

The Association of Coagulation Factor V (Leiden) and Factor II (Prothrombin) Mutations With Stroke

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Background: Epidemiological studies indicate that over the past forty years, the stroke incidence rates has increased. Factors V and II mutations are established genetic-variant risk factors for venous thrombosis; however, their contribution to stroke is a controversial issue.

Objectives: This study aimed to investigate the potential association of FV and FII mutations with stroke in an Iranian population.

Patients and Methods: The study population consisted of 153 patients of different stroke subtypes (except cryptogenic strokes), admitted to Ghaem Hospital, Mashhad, Iran. The control group included 153 age- and sex-matched subjects without a history of cerebrovascular or neurologic diseases. Mutations of FV and FII were determined by using a TaqMan SNP Genotyping technique. The chi-square and Exact Fisher tests were used to analyze the baseline characteristics. Results were as follows: The calculated P-value for sex and diabetes mellitus were 0.907 and 1.000, respectively. The case and control groups were also matched in low density lipoprotein (P = 0.816), high density lipoprotein (P = 0.323), triglyceride (P = 0.846), and total cholesterol (P = 0.079).

Results: Analysis of the FV showed that none of the study subjects were AA homozygous for this mutation and only 6 heterozygous subjects were detected in the case and control groups. Regarding FII variants, none of the study subjects were AG heterozygous and only 1 AA homozygous was detected in the control group.

Conclusions: The prevalence of both FV and FII variants are population based. Iran is an ethnically diverse country. Therefore, for a comprehensive analysis of a potential association of FV and/or FII mutations with stroke among Iranian population, epidemiological studies could be conducted among different ethnic groups.

Keywords: Stroke; Population; Incidence

1. Background

Epidemiological studies indicate that over the past forty years, the stroke incidence rate has increased from 52 out of 100,000 per year (1970-1979) to 117 (2000-2008) in the South Asian countries (1). The epidemiology of the stroke in the Middle East is not clear yet. During a 12-month period between 2006 and 2007, two teams of physicians from Iran and Australia, ascertained all strokes occurring in 450,229 Iranian adults in the city of Mashhad, located in the northeast of Iran (2). The results of this study showed that the incidence of stroke in Iran was considerably higher than Western countries; also the average age of the Iranian patients was about 10 years lower. Studies in twins and families demonstrate a substantial evidence of genetic background for stroke (3).

Among the most widely investigated genes are the ones involved in inflammation, coagulation, lipid metabolism, nitric oxide release, renin-angiotensin-aldosterone systems, and homeostasis (4, 5). Factor V is a large single-chain glycoprotein involved in the coagulation process and regulated by activated protein C. The FV gene's most studied variation is the single point mutation (c.1691G > A) leading to a p.Arg506Gln amino acid exchange, and finally FV resistance to activated protein C (6, 7). FII (the proenzyme of thrombin) is a vitamin K-dependent glycoprotein that converts fibrinogen into fibrin. A common genetic variation, g.20210G > A, in the 3'-untranslated region of the FII gene is associated with increased plasma prothrombin levels (8). Although the above mentioned FV and FII genetic

variants are established risk factors for venous thrombosis, their contribution to arterial atherothrombotic disease like stroke is controversial (9). Some epidemiological studies have shown that FV c.1691G > A and FII g.20210G > A, the two most common gene mutations responsible for thrombophilia, are also associated with an increased risk of stroke and coronary artery disease (10-12). However, some other large studies failed to find any significant association between these two genetic variants and stroke in unselected adult populations (6, 7, 10).

2. Objectives

This study aimed to investigate the association of FVL c.1691G > A and FII g.20210G > A, genetic variations with stroke in an Iranian population with a high incidence rate of stroke.

3. Patients and Methods

This study was a case-control type. The test population consisted of 153 patients with different stroke subtypes (except for cryptogenic strokes) admitted to the Ghaem Hospital, the main referral hospital for the eastern provinces of Iran, including Khorasan, from March 2012 to June 2013. The control group included 153 randomly selected age- and sex-matched subjects without any history of cerebrovascular or neurologic diseases. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution of Human Research Committee. Informed consents were obtained from all participants. The code and date of ethical approval was supported financially by Grant No: 1959 of Vice President for Research, Mashhad University of Medical Sciences, Mashhad, Iran.

Demographic variables and established stroke risk factors, including age, sex, history of hypertension, ischemic heart attack, diabetes mellitus, as well as the lipid profiles of the total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were collected by using Ghaem Hospital information system (HIS) software for stroke patients. Case and control groups were also matched in their baseline demographic and clinical features. The study was approved by the MUMS Ethics Committee and an informed consent was obtained from each participant.

The World Health Organization defines stroke as "rapidly developing signs of focal or global disturbance of cerebral function lasting 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin" (13). All of the participants enrolled in the study underwent a complete neurological examination. Diagnosis of stroke was confirmed by comput-

erized tomographic (CT) and/or magnetic resonance imaging (MRI) scans of the brain. The neuroimaging profiles were used to classify patients with definite FESs (First-ever stroke), into ischemic stroke (IS), intracerebral hemorrhage (ICH), and subarachnoid hemorrhage (SAH) subgroups. Overall, about 80% of the stroke cases were ischemic, which is due to arterial vascular occlusion. Among the remaining cases, about 15% were hemorrhagic strokes, which are due to vascular rupture, and 5% were of unknown etiology (14, 15). Participants were classified as having diabetes mellitus if they already had the diagnosis of diabetes mellitus, or their fasting plasma glucose was 126 mg/dl, or if the patient had a history of either oral hypoglycemic, hypoglycemic agent, or insulin use (16). Hypertension (HTN) was defined as a history of HTN or reported blood pressure greater than systolic 140 mmHg or diastolic 90 mmHg (17).

Genomic DNA was extracted from peripheral blood leukocytes using the PrimePrep Genomic DNA Isolation Kit (catalog No K-2000; Genet Bio). The FV c.1691G > A and FII g.20210G > A, genetic variants were determined by using a TaqMan SNP genotyping technique. Genotypes were detected by Rotor-Gene® qPCR machine. To analyze the baseline characteristics, we used the chi-square or Exact Fisher test for all categorical data (sex and diabetes mellitus) and the student t test for continuous data (age, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)) when comparing case and control baseline data. A P-value of <0.05 was considered statistically significant and the data analysis was conducted with the SPSS (version 11.5) software package.

4. Results

The demographic and clinical features of the case and control subjects included in this study are presented on Table 1. A total of 153 patients suffered from stroke (51.34 ± 13.7 y) and 153 control subjects (50.38 ± 12.494 y) were investigated. The calculated P-values for gender and diabetes mellitus were 0.900 and 1.000, respectively. The case and control groups were also matched for low-density lipoprotein ($P = 0.816$), high-density lipoprotein ($P = 0.323$), triglyceride ($P = 0.846$), and total cholesterol ($P = 0.0790$). None of the participants were AA homozygous for this FV c.1691 G>A mutation, but 6 heterozygous subjects were detected (4 AG heterozygous in the stroke group and 2 in the control group) (Table 2). As for FII g.20210G > A, none of the study subjects were AG heterozygous for this variation and only 1 AA homozygote subject was detected in the control group. The low frequencies of FV c.1691 G > A and FII g.20210G > A gene variants in our study population limits the power to detect a significant association with stroke in the present study (Table 3).

Table 1. The Demographic and Clinical Features of the Case Group and Control Group (n = 153)^a

Clinical Features	Case Group	Control Group	P Value
Age	51.3 ± 13.7	50.3 ± 12.4	0.522
Gender			0.907
Female	60	62	
Male	93	91	
Diabetes mellitus	40 (26.1)	41 (26.7)	1.000
LDL cholesterol, mg/dL	126.3 ± 33.7	125.4 ± 32.5	0.816
HDL cholesterol, mg/dL	40.7 ± 9.5	41.7 ± 9.0	0.323
Triglyceride, mg/dL	14.04 ± 93.0	142.3 ± 80.9	0.846
Total cholesterol, mg/dL	184.4 ± 45.0	193.0 ± 40.0	0.079

^a Data are presented as No. (%) or Mean ± SD.**Table 2.** The Mutation and the Allele Frequency for FV Leiden in Control Group and Stroke Patients^a

Group	Number of Patients	Factor V Leiden c.1691 G > A genotype				Allele Frequency	
		GG	AG	AA	P Value	G	A
Control	153	98.7	1.3	0.0	0.405	99.35	0.65
Stroke	153	97.4	2.6	0.0		98.7	1.3

^a Data are presented as %.**Table 3.** The Mutation and the Allele Frequency for Prothrombin in Control Group and Stroke Patients^a

Group	Number of Patients	Prothrombin g.20210G > A genotype, %				Allele Frequency, %	
		GG	AG	AA	P Value	G	A
Control	153	99.3	0.0	0.7	0.238	99.3	0.7
Stroke	153	100	0.0	0.0		100	0.0

^a Data are presented as %.

5. Discussion

The results of the present study is in agreement with some previous large studies, including prospective, case-control, and meta-analysis studies (18-22). FV c.1691 G > A, which is considered as an activated protein C resistance, is by far the most common form of familiar thrombophilia. Thrombophilia is defined as a hypercoagulability status leading to increased trend towards coagulation. Overall, individuals who are heterozygous for FV have a 5- to 10-fold increased risk of venous thromboembolism (VTE) and those who are homozygous for FV have a 50- to 100-fold increased risk of VTE (23-25). The causal relationship between FV c.1691 G > A and arterial thrombosis like stroke is controversial, most likely because of the limitation in patient recruitment methods. Studies like ours, recruited patients from consecutive neurology referrals or hospitalizations ('unselected' stroke studies). In most of these 'unselected' stroke studies no significant association has been reported between FVL c.1691 G > A and stroke (26-31). Some studies recruited cases from a subset

of patients referred to a laboratory for thrombophilia testing ('selected' stroke studies) (32-34). These studies were enriched by the presence of a predisposing suspicion of prothrombotic status. Another type of 'selected' stroke study is those which recruited cases from a subset of patients who had either cryptogenic strokes or criteria suggestive of cryptogenic strokes ('selected' stroke studies) (35-37). In both types of 'selected' stroke studies a significant association of FV with stroke has been reported. A recent 2011 meta-analysis (38) of 18 case-control studies of FV and ischemic stroke in young adults demonstrated an overall positive association of this genetic variation with stroke. Of 18 studies in this meta-analysis, 5 recruited cases from a subset of patients who were referred to a laboratory for thrombophilic work-up and 4 recruited cases from a subset of patients who had either cryptogenic strokes or criteria suggestive of cryptogenic strokes ('selected' stroke studies). The remaining 9 studies recruited patients from consecutive neurology referrals or hospi-

talizations ('unselected' stroke studies). However, when this meta-analysis was limited to 'unselected' stroke studies, the association was no longer significant. The results of our study are in an agreement with the remaining 9 studies in the above mentioned meta-analysis.

Some studies demonstrate that the combination of a FV allele and one or more traditional stroke risk factors such as age, sex, and using oral contraceptives was associated with increased risk for stroke. FV was associated with more than 3-fold increase in risk for stroke in women younger than 45-50 years (39). In studies by Slooter et al. (29) and Martinelli et al. (33), women younger than 50 years were investigated. However, in our study the mean age of studied subjects is around 50. The age difference might account for discrepancies in the results. Our findings demonstrate that Factor V c.1691 G > A genotype distribution was not significantly different between the stroke patients and control subjects (P value = 0.405) and a total of 4 FV AG heterozygous in stroke patients and 2 AG heterozygous in control subjects were observed, but none of the studied subjects were AA homozygous for FV the gene mutation. The FII g.20210G > A gene variant is the second most common inherited risk factor for thrombophilia. Although a 2-4 fold higher risk for venous thrombosis has been shown to correlate with FII g.20210G > A, its functional contribution in the development of arterial vascular disease, including ischemic stroke and myocardial infarction, is not clear (19, 21). It appears that the difference in the methods of patient recruitment accounts for the results discrepancy. In our study, we recruited the patients from unselected adult populations of consecutive neurology referrals or hospitalizations and as it was mentioned earlier, no cryptogenic stroke subtypes was among them.

To the best of our knowledge, this is the first study to examine the association between FIIg.20210G > A variant and stroke in an Iranian population from the North-eastern part of the country. Of 153 stroke patients and 153 controls, none were AG heterozygous for the prothrombin g.20210G > A variant and only one AA homozygote individual was detected in the control group. We did not find any association between FII g.20210G > A variant and stroke in our case and control groups. This is consistent with many large studies that recruited their patients from unselected adult populations (20, 22). In these studies unlike ours, patients were mostly recruited from selected populations. Age is another conventional risk factor that plays an important role in investigating the association of FII g.20210G > A with Stroke. In two separate studies, De Stefano et al. (40) and Aznar et al. (39) found that FII g.20210G > A was significantly more common in the stroke cases than controls. However, unlike our study, in both studies, the stroke patients were younger than 50 years.

To summarize, our findings demonstrate that there are no meaningful associations between either FV c.1691 G > A or FII g.20210G > A variant and stroke in an Irani-

an population with a high incidence rate of stroke. The prevalence of FV and FII g.20210G > A are basically population based. Especially for FV, a significant variation in prevalence can occur within ethnically different populations even within the same country. Iran is an ethnically diverse country. To determine the exact role of FV and FII variations with stroke disease, more epidemiological studies should be conducted.

Authors' Contributions

Study concept and design: Ariane Sadr Nabavi; analysis and interpretation of data: Ariane Sadr-Nabavi, Javad Hami and Maryam Pirhoushiaran; drafting of the manuscript: Maryam Pirhoushiaran; clinical Analysis: Payam Sasan nezhad and Mahmood Reza Azarpazhooh; patient sampling and laboratory work: Mohammad Reza Ghaseemi, Javad Hami and Peyman Zargari.

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