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FII G20210A and FV Leiden G1691A Polymorphisms in Patients with Atherothrom botic and Cardioembolic Ischemic Stroke from Ukraine.

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ABSTRACT

Factor V Leiden and prothrombin G20210A mutations are the most common inherited thrombophilias that predispose to thrombotic events which can result in ischemic stroke development. These mutations lead to increased blood coagulation due to elevated plasma prothrombin levels and increased coagulation factor V stability because of its resistance to inactivation by Protein C. A tendency toward a higher frequency of FII G20210A heterozygotes in atherothrombotic ischemic stroke patients (8 %) compared with cardioembolic ischemic stroke patients (2 %) and donors (0 %) was observed. We also observed that FV Leiden heterozygotes are more frequent in cardioembolic stroke. 3 (6 %) and 0 individuals with FV G1691A heterozygous mutations were found among cardioembolic stroke patients and in patients with atherothrombotic stroke, respectively. However, no significant difference between both study groups and controls was found. Both ischemic stroke subtypes were accompanied by elevated prothrombin pool and decreased protein C activity.

KEYWORDS: atherothrombotic ischemic stroke; cardioembolic ischemic stroke; genetic polymorphism; Factor II; Factor V; Leiden mutation.

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INTRODUCTION

Stroke is the second leading cause of death worldwide and in Ukraine. About 87 % of strokes are ischemic, the rest being hemorrhagic. The most common subtypes of ischemic stroke are atherothrombotic (34 %) and cardioembolic (22 %) stroke [1, 2]. Stroke is often caused by disorders of coagulation like thrombophilia or hypercoagulability [3]. Coagulation factors II (prothrombin, FII) and V (FV) are among of the key components of blood clotting. Under the influence of activated prothrombinase (FXa), which forms a complex with activated factor V (FVa) and calcium ions, prothrombin (FII) is converted to thrombin (FIIa), which stimulates the conversion of fibrinogen (FI) to fibrin (FIa) following by the formation of a blood clot [4].

Thrombophilia is a pathological condition that is characterized by abnormal blood coagulation that increases the risk of thrombosis. This condition can be either inherited or acquired through situations such as surgery, cancer, pregnancy or certain medications. Deficiencies in the natural anticoagulants, protein C, protein S, and antithrombin may result in either hereditary or acquired thrombophilia. The two most common hereditary thrombophilia conditions are activated protein C (APC) resistance (FV Leiden mutation) and FII (prothrombin) G20210A mutation [5].

FII G20210A polymorphism involves a guanine to adenine substitution at position 20210 located in the 3'-untranslated region of the FII gene. Heterozygote (GA) and mutant homozygote (AA) variants cause elevated plasma prothrombin levels, possibly due to increased pre-mRNA stability. As prothrombin is the precursor to thrombin, which plays a key role in causing blood coagulation, G20120A makes a contribution to hypercoagulability state [6].

FV G1691A polymorphism or Leiden mutation is located in exon 10 and involves a specific guanine to adenine substitution at nucleotide 1691 in the FV gene, which predicts the substitution of glutamine for arginine at the Arg506 activated Protein C cleavage site. Because of this single amino acid substitution, Factor Va is resistant to activated Protein C and is inactivated at a 10-fold slower rate than normal, resulting in increased thrombin generation [7].

Since there is not enough data on molecular-genetic aspects of stroke subtypes in Ukraine, we conducted a case-control study to examine whether there were different distributions of alleles and genotypes of coagulation factors FII (prothrombin) G20210A polymorphism and FV G1691A polymorphism (Leiden mutation) in Ukrainian patients with atherothrombotic ischemic stroke, cardioembolic ischemic stroke and atrial fibrillation (AF) and healthy donors without previous history of stroke. Laboratory screening for protein C activity and prothrombin pool was also performed.

MATERIALS AND METHODS

Groups

122 patients with acute ischemic stroke (mean age 73, 62 ± 8,9 years, range 43 to 91) and 40 healthy donors without previous history of stroke were enrolled in this study. All the study participants came from Ukraine and had similar socioeconomic and ethnic backgrounds. Stroke patients were admitted to the 1st and 2nd neurological units of Kyiv City Hospital № 4. Depending on the stroke subtype, patients were divided into 2 groups: atherothrombotic ischemic stroke patients and cardioembolic ischemic stroke patients with AF.

Diagnosis

The diagnosis of ischemic stroke was confirmed by neuroimaging (X-ray CT or brain MRI). Cardioembolic ischemic stroke was diagnosed by the presence of a constant and/or paroxysmal form of atrial fibrillation and/or acute myocardial infarction. The diagnosis of atrial fibrillation was confirmed by the electrocardiogram or according to the hospital records made before the stroke attack. Myocardial infarction was also confirmed by the electrocardiogram as a postinfarction cardiosclerosis. The study did not include patients in coma, patients with severe respiratory failure or suspected cancer. All patients received a single orally administered dose of aspirin (325 mg) within 24 hours after admission. The investigation was approved by Institutional Human Ethics Committee and written informed consent was obtained from all donors and patients or their relatives.

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DNA extraction

Blood samples were collected from both patients and donors by venepuncture into vacutainer tube with EDTA. Total genomic DNA was extracted from peripheral blood lymphocytes by the phenol-chloroform extraction. Extracted DNA samples were frozen at -20ºC until further assessment. FII G20210A and FV G1691A genotyping was performed as previously described [8]. PCR products were analyzed by electrophoresis in 2% agarose gel. Statistical analysis of the results was performed using Statistica 6.0 program by chi-square and Exact Fisher tests. A P-value of less than 0.05 was considered statistically significant.

Prothrombin pool measurement was performed in citrated blood plasma by ELISA [9]. Anti-prothrombin rabbit polyclonal antibodies (Shijin International, Mongolia) and o-phenylenediamine dihydrochloride substrate were used.

Protein C activity assay was performed in citrated blood plasma using protein C activator, chromogenic substrate and lyophilized calibration plasma supplied by "Renam", Republic of Belarus.

RESULTS AND DISCUSSION

We have performed molecular-genetic analysis of polymorphic variants of FII (G20210A) and FV (G1691A, Leiden mutation) genes in both study and control groups. The genotyping data for all case patients and controls is shown in Table 1.

Table 1: Genotypes and allele frequencies for polymorphisms in the FII and FV genes in both study and control groups.

Group Genotypes and allels	Patients with cardioembolic ischemic stroke and atrial fibrillation, n=54	Patients with atherothrombotic ischemic stroke, n=60	Control group (donors), n=40
FII (G20210A) Genotypes, n (%)			
GG	53 (98)	55 (92)	40 (100)
GA	1 (2)	5 (8)	0 (0)
AA	0 (0)	0 (0)	0 (0)
Allels (%)			
G	99	96	100
A	1	4	0
FV (G1691A, Leiden mutation) Genotypes, n (%)			
GG	51 (94)	60 (100)	38 (95)
GA	3 (6)	0 (0)	2 (5)
AA	0 (0)	0 (0)	0 (0)
Allels (%)			
G	97	100	98
A	3	0	2

The allele and genotype frequencies were consistent with those predicted by Hardy-Weinberg equilibrium. Genotype distributions and allele frequencies of FII G20210A or FV G1691A polymorphisms were not significantly different between both study groups and controls. However, we observed a tendency toward a higher frequency of FII G20210A heterozygotes in atherothrombotic ischemic stroke patients compared with cardioembolic ischemic stroke patients and AF. 1 (2%) and 5 (8%) individuals with heterozygous mutation of FII G20210A were found in cardioembolic stroke patients with AF and in patients with atherothrombotic stroke, respectively. We also observed that FV Leiden heterozygotes are more frequent in cardioembolic stroke with AF. 3 (6%) and 0 individuals with FV G1691A heterozygous mutations were found among cardioembolic stroke patients with AF and in patients with atherothrombotic stroke, respectively. In control group, 0 FII and 2 (5%)

FV heterozygous mutations were found. No individuals with FII A1691 and FV A1691 homozygous mutations were found in both study groups.

In the present study, we also investigated plasma prothrombin pool and protein C activity in all study groups. The results are shown in Table 2.

Table 2: Plasma prothrombin pool and protein C activity in both study and control groups.

Group Biochemical markers	Patients with cardioembolic ischemic stroke and atrial fibrillation	Patients with atherothrombotic ischemic stroke	Control group (donors), n=40
prothrombin pool, units	0,79±0,03 (n=56)	0,69±0,03 (n=66)	0,63±0,02
protein C activity, %	67,8 ± 19,1 (n=40)	70,1 ± 22,9 (n=33)	100 ± 5

Patients with both subtypes of stroke had increased prothrombin pool, which confirmed the development of prothrombotic state. In atherothrombotic stroke patients prothrombin level was elevated by 10%, while in cardioembolic ischemic stroke patients - by 25% relative to donors. We have also shown that protein C activity in 72% of atherothrombotic ischemic stroke patients was reduced to 70,1 ± 22,9%, whereas 85% of cardioembolic ischemic stroke patients had protein C activity of 67,8 ± 19,1%. Donor rate was at the level of 100 ± 5%.

CONCLUSIONS

Our findings suggest that there is a tendency toward a higher frequency of FII G20210A heterozygotes in atherothrombotic ischemic stroke patients compared to cardioembolic ischemic stroke patients and AF. We also observed that FV Leiden heterozygotes are more frequent in cardioembolic stroke with AF. Both subtypes of ischemic stroke were accompanied by elevated prothrombin pool and decreased protein C activity, however patients with cardioembolic ischemic stroke and atrial fibrillation had more warning prognosis.

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