethnicity in SPYW-2 and SPYM. The number of cases and controls recruited in each study is as follows: 115 cases and 198 controls from SPYW-1, 234 cases and 209 controls from SPYW-2, and 478 cases and 500 controls from SPYM.

The abstracted hospital records of potential cases were reviewed and adjudicated for ischemic stroke subtype by a pair of vascular neurologists according to previously published procedures [22–23], with disagreements resolved by a third vascular neurologist. The ischemic stroke subtype classification system retains information on all probable and possible causes, and is reducible to the more widely used TOAST [24] system that assigns each case to a single category. Ischemic strokes with the following characteristics were excluded from participation: stroke occurring as an immediate consequence of trauma, stroke within 48 hours after a hospital procedure, stroke within 60 days after the onset of a non-traumatic subarachnoid hemorrhage, and cerebral venous thrombosis. Additional exclusions for these genetic analyses were as follows: known single-gene or mitochondrial disorders recognized by a distinctive phenotype (e.g. cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), homocystinuria, Fabry disease, or sickle cell anemia); mechanical aortic or mitral valve at the time of index stroke; untreated or actively treated bacterial endocarditis at the time of the index stroke; neurosyphilis or other CNS infections; neurosarcoidosis; severe sepsis with hypotension at the time of the index stroke; cerebral vasculitis by angiogram and clinical criteria; post-radiation arteriopathy; left atrial myxoma; major congenital heart disease; and cocaine use in the 48 hours prior to their stroke.

Demographic variables, including age, ancestry, ethnicity, and established stroke risk factors including history of hypertension, diabetes, myocardial infarction (MI), current smoking status, and current oral contraceptive (OC) use (both defined as use within one month prior to event for cases and at a comparable reference time for controls), were collected during a standardized interview and were included as covariates in our analyses. Hyperlipidemia was

not included since it was not a study measurement and self-reporting by participants was inadequate.

Rs6025 genotype, which corresponds to the G1691A FVL mutation, was obtained in all 830 cases and 907 controls of the GEOS study using the Illumina custom cardiovascular gene-centric 50K SNP array (ITMAT-Broad-CARE array), which included 49,094 SNPs from ~2,000 loci. Genotyping was carried out at the Institute for Translational Medicine and Therapeutics, University of Pennsylvania. Genotype call rate was 99.9% for this SNP.

Statistical analysis was performed using SAS version 9.1 (SAS Institute, Cary, NC). The presence of Hardy-Weinberg equilibrium in controls was determined for FVL by the permutation version of the exact chi-square test using the PROC ALLELE procedure. The distribution of FVL and other characteristics among cases and controls was compared using *t* tests for continuous variables and Mantel-Haenszel chi-square tests for categorical variables. The association between FVL and ischemic stroke was then examined within predefined subgroups using chi-square tests or Fisher's exact tests in the event of small sample sizes. Whenever possible, comparisons were repeated using a logistic regression model adjusted for age, ethnicity, and gender. We assessed whether the effect of FVL on stroke differed between those with and without vascular risk factors by including a FVL*risk factor interaction term in the model. Two-tailed probability values of <0.05 were considered statistically significant. Among persons of European ancestry, based on a frequency of 5% for FVL [25], we had 80% power in our sample to detect an odds ratio of 2.4 or higher for all first ischemic stroke and an odds ratio of 2.5 or higher for first ischemic stroke of undetermined etiology. Among persons of African ancestry, the current study had minimal power to detect even strong associations between FVL and stroke due to the low prevalence of FVL ($\approx 1\%$) in this group.

RESULTS

The clinical characteristics of cases ($n = 830$) and controls ($n = 907$) are summarized in Tables 1 and 2. Controls were in Hardy-Weinberg equilibrium for the FVL mutation, both when taken as a whole and when stratified by ethnicity ($p > 0.05$). The proportion of subjects carrying the FVL mutation in the combined case-control sample was 5.37% in participants of European ancestry and 1.37% in participants of African ancestry. There was only one homozygote in the entire sample. Cases were significantly older than controls and were more likely to report a history of hypertension, diabetes, myocardial infarction, oral contraceptive use, and current cigarette smoking. However, there was no significant difference between the proportion of cases (3.6%, 95% CI: 2.5%–5.1%) and controls (3.8%, 95% CI: 2.7%–5.2%) with FVL (OR = 1.00, 95% CI: 0.60–1.66, $p = 1.00$). This result did not change when carrying out stroke-specific analyses (e.g., large artery stroke, small artery stroke, cardioembolic stroke, stroke of other determined etiology, and stroke of undetermined etiology).

Analyses of FVL and ischemic stroke risk are presented in Table 3 stratified by demographic and vascular risk factors. There was no association of FVL in either ancestry group or gender. Due to small numbers of patients with FVL in any vascular risk group, we examined the association between FVL and ischemic stroke risk among those with one or more vascular risk factors, defined as having a history of hypertension, diabetes, myocardial infarction, oral contraceptive use, or current smoking. Within this subgroup, cases were significantly more likely to have FVL than controls (22/604 cases vs. 6/416 controls; OR = 2.6, 95% CI: 1.04–6.0, $p = 0.04$). Of the individual vascular risk factors, the point estimates for the association of FVL and ischemic stroke were higher among those with hypertension, diabetes, cigarette smoking and oral contraceptive use, but not among those with MI. While

the association in the hypertension subgroup (OR = 13.6, $p=0.01$) was marginally statistically significant, the numbers with FVL were very small and this association would not have been significant after correction for multiple comparisons.

DISCUSSION

Recently, we performed a meta-analysis of case-control studies of Factor V Leiden and ischemic stroke in young adults and found an overall positive association, although a number of these studies were enriched for the presence of a prothrombotic genetic predisposition due to the manner of patient selection [4]. For example, five of eighteen studies recruited cases from a subset of patients who were referred for thrombophilic work-up [5–9], suggesting that there was a pre-existing suspicion of prothrombotic status in these patients. Another four studies either specified that cases were limited to cryptogenic strokes or utilized criteria suggestive of cryptogenic strokes [10–13]. When we limited our meta-analysis to studies that recruited patients from consecutive hospital admissions or neurology referrals [14–21], the association was no longer significant.

To our knowledge, this is the largest study to date that has examined the association between FVL and ischemic stroke in young adults. We failed to find an overall association between the FVL mutation and either total ischemic stroke or stroke of undetermined etiology. This is in agreement with previous studies that, like ours, recruited cases from consecutive hospitalizations or referrals, rather than from a subset of patients who were suspected to have a prothrombotic genetic predisposition [14–21].

To our knowledge, no prior studies examined whether the presence or absence of other cardiovascular risk factors modified the association between FVL and ischemic stroke. Although our data suggests an association between FVL among those with hypertension or more than one vascular risk factor, we suspect that this may be due to chance. In part, our skepticism stems from the fact that the point estimate for the association of FVL with stroke was less than one (i.e. protective) among those without hypertension or among those without any vascular risk factors. A protective effect for FVL mutation does not seem biologically plausible. Thus, this observation will require independent replication in other studies.

Our study has several limitations. First, the low frequency of FVL in our study population limits our power to detect an association with ischemic stroke. This limitation is even more pronounced in the stratified analyses. Due to the very low frequency of FVL in persons of African ancestry, our study also does not provide useful information about this subgroup. Second, because all but one of those with the FVL mutation were heterozygotes, our results are not applicable to homozygotes. Third, the population-based design of the study with recruitment at over 50 regional hospitals precluded consistent assessment of the presence of patent foramen ovale (PFO) and potential paradoxical embolism among cases. This is important because an important mechanism by which FVL could cause ischemic stroke is via venous thrombosis and paradoxical embolization. Some studies have looked specifically at the association between FVL and ischemic stroke in those with PFO; a recent meta-analysis of studies examining the relationship between FVL and PFO-positive ischemic stroke found a trend towards a positive association, though it did not meet the threshold for significance [26]. Fourth, our study was limited to non-fatal ischemic strokes, so a role for FVL in causing fatal strokes cannot be ruled out; though this is unlikely given the low case-fatality rate in this population [27]. Finally, our study provides no information about the role of FVL in ischemic stroke among young adults with a personal or family history of prior early-onset thrombotic events.

In conclusion, we report that Factor V Leiden is not associated with ischemic stroke in young adults. To further address our findings of a possible association between FVL and ischemic stroke among persons with vascular risk factors, new large sample size studies or meta-analysis of prior studies would be needed. Consistent with recent recommendations [28], our results do not support routine screening for FVL in patients with first-ever ischemic stroke.

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Table 1

Demographic and clinical characteristics of cases and controls in the Genetics of Early Onset Stroke (GEOS) Study

	Cases (n=830)	Controls (n=907)	p-value
ethnicity (African ancestry)*	365 (44.0%)	366 (40.4%)	0.13
gender (male)	476 (57.4%)	500 (55.1%)	0.35
age (years)	41.4 (40.9–41.9)	39.5 (39.1–40.0)	<0.0001
hypertension	355 (42.9%)	172 (19.0%)	<0.0001
diabetes mellitus	139 (16.8%)	47 (5.2%)	<0.0001
previous myocardial infarction	46 (5.56%)	6 (0.66%)	<0.0001
current oral contraceptive use ^ψ	57 (16.4%)	34 (8.37%)	<0.001
history of current smoking	348 (41.3%)	257 (28.3%)	<0.0001
packs/day of current smoking	0.93 (0.86–0.99)	0.69 (0.63–0.75)	<0.0001
factor V Leiden [†]	30 (3.62%)	34 (3.75%)	0.89

* There were 24 subjects who self-identified as Hispanic. They were divided evenly between the European ancestry and African ancestry groups. The European ancestry population also included 14 subjects self-identifying as American-Indian and 27 subjects self-identifying as Asian.

[†] Includes heterozygotes and homozygotes.

^ψ Among female cases and controls only.

Table 2

Distribution of TOAST subtypes among stroke cases

TOAST subtype	No. of cases
undetermined etiology	419 (50.4%)
small vessel	130 (15.7%)
cardioembolism - possible	101 (12.2%)
cardioembolism - probable	65 (7.8%)
large artery	59 (7.1%)
other determined etiology	56 (6.7%)

Table 3

Odds ratios for Factor V Leiden and ischemic stroke in young adults, stratified by presence or absence of risk factors

	#FVL /# cases ^ψ	#FVL / #controls ^ψ	odds ratio	p-value
overall ancestry	30/830	34/907	1.00 (0.60–1.66)	1.00
European	25/464	29/541	1.0 (0.6–1.7)	0.98
African	5/365	5/366	1.0 (0.3–3.6)	1.00
gender				
male	19/476	22/500	1.0 (0.5–1.9)	0.98
female	11/353	12/407	1.1 (0.5–2.5)	0.89
vascular risk factors				
1	22/604	6/416	2.6 (1.0–6.0)	0.04
0	7/215	28/460	0.5 (0.2–1.2)	0.11
hypertension				
yes [*]	13/355	0/172	13.6	0.01
no	16/472	34/734	0.7 (0.4–1.3)	0.23
diabetes mellitus				
yes [*]	3/139	0/47	2.4	0.31
no	27/689	34/859	1.00 (0.6–1.7)	1.00
myocardial infarction				
yes [*]	2/45	0/6	0.7	0.60
no	28/782	34/900	1.00 (0.6–1.6)	0.94
current oral contraceptive use				
yes [*]	1/57	0/34	1.8	0.43
no	10/290	12/372	1.10 (0.5–2.6)	0.83
history of current smoking				
yes	17/347	6/257	1.9 (0.7–5.0)	0.20
no	13/482	28/650	0.7 (0.3–1.3)	0.22

^{*}Fisher's exact test (confidence interval not available)

^ψMinor discrepancies in the total number of cases and controls among the different stratified analyses due to the presence of missing values.