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THE ROLE OF CLASSIC RISK FACTORS AND PROTHROMBOTIC FACTOR GENE MUTATIONS IN ISCHEMIC STROKE RISK DEVELOPMENT IN YOUNG AND MIDDLE-AGED INDIVIDUALS

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ABSTRACT

Background. In young individuals, a genetically predisposing hypercoagulability and classic modifying risk factors can act synergistically on the ischemic stroke risk development. The aim of the study was to compare the prevalence of classic vascular risk factors and polymorphisms of the G20210A coagulation factor II (prothrombin), Arg506Glu coagulation factor V Leiden, C677T methylenetetrahydrofolate reductase (MTHFR) and 4G/5G plasminogen activator inhibitor-1 (PAI-1) and the impact of these gene mutations and classic vascular risk factors on the overall stroke risk in individuals aged <55.

Methods. The study included 155 stroke patients aged ≤55 and 150 control subjects. Stroke prevalence and odds ratio (OR) were assessed for the following parameters: G20210A prothrombin, Arg506Glu factor V Leiden, C677T MTHFR and 4G/5G PAI-1 polymorphisms; total number of study polymorphisms in a particular subject (genetic sum), and classic vascular risk factors of hypertension, obesity, diabetes mellitus, cigarette smoking, hypercholesterolemia, hypertriglyceridemia, and elevated levels of LDL-cholesterol and VLDL-cholesterol.

Results. The prevalence of hypertension ($p<0.001$), smoking ($p<0.001$), decreased HDL-cholesterol levels ($p<0.001$), obesity ($p=0.001$), elevated LDL-cholesterol ($p=0.036$), C677T MTHFR polymorphism ($p<0.001$) and genetic sum was significantly higher in the group of stroke patients. The following parameters were found to act as independent risk factors for ischemic stroke: decreased HDL-cholesterol level ($p<0.001$; OR 4.618; 95%CI 2.381-8.957); hypertension ($p=0.001$; OR 2.839; 95%CI 1.519-5.305); obesity ($p=0.040$; OR 2.148; 95%CI

1.036-4.457); smoking (p=0.001; OR 2.502; 95%CI 1.436-4.359); and genetic sum as a continuous variable (p<0.01; OR 2.307; 95%CI 1.638-3.250).

Conclusions. Gene mutations of the procoagulable and proatherosclerotic factors investigated exerted a synergistic action in the development of overall risk of ischemic stroke in young and middle-aged individuals.

Introduction

Although ischemic stroke occurs mainly in elderly individuals aged ≥65, nowadays it is increasingly found in young individuals, even in children. According to various epidemiological studies, the incidence of stroke in subjects aged <55 ranges from 7 to 11 per 100,000 [1,2].

In most cases, ischemic stroke is a multifactorial disease with genetic predisposition, while the inherited risk is likely to be multigenic [3-5]. Research results suggest that the genetic predisposition might primarily influence the etiopathogenesis of premature ischemic stroke in young and middle-aged individuals [6-8].

Recent studies have confirmed the role of the prothrombotic factor gene mutations in the development of venous thrombosis, whereas their impact on the occurrence of arterial events has not yet been clarified. The role of different prothrombotic and proatherogenic polymorphisms in the pathogenesis of ischemic stroke has been investigated [9-13]. Among them, the G20210A polymorphism of the prothrombin gene, the G1691A polymorphism of the factor V gene, the C677T polymorphism of the methylentetrahydrofolate reductase (MTHDR) gene and the 4G/5G polymorphism of the plasminogen activator inhibitor-1 (PAI-1) are the most frequently studied. Some studies failed, but others did demonstrate the association of these polymorphisms with the risk of ischemic stroke [6-12]. Results from these studies address the weak effect of single genetic marker on the stroke risk development;

it might be stronger in younger individuals and in mutually cumulative interactions of different polymorphisms. To date, we are aware of just few studies that have investigated cumulative effect of these prothrombotic and proatherogenic polymorphisms on the stroke risk development [6,10].

The aim of the present study was to determine and compare the prevalence of classic vascular risk factors and polymorphisms of the G20210A coagulation factor II, Arg506Glu coagulation factor V Leiden, C677T MTHFR and 4G/5G plasminogen activator inhibitor-1 in the group of younger stroke patients and age- and sex-matched control group. In addition, the impact of these gene mutations and classic vascular risk factors on the overall stroke risk in individuals aged <55 was assessed.

Subjects and Methods

This prospective study was conducted at University Department of Neurology, Sestre milosrdnice University Hospital Center in Zagreb, Croatia, from June 2009 to January 2012.

Study protocol was consistent with ethical principles of medical profession and was approved by the Ethics committee Sestre milosrdnice University Hospital Center. All study subjects were informed on all the procedures to be used during the study and provided their written informed consent on participation in the study.

The study included 155 successive ischemic stroke patients younger than 55 and 150 age- and sex-matched control subjects. Clinical diagnosis of ischemic stroke was based on the computed tomography (CT) and magnetic resonance (MR) evidence for cerebral infarct.

Complete demographic and history data on age, sex and classic vascular risk factors for stroke, including hypertension, diabetes mellitus, cigarette smoking, hyperlipidemia and body weight were collected from both stroke patients and control subjects. Hypertension was determined on the basis of systolic blood pressure greater than 160 mm Hg or/and diastolic

blood pressure greater than 95 mm Hg measured at two time points at least two weeks after acute phase of ischemic stroke, or if the patient had been on antihypertensive therapy before inclusion in the study. Diabetes mellitus was diagnosed according to the World Health Organization (WHO) criteria [14]. According to smoking habits, study subjects were divided into two groups of current smokers and nonsmokers, the latter including those that had ceased smoking more than six months before. Hypercholesterolemia was defined as cholesterol levels exceeding 5 mmol/L or taking cholesterol lowering agents. Decreased HDL-cholesterol levels were defined as <1.2 mmol/L in female and <1.0 mmol/L in male subjects. Elevated LDL-cholesterol levels were defined as >3.0 mmol/L. Elevated triglyceride levels were defined as >1.7 mmol/L. Body mass index (BMI; body weight divided by square body height) was calculated in all study subjects, classifying them into three groups of normal weight (BMI <25), overweight (BMI 25-29.99) and obese (BMI >30) subjects.

Study patients underwent brain CT, color Doppler sonography and transcranial Doppler of carotid arteries, brain MR, cerebral MR panangiography, electrocardiography, lung x-ray, transthoracic and/or transesophageal echocardiography, and urine biochemistry. Blood samples were obtained for determination of complete blood count (CBC), erythrocyte sedimentation rate (ESR), coagulation tests and fibrinogen, blood glucose, lipidogram, and coagulation factors II and V, MTHFR and PAI-1 polymorphisms.

To investigate any potential association between the studied polymorphisms and specific subtypes of infarct, we created modified Trial of ORG 10172 in Acute Stroke Treatment classification accommodated for stroke in the young; accordingly patients were divided in five major etiologic categories: (1) atherosclerosis; (2) cardiac embolism; (3) cervical artery dissection; (4) other known, rare causes and (5) cryptogenic stroke.

Control group included 150 subjects treated for vertebrogenic disorders as part of the cervicobrachial and lumbosacral syndrome at Pain Clinic, University Department of

Neurology, Sestre milosrdnice University Hospital Center from June 2009 to January 2012. Control subjects were age- and sex-matched to ischemic stroke patients, had no known history of vascular or thromboembolic disease, and had normal neurologic status. All study subjects were Caucasians, originating from the same geographical area, and were of comparable social status.

Venous blood samples were obtained from both stroke patients and control subjects for molecular diagnosis and determination of coagulation factor II, coagulation factor V, PAI-1 and MTHFR polymorphisms. All study polymorphisms were determined by the standardized polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Total number of classic vascular risk factors (range 0-8) and total number of gene polymorphisms under study (range 0-4) as genetic sum were calculated in each study subject.

The normality of numerical data distribution was tested by Kolmogorov-Smirnov test. As most of the numerical data did not follow normal distribution, nonparametric statistical tests were employed in the analysis.

Mann-Whitney test was used to compare numerical data between two independent groups of patients and control subjects. Kruskal-Wallis test was used to compare more than two independent groups of numerical data divided according to the genetic sum into five groups. In case of statistically significant difference, multiple testing of particular group pairs was performed using Mann-Whitney test with Bonferroni correction for multiple testing.

Categorical characteristics were tested by χ^2-test whenever possible; otherwise, Fisher exact test was employed. In addition to testing the existence of associations, the strength of associations was assessed using odds ratio with the corresponding 95% confidence interval (95%CI).

Ischemic stroke prediction with the potential classic and genetic predictors and their interactions were analyzed by use of multivariate logistic regression as follows: a wider set of predictors defined by clinical and statistical evaluation, included in the initial model of logistic regression. All predictors at the level of statistical significance of $p<0.2$ were chosen from the initial model of logistic regression. Then, nonsignificant predictors at the level of statistical significance of $p<0.05$ were excluded from the logistic model. The potentially final model of logistic regression was analyzed for the possible interactions and impact of all excluded predictors, so that every excluded predictor was reintroduced in the model to analyze its impact on the significance of predictors in the potentially final model. If a previously excluded predictor modified the significance of chosen predictors by more than 20%, the excluded predictor was returned to the final model.

The Microsoft Excel 2003 software was used on data processing and R Version 2.10.1 software on statistical analysis.

Results

The study included 305 subjects in total, i.e. 155 ischemic stroke patients and 150 control subjects. There were no statistically significant between-group age ($p=0.121$; Mann-Whitney test) and sex ($p=0.778$; χ^2-test) differences.

Patient/subject distribution according to the presence of classic vascular stroke risk factors and studied polymorphisms is illustrated in Table 1. Hypertension, current smoking, decreased HDL-cholesterol, obesity ($p=0.001$; χ^2-test all) and elevated LDL-cholesterol ($p=0.036$; χ^2-test) were statistically significantly more frequently recorded in the group of ischemic stroke patients. A statistically significant between-group difference was found in the distribution of MTHFR genotype ($p<0.001$; χ^2-test).

There was a statistically significant between-group difference in the total number of study polymorphisms (genetic sum) in individual subjects ($p<0.001$; Mann-Whitney test) (Table 2). Concurrence of all four single nucleotide polymorphisms under study was not recorded in any of the study patients/control subjects.

There were 99 (63,87%) patients with established cause of stroke: atherosclerotic vasculopathy in 46 (29,67%) patients, cardiac embolism in 30 (19,36%) patients, cervical artery dissection in 13 (8,39%) patients, other rare causes of stroke were established in 10 patients (6,45%); while in 56 (36,13%) patients cause of stroke remains undetermined and they formed a group of cryptogenic stroke. Patients with cryptogenic stroke were somewhat younger, but the difference is on the border of statistical significance ($p=0,050$; Kruskal-Wallis test).

Hypertension ($p<0,001$; χ^2-test), current smoking ($p<0,001$; χ^2-test), obesity ($p=0.001$; χ^2-test), elevated LDL-cholesterol ($p=0.036$; χ^2-test) and decreased HLD-cholesterol ($p<0,001$; χ^2-test) were statistically significantly more frequently recorded in the group of noncryptogenic stroke patients. There was a statistically significant between-group difference in the total number of classic vascular risk factors ($p=0,002$; Mann-Whitney test) and in the distribution of MTHFR ($p<0.001$; χ^2-test) and PAI-1 genotype ($p<0.001$; χ^2-test). There was a statistically significant between-group difference in the total number of study polymorphisms (genetic sum) in individual subjects ($p<0.001$; χ^2-test) (table 3).

The following variables were included in the initial model of multivariate logistic regression: age, sex, prothrombin G20210A, factor V Leiden Arg506Glu, MTHFR C677T and PAI-1 4G/5G polymorphisms, genetic sum = 3, genetic sum = 1, genetic sum = 2, elevated total cholesterol, decreased HDL-cholesterol, elevated LDL-cholesterol, elevated triglycerides, hypertension, obesity, diabetes and cigarette smoking as potential predictors of the ischemic stroke risk significance.

Based on the initial logistic regression with the criterion of variable significance in the model of >0.2, the second model of logistic regression was designed in two versions. The following variables were included in model version (A): age, prothrombin G20210A, factor V Leiden Arg506Glu, MTHFR C677T and PAI-1 4G/5G polymorphisms, elevated total cholesterol, decreased HDL-cholesterol, hypertension, obesity and smoking. The other model version (B), following the same procedure, included the variables of age, elevated total cholesterol, decreased HDL-cholesterol, hypertension, obesity, smoking, and genetic sum as a continuous variable instead of particular polymorphisms. Classification accuracy at the univariate level of 72.1% and 71.5%, respectively, was determined for both versions of the second multivariate logistic model. The model was statistically significant ($p<0.001$).

Based on the final logistic model results, nonsignificant variables were excluded; these were elevated total cholesterol levels, prothrombin G20210A polymorphism, factor V Leiden Arg506Glu polymorphism and PAI-1 4G/5G polymorphism in the (A) version, and elevated total cholesterol and obesity in the (B) version of the second multivariate logistic model. As either particular polymorphisms or genetic sum were included in the model of multivariate logistic regression, and the genetic sum proved to be superior to particular polymorphisms as a predictor, the latter was left in the final model. The MTHFR C677T polymorphism was not included in the final model due to nonsignificance of other polymorphisms making the whole of prediction system; therefore, inclusion of the genetic sum was concluded to be more useful. Since the MTHFR C677T polymorphism plays a role in the genetic sum formation, their concurrence in the model would pose a problem of multi-colinearity and possible separation of nonsignificant predictors as significant ones with their taking over the predictive role.

The final model of logistic regression of the risk factor significance in the development of ischemic stroke included the following variables: age, decreased HDL-cholesterol, hypertension, obesity, cigarette smoking, and genetic sum as a continuous

variable (Table 4). This multivariate logistic model yielded a classification accuracy of 69.8%. The model was statistically significant ($p<0.001$). The impact of excluded variables on the final regression model was assessed by a series of statistical testing for each individual variable, and their contribution to model modification did not exceed 20% of the statistical significance (p value). The suspicion of the possible effect of these variables was thus dissipated and there was no need to return them in the final model.

Discussion

Our study results indicated the classic vascular risk factors of elevated LDL-cholesterol, cigarette smoking, hypertension, decreased HDL-cholesterol and obesity to be statistically significantly more common in the group of young and middle-aged ischemic stroke patients. A very similar profile of classic vascular risk factors in young ischemic stroke patients has also been reported elsewhere, however, differing in the order of frequency [3-7,15-19]. These results show that conventional vascular risk factors are significant risk factors for ischemic stroke not only in the >55 age group, but also in younger population.

Like most other studies, the present study was a pair study with the candidate gene concept. A drawback of the studies employing such a methodology always lies in fear that positive associations may be apparent and negative results a consequence of inadequate statistical power.

In the present study, it was only the MTHFR C677T polymorphism that proved to be an independent risk factor for ischemic stroke in multivariate logistic regression; however, as genetic sum was superior to particular polymorphisms in predicting ischemic stroke, the MTHFR C677T polymorphism was not included in the final model due to nonsignificance of other polymorphisms constituting a unified prediction system. Other studies of the MTHFR C677T polymorphism association with ischemic stroke have produced contradictory results.

Most of the studies found mild yet statistically significant association with ischemic stroke, and it seems that the risk increase was mainly mediated by elevated homocysteine levels [10,20].

Multivariate logistic regression confirmed the genetic sum as a continuous variable to be a statistically significant and independent risk factor for ischemic stroke (OR 2.315; 95%CI 1.643-3.260). This result is important for confirming the hypothesis on the synergistic gene interplay. Similar analyses have rarely been reported in published studies. Pezzini *et al*. found that odds ratio for ischemic stroke was 1.73 with 95%CI 1.20-2.51 in subjects with one prothrombotic polymorphism and 3.00 with 95%CI 1.43-6.30 in those with two or more polymorphisms [6].

The possibility of systematic error was obviated by double blind study protocol, so that the neurologist was blinded for the molecular diagnosis results on the study polymorphisms, whereas laboratory personnel were blinded for the rest of diagnostic work-up and classification of study subjects.

A drawback of this and other similar studies is the fact that it was a hospital based pair study. As the control group consisted of subjects treated at the same institution for other health problems, the possibility of systematic error in pair matching due to population complexity cannot be excluded. Yet, as the frequency of study polymorphisms recorded in control group corresponded to those in other groups of healthy individuals from a wider area of south-east Europe, we believe that the possible systematic error had no impact in our study.

Like most of the previous studies, our study results do not support genetic testing for susceptibility to hypercoagulability in unselected patients, but suggest its possible use and benefit in some younger age patients, primarily those where the etiology of ischemic stroke remains obscure even after comprehensive and thorough diagnostic work-up [21-24]. Our study pointed to the importance and impact of genetic burden, i.e. presence of multiple gene

polymorphisms in a particular patient, as an independent risk factor for overall ischemic stroke risk development. This opens the possibility of further research and use of novel preventive and therapeutic strategies in the management of ischemic stroke.

Conflict of interest: None.

References

1. Feigin VL, Lawes CMM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol. 2009;8:355-69.
2. European Registers of Stroke (EROS) Investigators, Heuschmann PU, Di Carlo A, Bejot Y, Rastenyte D, Ryglewicz D, Sarti C, Torrent M, Wolfe CD. Incidence of stroke in Europe at the beginning of the 21st century. Stroke. 2009;40:1557-63.
3. Putaala J, Metso AJ, Metso TM, Konkola N, Kraemer Y, Haapaniemi E, *et al*. Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke. The Helsinki Young Stroke Registry. Stroke. 2009;40:1195-203.
4. Spengos K, Vemmos K. Risk factors, etiology, and outcome of first-ever ischemic stroke in young adults aged 15 to 45 – the Athens Young Stroke Registry. Eur J Neurol. 2010;17:1358-64.
5. Bi Q, Wang L, Li X, Song Z. Risk factors and treatment of stroke in Chinese young adults. Neurol Res. 2010;32:366-70.
6. Pezzini A, Grassi M, Del Zotto E, Archetti S, Spezi R, Vergani V, *et al*. Cumulative effect of predisposing genotypes and their interaction with modifiable factors on the risk of ischemic stroke in young adults. Stroke. 2005;36:533-9.

7. Pezzini A, Grassi M, Del Zotto E, Lodigiani C, Ferrazzi P, Spalloni A, *et al*. Common genetic markers and prediction of recurrent events after ischemic stroke in young adults. Neurology. 2009;73:717-23.
8. Calabrò RS, La Spina P, Serra S, Laganà A, Postorino P, Savica R, *et al*. Prevalence of prothrombotic polymorphisms in a selected cohort of cryptogenic and noncryptogenic ischemic stroke patients. Neurol India. 2009;57:636-7.
9. Moskau S, Smolka K, Semmler A, Schweichel D, Harbrecht U, Müller J, *et al*. Common genetic coagulation variants are not associated with ischemic stroke in a case-control study. Neurol Res. 2010;32:519-22.
10. Weber R, Goertler M, Benemann J, Diener HC, Weimar C, German Stroke Study Collaboration. Prognosis after cryptogenic cerebral ischemia in patients with coagulopathies. Cerebrovasc Dis. 2009;28:611-7.
11. Ariyaratnam R, Casas JP, Whittaker J, Smeeth L, Hingorani AD, Sharma P. Genetics of ischaemic stroke among persons of non-European descent: a meta-analysis of eight genes involving approximately 32,500 individuals. PLoS Med. 2007;4:e131.
12. Xin XY, Song YY, Ma JF, Fan CN, Ding JQ, Yang GY, Chen SD. Gene polymorphisms and risk of adult early-onset ischemic stroke: a meta-analysis. Thromb Res. 2009;124:619-24.
13. Wang X, Cheng S, Brophy VH, Erlich HA, Mannhalter C, Berger K, *et al*.; RMS Stroke SNP Consortium. A meta-analysis of candidate gene polymorphisms and ischemic stroke in 6 study populations: association of lymphotoxin-alpha in nonhypertensive patients. Stroke. 2009;40:683-95.
14. WHO Study Group on Diabetes Mellitus. World Health Organization Technical Report, Series 727. Geneva, Switzerland: World Health Organization; 1985.

15. Brouns R, Thijs V, Eyskens F, Van den Broeck M, Belachew S, Van Broeckhoven C, *et al*.; BeFaS Investigators. Belgian Fabry Study: prevalence of Fabry disease in a cohort of 1000 young patients with cerebrovascular disease. Stroke. 2010;41:863-8.
16. Janssen AW, de Leeuw FE, Janssen MC. Risk factors for ischemic stroke and transient ischemic attack in patients under age 50. J Thromb Thrombolysis. 2011;31:85-91.
17. Putaala J, Yesilot N, Waje-Andreassen U, Pitkäniemi J, Vassilopoulou S, Nardi K, *et al*. Demographic and geographic vascular risk factor differences in European young adults with ischemic stroke: the 15 Cities Young Stroke Study. Stroke. 2012;43:2624-30.
18. Isordia-Salas I, Barinagarrementería-Aldatz F, Leaños-Miranda A, Borrayo-Sánchez G, Vela-Ojeda J, García-Chávez J, *et al*. The C677T polymorphism of the methylenetetrahydrofolate reductase gene is associated with idiopathic ischemic stroke in the young Mexican-Mestizo population. Cerebrovasc Dis. 2010;29:454-9.
19. Babu MS, Prabha TS, Kaul S, Al-Hazzani A, Shafi G, Roy S, *et al*. Association of genetic variants of fibrinolytic system with stroke and stroke subtypes. Gene. 2012;495:76-80.
20. Dragoni F, Chiarotti F, Rosano G, Simioni P, Tormene D, Mazzucconi MG, Cafolla A, Avvisati G. Thrombophilic screening in young patients (<40 years) with idiopathic ischemic stroke: a controlled study. Thromb Res. 2011;127:85-90.
21. Hamedani AG, Cole JW, Cheng Y, Sparks MJ, O'Connell JR, Stine OC, *et al*. Factor V Leiden and ischemic stroke risk: the Genetics of Early Onset Stroke (GEOS) Study. J Stroke Cerebrovasc Dis. 2011 Nov 17. Epub ahead of print
22. Naess H, Tatlisumak T, Kõrv J. Stroke in the young 2012. Stroke Res Treat. 2012;2012:656913. Epub 2012 Sep 5.

23. Putaala J, Yesilot N, Waje-Andreassen U, Pitkäniemi J, Vassilopoulou S, Nardi K, et al. Demographic and geographic vascular risk factor differences in European young adults with ischemic stroke: the 15 cities young stroke study. Stroke. 2012;43:2624-30.

24. Rolfs A, Fazekas F, Grittner U, Dichgans M, Martus P, Holzhausen M, et al.; Stroke in Young Fabry Patients (sifap) Investigators. Acute cerebrovascular disease in the young: the Stroke in Young Fabry Patients study. Stroke. 2013;44:340-9.

Table 1. Distribution of classic vascular risk factors and studied polymorphisms in ischemic stroke patients and control subjects

Risk factor/studied polymorphisms		Ischemic stroke patients		Control subjects		p-value	OR	95%CI
		n	%	n	%			
Hypertension		58	37.4	28	18.7	**p<0.001**	2.604	1.543-4.405
Obesity		31	20.0	17	11.3	**p=0.001**	1.957	1.031-3.704
Diabetes		12	3.9	9	3.0	p=0.548	1.314	0.537-3.215
Smoking		70	45.2	36	24.0	**p<0.001**	2.611	1.597-4.255
Elevated total cholesterol		97	62.6	79	52.7	p=0.080	1.504	0.952-2.375
Elevated LDL-cholesterol		99	63.9	78	52.0	**p=0.036**	1.631	1.032-2.577
Decreased HDL-cholesterol		55	35.5	19	12.7	**p<0.001**	3.792	2.117-6.792
Elevated triglycerides		57	37.0	51	34.0	p=0.583	1.140	0.713-1.825
F II	GG	148	95.5	148	98.7	p=0.174	3.496	0.715-17.241
	GA	7	4.5	2	1.3			
	AA	0	0	0	0			
F V	GG	141	91.0	145	96.7	p=0.40	2.882	1.010-8.197
	GA	12	7.7	5	3.3			
	AA	2	1.3	0	0			
MTHFR	CC	41	26.5	75	50	**p<0.001**	2.778	1.721-4.484
	CT	78	50.3	59	39.3			
	TT	36	23.2	16	10.7			
PAI-1	5G/5G	60	38.7	76	50.7	p=0.060	1.626	1.032-2.564
	4G/5G	51	32.9	46	30.7			
	4G/4G	44	28.4	28	18.7			

F II = prothrombin; F V = coagulation factor V Leiden; MTHFR = methylenetetrahydrofolate reductase; PAI-1 = plasminogen activator inhibitor-1; GG = genotype guanine guanine; GA = genotype guanine adenine; AA = genotype adenine adenine; CC = genotype cytosine cytosine; CT = genotype cytosine thymine;

TT = genotype thymine thymine; 5G = genotype 5 guanine in a sequence; 4G = genotype 4 guanine in a sequence

Table 2. Total number of study factor polymorphisms (genetic sum) in individual ischemic stroke patients and control subjects

Genetic sum	Ischemic stroke patients		Control subjects	
	n	%	n	%
0	15	9.7	40	26.7
1	64	41.3	65	43.3
2	62	40.0	44	29.3
3	14	9.0	1	0.7
4	0	0	0	0
$p<0.001$				

Table 3. Total number of study factor polymorphisms (genetic sum) in group of patients with non-cryptogenic stroke, cryptogenic stroke and control group.

Genetic sum	Patients				Control subjects (n=150)	
	Non-crpytogenic stroke (n=99)		Cryptogenic stroke (n=56)			
	n	%	n	%	n	%
0	14	14,1	1	1,8	40	26,7
1	49	49,5	15	26,8	65	43,3
2	31	31,3	31	55,4	44	29,3
3	5	5,1	9	16,1	1	0,7
p<0,001						

Table 4. Final model of logistic regression of the risk factor significance in ischemic stroke development

Variable	p	OR	95%CI	
			Lower	Upper
Age	0.028	0.967	0.938	0.996
Decreased HDL-cholesterol	0.000	4.618	2.381	8.957
Hypertension	0.001	2.839	1.519	5.305
Obesity	0.040	2.148	1.036	4.457
Smoking	0.001	2.502	1.436	4.359
Genetic sum	0.000	2.307	1.638	3.250
Constant	0.253	0.478		

OR = odds ratio; 95%CI = 95% confidence interval