Original Article

Bosnian Study on Markers of Ischaemic Stroke in Adults 20–50 Years Old (SMISAO): Preliminary Report

(stroke / young adults / risk factors / SNPs)

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Abstract. Previous research suggested that several genetic polymorphisms are associated with increased risk of ischaemic stroke (IS) in young adults. However, the predictive biomarkers of IS in young adults are still unclear. Our aim was to assess the contribution of modifiable and genetic factors in IS in young adults. In total, 40 stroke patients and 40 healthy controls aged 20 to 50 years were recruited. Data on modifiable factors were collected, then participants were genotyped for seven SNPs linked to thrombophilia: *ACE* rs1799752, *PAI-1* rs1799889, *APOE* rs1412 and rs429358, *FV* rs6025 and rs1800595, and *FII* rs62623459. Significantly increased risk factors: hypertension and dyslipidaemia in stroke patients compared with the controls: 50.0 % *vs* 27.5 % and 75.0 % *vs* 40.0% (P = 0.039 and P = 0.002, respectively) were observed. Stroke patients compared with controls did not differ in distribution of *ACE, APOE*, *FV*, and *FII* variants. The 4G4G homozygotes of the *PAI-1* gene were significantly more prevalent in stroke patients compared to the controls: 42.5 % *vs* 17.5 %, (P = 0.033). In the group with the small vessel occlusion subtype of stroke, statistically significant overrepresentation of 4G4G homozygotes and frequency of the 4G allele compared with controls: 57.1 % *vs* 17.5 % and 0.7 *vs* 0.45 (P = 0.026 and P = 0.03, respectively) were observed. Independent predictors of stroke incident were: dyslipidaemia (OR (95% CI) = 4.2 (1.4–12.4)) and 4G4G genotype (OR (95% CI) = 3.9 (1.1–13.7)). These results confirm the contribution of dyslipidaemia and 4G4G genotype in the increased risk of IS in young Bosnian adults.

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Abbreviations: ACC – American College of Cardiology, ACSM – American College of Sports Medicine, AHA – American Heart Association, ASA – American Stroke Association, B&H – Bosnia and Herzegovina, BMI – body mass index, CI – confidence interval, HWE – Hardy-Weinberg equilibrium, IQR – interquartile range, IS – ischaemic stroke, MED – median, NIHSS – National Institutes of Health Stroke Scale, OR – odds ratio, PFO – patent foramen ovale, PCR – polymerase chain reaction, SD – standard deviation, SNPs – single-nucleotide polymorphisms, TEE – transoesophageal echocardiography, TIA – transient ischaemic attack, TOAST – trial of ORG 10172 in acute stroke treatment, TTE – transthoracic echocardiography.

Introduction

Large studies have observed an expanding rise in prevalence of standard modifiable risk factors at younger ages, and so far, research on the role of heredity in the development of cerebrovascular diseases has revealed a number of clinical conditions that may be linked to the genetic background and interaction with variable risk factors including environmental factors (Putaala et al., 2012; von Sarnowski et al., 2013; Cheng et al., 2014; Supanc et al., 2014; Feigin et al., 2016).

Although genetic factors of thrombophilia are quite well known and linked to venous thromboembolism and

pregnancy loss, their role in arterial ischaemic stroke (IS) remains unclear. Several SNPs (single-nucleotide polymorphisms), e.g., I/D *ACE* (rs1799752), −675 I/D, 4G5G *PAI-1* (rs1799889), *APOE* (rs1412 and rs429358), 1691G > A and 4070A > G *FV* (rs6025 and rs1800595, respectively), and 20210G > A *FII* (rs1799963) have been linked to arterial thrombotic events. It has been suggested that the above-mentioned genetic variants may contribute to thrombus formation by increasing the levels of prothrombotic factors and decreasing the levels of inhibitors, and consequently lead to hypercoagulability (Opstad et al., 2010; Campo et al., 2013; Turfan et al., 2014). On the one hand, the 2018 American Heart Association/American Stroke Association (AHA/ASA) clinical practice guideline recommends against thrombophilia testing in patients with IS (Cox et al., 2017; Powers et al., 2018; Chiasakul et al., 2019). On the other hand, testing the genetic factors of thrombophilia in clinical practice remains common, because some of the published results suggest that thrombophilia factors may contribute to IS (Cox et al., 2017; Powers et al., 2018; Chiasakul et al., 2019).

Thus, we sought to assess the potential association between the above-mentioned SNPs, modifiable factors and ischaemic stroke in young subjects from Bosnia and Herzegovina (B&H).

Material and Methods

Subjects

This prospective case-control study was initiated in 2018 at the Neurology Clinic in Sarajevo. All study participants provided written informed consent before the enrollment. We consecutively recruited 40 patients, mean age 40.5 (± 7.6) years, with clinical symptoms of first or recurrent IS. The control group consisted of 40 healthy subjects, mean age 41.8 (± 5.5) years. The IS subtypes were classified according to the published criteria in the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) (Adams et al., 1993). Stroke location included vascular territory of: anterior, medial, posterior, and vertebrobasilar cerebral circulation. Inclusion criteria were: age 20–50 years and ischaemic cerebrovascular insult confirmed by computerized tomography or magnetic resonance. Patients with haemorrhagic stroke, cerebral venous sinus thrombosis, previous history of immunological, thyroid diseases, neoplastic process, and active infectious or inflammatory diseases were excluded.

Medical examination and survey of drinking

In both groups, the examination process consisted of: anamnesis, objective physical and neurological examination. Furthermore, dyslipidaemia, diabetes, cardiovascular disease, atrial fibrillation, hypertension, body mass index (BMI) and physical activity, use of oral contraceptives, smoking, alcohol consumption, and drug abuse were examined.

Severity of stroke according to the National Institutes of Health Stroke scale (NIHSS) was assessed within 24 h upon admission to the clinic and at the time of discharge from the hospital. Dyslipidaemia and hypertension were defined according to the American College of Cardiology (ACC)/ AHA (Carey and Whelton, 2018; Grundy et al., 2019).

Body mass index (BMI) was calculated as a weight divided by height squared (kg/m^2), and BMI ≥ 30 was defined as obesity. To assess the physical activities, AHA or the American College of Sports Medicine (ACSM) ranges were used as follows: activity < 30 min per day; 30 min daily; daily activities for 20 min, three per week (e.g., walking, fast walking, cycling) (Haskell et al., 2007). Smoking status was categorized into: current, former, or never smoker. Regular drinking was defined as a consumption of at least three drinks per week (National Institute on Alcohol Abuse and Alcoholism, 2010).

All procedures performed in the studies involving human participants were conducted according to the standards of the Declaration of Helsinki (1975, revised 2000). The study was approved by the Local Ethics Committee (decision ref. No. 0302-27982). Informed consent was obtained from all individual participants included in the study.

DNA extraction and genotyping

A QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from buccal swabs. The extraction was performed according to the manufacturer's instructions, and following DNA isolation, polymerase chain reactions (PCR) of selected polymorphisms were performed. The protocol for 4070 A > G *FV* (rs1800595) followed the previously described PCR-RFLP (restriction fragment length polymorphism) technique by Poort et al. (1996), and I/D *ACE* (rs1799752) followed the previously described PCR technique by Glenn et al. (2009). The above-mentioned SNP genotyping was performed in a Hightech Thermocycler Cycler-Technology for Life (Senso Quest, Gottingen, Germany). Note that in the studied population, we found overrepresentation of DD homozygotes. Therefore, an independent person verified all DD homozygotes using a previously described technique by Lindpaintner et al. (1995) for the presence of the insertion allele (I). The same results as previously were obtained. Then, two *APOE* SNPs: TGC → CGC (rs429358) and CGC → TGC (rs7412); and 20210G > A *FII* (rs62623459) and 1691 G > A *FV* (rs6025) were determined by real-time PCR using TaqMan® SNP Genotyping Assays (Assay ID: C_3084793_20, C_904973, C_64636124_10, and C_11975250_10, respectively) (Thermo Fisher Scientific, Waltham, MA). The −675 I/D, 4G5G *PAI-1* (rs1799752) was determined by a separate Genotyping PCR Kit, Gene Proof (Imogena, Brno, Czech Republic). For quality control purposes, approximately 10 % of the samples were re-genotyped in a blinded fashion and the same results were obtained. For genotyping by real-time PCR, a LightCycler®96 was

used, and data were analysed with LightCycler®96 Software v.1.1.0.1320 (Roche Diagnostics, Warsaw, Poland).

Statistical analysis

All statistical calculations were performed with the SPSS 19 software (version 19.0, SPSS Inc, Chicago, Illinois, IL). Each value was expressed as the mean standard deviation (±SD), median (MED) and interquartile range (IQR), or absolute number and corresponding percentages. The distributions of variables were tested for deviations from Gaussian distribution by the Shapiro-Wilk test. The differences between two groups were analysed by the Mann-Whitney test or *t*-test depending on data distribution. Significant changes in the NIHS score from admission to discharge between different *PAI-1* genotypes were tested with the Kruskal-Wallis test.

The allelic frequencies were estimated by gene counting, and genotypes were scored. The observed numbers of each genotype were compared with the expected numbers for a population in Hardy-Weinberg equilibrium (HWE) by using a Chi-square (χ^2) test. The significance of the differences of observed alleles and genotypes between the groups was tested using the χ^2 or Fisher exact test. Differences in subject characteristics among the genotypes were tested with ANOVA or Kruskal-Wallis test (continuous variables), where appropriate, or χ^2 test (categorical variables).

A logistic regression analysis was applied to identify predictors of stroke. Baseline and clinical parameters and genotypes that were associated with stroke in univariate analysis were accounted in regression analysis and their odds ratio (OR) with corresponding 95% confidence intervals (95% CI) were presented. P values < 0.05 were considered statistically significant.

Results

In the presented study, stroke subjects compared with the controls had significantly higher frequency of hypertension: 50.0 % *vs* 27.5 % and hyperlipidaemia: 75.0 % *vs* 40.0 % (P = 0.039 and P = 0.002, respectively). No significant differences in age, gender, family history, and obesity or lipid levels between the stroke subjects and controls were observed. Characteristics of stroke subjects and controls are shown in Table 1.

In our study, the most common stroke subtype was a stroke of undetermined aetiology (N = 19; 47.5 %), followed by linked to small vessel occlusion (N = 14; 35.0 %), large-artery atherosclerosis (N = 4; 10.0 %), cardioembolism (N = 1; 2.5 %) and other aetiology (N = 2; 5.0 %). The most common stroke localization was left (N = 22; 55.0 %) and right (N = 17; 42.5 %) hemisphere, and most affected vascular territory was middle cerebral artery (N = 27; 67.5 %) (Table 2).

Table 1. Baseline characteristics of stroke subjects and controls

Characteristic		Stroke patients, N = 40	Controls, N = 40	P value
Age, years		40.5 ± 7.6	41.8 ± 5.5	0.37
Gender (M), n (%)		19 (47.5)	23 (57.5)	0.37
Smoking, n (%)		25 (62.5)	20 (50.0)	0.26
Alcohol, n (%)		12 (30.0)	13 (32.5)	0.81
Positive family history, n (%)		14 (35.0)	7 (17.5)	0.075
Physical activity, n (%)	< 30 min/day	6 (15.0)	1 (2.5)	0.14
	30 min/day	20 (50.0)	20 (50.0)	
	3× weekly	14 (35.0)	19 (47.0)	
Marital status (married), n (%)		29 (72.5)	37 (92.5)	**0.019**
Hypertension, n (%)		20 (50.0)	11 (27.5)	**0.039**
Hyperlipidaemia n (%)		30 (75.0)	16 (40.0)	**0.002**
Diabetes mellitus, n (%)		1 (2.5)	0	
Heart disease, n (%)		3 (7.5)	0	
Oral contraceptives, n (%)		5 (12.5)	0	
Antiphospholipid syndrome, n (%)		5 (12.5)	0	
Atrial fibrillation, n (%)		4 (10.0)	0	
Patent foramen ovale, n (%)		1 (2.5)	0	
BMI (kg/m^2)		25.5 [23.5–28.5]	24.5 [23.6–28.2]	0.58
Cholesterol (mmol/l)		5.4 [4.5–6.2]	4.8 [4.5–5.2]	0.07
Triglycerides (mmol/l)		1.9 [1.5–2.7]	1.6 [1.4–2.4]	0.15
LDLc (mmol/l)		3.4 [2.5–3.9]	2.9 [2.6–3.6]	0.20

↑ Statistically significant P values (P < 0.05) are bolded

Table 2. Clinical characteristics of stroke subjects

Characteristic	Stroke patients N = 40	
TOAST	Stroke of other determined aetiology, n (%)	2 (5.0)
	Stroke of undetermined aetiology, n (%)	19 (47.5)
	Large-artery atherosclerosis, n (%)	4 (10.0)
	Small-vessel occlusion, n (%)	14 (35.0)
	Cardioembolism, n (%)	1 (2.5)
Previous TIA, N (%)		9 (22.5)
Localization	Right hemisphere, n (%)	17 (42.5)
	Left hemisphere, n (%)	22 (55.0)
	Cerebellum, n (%)	3 (7.5)
	Brain stem, n (%)	6 (15.0)
Vascular territory	Anterior cerebral artery, n (%)	4 (10.0)
	Middle cerebral artery, n (%)	27 (67.5)
	Posterior cerebral artery, n (%)	3 (7.5)
	Vertebrobasilar artery, n (%)	9 (22.5)
Number of lesions (SD)		1.76 ± 0.88
NIHSS score at admission		4.0 [1.25–4.75]
NIHSS score at discharge		1.5 [0.0–3.75]

Distributions of genotypes: *ACE* rs1799752, *PAI-1* rs1799889, *FV* rs6025, and *FII* rs62623459 in stroke patients were not in a state of HWE ($\chi^2 = 5.8$, P = 0.016; $\chi^2 = 4.3$, P = 0.037; $\chi^2 = 21$, P < 0.001 and $\chi^2 = 36$, P < 0.001, respectively).

Our results did not show any significant differences between the stroke patients and controls in the prevalence of genotypes or allele distributions: rs1799752 *ACE*, rs1412 and rs429358 *APOE*, rs6025 and rs1800595 *FV,* and rs62623459 *FII* (each P > 0.05) (Table 3).

However, the homozygous 4G *PAI-1* gene occurred significantly more often in stroke patients compared to the controls, 42.5 % *vs* 17.5 % (P = 0.033). Moreover, when cases were grouped according to the stroke subtype, overrepresentation of 4G homozygotes in the small vessel occlusion subtype of stroke compared with the controls, 57.1 % *vs* 17.5 % (P = 0.026), was observed. Additionally, the frequency of the 4G allele was significantly higher in the small vessel occlusion group compared with controls, 0.7 % *vs* 0.45 % (P = 0.03). Since other stroke subtypes occurred with very low frequency, those were not included in the analysis. However, we did not find significant differences in the prevalence of rs1799752 *PAI-1* genotypes compared with baseline and clinical characteristics of stroke patients (Table 4).

Logistic regression analysis was performed in order to examine which factors were significant predictors of stroke. The model was statistically significant ($\chi^2 = 24.0$; P < 0.001) and could explain between 26.0 % (R2 Cox and Snell) and 35.0 % (R2 Nagelkerke) of results' variance and correctly classify 72.5 % of cases. Independent positive predictors of incident stroke were dyslipidaemia (OR (95% CI) = 4.2 (1.4–12.4)) and 4G4G genotype (OR (95% CI) = 3.9 (1.1–13.7)) (Table 5).

Discussion

Our results showed that the vascular risk factors with statistical significance in stroke were hypertension and dyslipidaemia, but only dyslipidaemia was identified as an independent positive predictor of IS (OR (95 % CI) = 4.2 (1.4–12.4)). Other significant differences between the stroke patients and controls were not found, which confirms the findings of other researchers (Putaala et al., 2009; Aigner et al., 2017; Ekker et al., 2018). Moreover, other authors report that the prevalence of modifiable vascular risk factors in young adults tends to increase, i.a.: hypertension (4–11 %), hypercholesterolaemia (12–21 %), diabetes mellitus (4–7 %), smoking (5–16 %), and obesity (4–9 %) (von Sarnowski et al., 2013; George et al., 2017). Many vascular risk factors may often coexist together, leading to an exponential increase in stroke risk (Ji et al., 2013).

In the presented study, the most common stroke subtype was a stroke of undetermined aetiology (N = 19; 47.5 %), followed by linked to small vessel occlusion (N = 14; 35.0 %), large-artery atherosclerosis (N = 4; 10.0 %), cardioembolism (N = 1; 2.5 %) and other aetiology (N = 2; 5.0 %) (Table 2).

Smajlovic et al. (2013) showed that 4 % of Bosnian patients with cerebrovascular insult aged 18–45 years (N = 3864) had a cerebrovascular insult with the highest prevalence of lacunar stroke (26.6 % *vs.* 16.1 %, P = 0.01). Croatian authors reported cryptogenic stroke in 36.13 % (N = 56) of young patients, while most of their

Table 3. ACE, PAI-1, APOE, FV, FII and allele frequencies in stroke patients and controls

Polymorphism/ Reference sequence	Genotype/ Allele	Stroke n, 40	Controls n, 40	P value[T]	Stroke of undetermined aetiology n, 19	Small-vessel occlusion n, 14	P value
I/D *ACE* genotype n (%)	DD	18, 45 %	17, 42.5 %	0.07	9, 47.4 %	5, 35.7 %	*0.35 **0.3
	ID	22, 55 %	18, 45.0 %		10, 52.6 %	9, 64.3 %	
	II	0	5, 12.5 %		0	0	
I/D *ACE* allele n (%)	D	58, 0.73	52, 0.65	0.39	28, 0.74	19, 0.68	NS
	I	22, 0.27	28, 0.35		10, 0.26	9, 0.32	
PAI-1 genotype n (%)	4G4G	17, 42.5 %	7, 17.5 %	**0.033**	9, 47.4 %	8, 57.1 %	*0.057 **0.026
	4G5G	13, 32. 5%	22, 55.0 %		6, 31.6 %	4, 28.6 %	
	5G5G	10, 25.0 %	11, 27.5 %		4, 21.1 %	2, 14.3 %	
PAI-1 allele n (%)	4G	47, 0.59	36, 0.45	0.11	24, 0.63	20, 0.70	*0.08 **P = 0.03
	5G	33, 0.41	44, 0.55		14, 0.37	8, 0.30	
APOE genotype n (%)	E2E2/E2E3	3, 7.5 %	7, 17.5 %	0.41	0	0	*0.16 **0.23
	E3E3	31, 77.5 %	26, 65.0 %		15, 78.9 %	12, 85.7 %	
	E3E4/E4E4	6, 16.0 %	7, 17.5 %		4, 21.1 %	2, 14.2 %	
APOE allele n (%)	E2	4, 0.05	9, 0.11	0.38	0	0	*P = 0.07 **P = 0.2
	E3	68, 0.85	64, 0.80		33, 0.87	25, 0.89	
	E4	8, 0.10	7, 0.09		5, 0.13	3, 0.11	
16910 G>A *FV* genotype n (%)	GA	6, 15.0 %	5, 12.5 %	0.75	2, 10.5 %	4, 28.6 %	*NS **0.22
	GG	34, 85.0 %	35, 87.5 %		17, 89.5	10, 71.4 %	
16910 G>A *FV* allele n (%)	G	74, 0.93	75, 0.94	NS	36, 0.95	24, 0.86	*NS **0.23
	A	6, 0.07	5, 0.06		2, 0.05	4, 0.14	
4070A>G *FV* genotype n (%)	AA	36, 90.0 %	33, 82.5 %	0.52	17, 89.5 %	12, 85.7 %	NS
	GA	4, 10.0 %	6, 15.0 %		2, 10.5	2, 14.3 %	
	GG	0	1, 2.5%		0	0	
4070A>G *FV* allele n (%)	A	76, 0.95	72, 0.90	0.37	36, 0.95	26, 0.93	NS
	G	4, 0.50	8, 0.10		2, 0.05	2, 0.07	
20210 G>A *FII* genotype n (%)	GA	1, 2.5 %	0	NS	0	1, 7.1 %	NS
	GG	39, 97.5 %	40, 100 %		19, 100 %	13, 92.9 %	
20210 G>A *FII* allele n (%)	A	1, 0.01	0	NS	0	1, 0.04	NS
	G	79, 0.99	80, 1		38, 1	27, 0.96	

NS – not significant; [T] – comparison between stroke patients and controls, * between stroke of undetermined aetiology and controls, ** between small-vessel occlusion stroke and controls; statistically significant P values (P < 0. 05) are bolded.

stroke cases were due to atherosclerotic disease (N = 99; 63.87 %) (Supanc et al., 2014). Unlike them, we found surprisingly low prevalence of cervical artery dissection as a leading cause of stroke of other determined aetiology (N = 2; 5.0 %) (Table 2). Furthermore, the prevalence of cardioembolic stroke in our study was lower than reported in neighbouring populations, and we detected only a single case of patent foramen ovale (PFO) (N = 1; 2.5 %), which indicates a need for larger use of transoesophageal echocardiography (TEE) rather than transthoracic echocardiography (TTE) (Jovanovic et al., 2008; Supanc et al., 2014).

Recently published results suggest that genetic factors may also contribute to the risk of stroke, i.a., by their interaction with vascular risk factors (Hu et al., 2017; Omran et al., 2019). However, the relationship between genetic factors and stroke, especially in young adults, has not been fully explained (Konialis et al., 2016; Pahus et al., 2016; Wei et al., 2017).

In 2019, Omran et al. (2019) published a retrospective cohort study of subjects aged 18–65 years and reported that two out of five young subjects with IS who underwent thrombophilia screening had at least one positive test, but none of the studied clinical factors or stroke subtypes were associated with a prothrombotic state. Other authors in a retrospective review of 171 patients with stroke and 189 transient ischaemic attack ≤ 60 years (genetic tests included *FV* and *FII*) suggested that thrombophilia testing was positive in 50 (14 %) subjects, of whom 24 were < 50 years. Positive results were found in 36 (10 %) with acute IS, 4 (1 %) with haemorrhagic stroke and 10 (3 %) with transient ischaemic attack (Alakbarzade et al., 2018). Danish authors performed a population study on 685 subjects, and posi-

Table 4. Baseline and clinical characteristics of stroke subjects with different PAI-1 genotypes

Characteristic		4G4G, n = 17	4G5G, n = 13	5G5G, n = 10	P value
Smoking (n = 25)	No, n (%)	5 (33.3)	7 (46.7)	3 (20.0)	0.33
	Yes, n (%)	12 (48.5)	6 (24.0)	7 (28.0)	
Hypertension (n = 20)	No, n (%)	9 (45.0)	5 (25.0)	6 (30.0)	0.54
	Yes, n (%)	8 (40)	8 (40)	4 (20)	
Dyslipidaemia n = 30)	No, n (%)	4 (40)	4 (40)	2 (40)	0.80
	Yes, n (%)	13 (43.3)	9 (30.0)	8 (26.7)	
Positive family history (n = 14)	No, n (%)	11 (42.3)	10 (38.5)	5 (19.5)	0.40
	Yes, n (%)	6 (42.9)	3 (21.4)	5 (35.7)	
Cholesterol (mmol/l)		5.1 [4.5–6.1]	4.8 [4.3–5.4]	5.3 [4.3–5.4]	0.36
Triglycerides (mmol/l)		1.6 [1.2–2.8]	1.8 [1.5–2.6]	1.7 [1.4–2.5]	0.74
LDLc (mmol/l)		3.1 [2.7–3.9]	2.9 [2.5–3.7]	3.4 [2.7–3.8]	0.60
BMI (kg/m^2)		24.9 [23.8–28.2]	24.5 [23.4–28.3]	25.6 [23.6–28.9]	0.57
Previous TIA	No, n (%)	12 (38.7)	11 (35.5)	8 (25.8)	0.64
	Yes, n (%)	5 (55.6)	2 (22.2)	2 (22.2)	
Previous stroke	No, n (%)	14 (45.2)	11 (35.5)	6 (19.4)	0.30
	Yes, n (%)	3 (33.3)	2 (22.2)	4 (44.4)	
Number of lesions (SD)		1.7 ± 0.8	1.8 ± 0.8	1.8 ± 1.0	0.85
NIHSS score at admission		4.0 [2.0–6–0]	2.0 [0.0–4.0]	4.0 [2.8–6.0]	0.20
NIHSS score at discharge		2.0 [0.0–4.0]	0.0 [0.0–3.5]	1.5 [0–0–4.5]	0.80
ΔNIHSS score from admission to discharge		−2.0 [−2.0–0.0]	0.0 [−2.0–0.0]	−1.5 [−3.5–0.0]	0.25

Table 5. Logistic regression analysis and risk factors independently associated with stroke

Model	B	Standard error	P value	OR	95% confidence interval
Hypertension	1.09	0.60	0.060	2.97	(0.95–9.30)
Dyslipidaemia	**1.42**	**0.60**	**0.012**	**4.13**	**(1.40–12.40)**
PA-1 4G4G genotype	**1.37**	**0.64**	**0.032**	**3.92**	**(1.10–13.70)**
Gender: males	−0.80	0.50	0.140	0.44	(0.15–1.30)
Dependent variable: stroke					

tive association only of transient ischaemic attack (TIA)/amaurosis fugax and heterozygotes *FV* rs6025 (12%) (OR (95 % CI) = 1.99 (1.14–3.28)) was observed (Pahus et al., 2016).

Because the *ACE* gene is a candidate for atherosclerotic-related diseases, similarly to our study, several authors investigated the association between *ACE* variants and IS. Isordia-Salas et al. (2019) reported results similar to ours and lack of association between the *ACE* variants in 224 Mexicans ≤ 45 years and idiopathic IS. Also, in 100 Greeks with cerebral infarction, no association between the *ACE* variants and the presence of IS was found (OR (95 % CI) = 0.874 (0.4–1.97)). Authors suggested to examine the possibility of an increased risk of IS in DD homozygotes (Karagiannis et al., 2004). Furthermore, in 531 Spaniards this relationship was not confirmed as well. Additionally, the authors reported that both ACE protein levels and activity did not differ between IS aetiologies (Domingues-Montanari et al., 2010).

Schellekens et al. (2018) studied, i.a., genetic prothrombotic factors *FV*, *FIII,* and *FVIII* and did not report any difference in the prevalence of prothrombotic factors in 18–50 years aged subjects with a cryptogenic stroke compared to the subjects with established cause. Additionally, the presence of any coagulation disorder was not associated with an increased risk of recurrent cerebral ischaemia (Schellekens et al., 2018).

So far published results with higher numbers of genetic risk factors, i.a., of thrombophilia, in the stroke predisposition are scarce; most often, authors study one to four SNPs. This study is the first report on the contribution of genetic risk factors of thrombophilia SNPs rs1799752, rs62623459, rs1412, rs429358, rs6025,

rs1800595, rs1799963 and IS in young Bosnian adults. Also, the authors investigated the association of the above-mentioned SNPs with the stroke subtype. We could not observe any differences in the distribution of rs1799752, rs1412, rs429358, rs6025, rs1800595, and rs62623459 between the stroke subjects and controls. When the cases were stratified according to the stroke subtype, the overrepresentation of 4G4G homozygotes in the small vessel occlusion subtype of IS was significantly higher compared with controls (57.1 % *vs* 17.5 %; P = 0.026), and the 4G allele frequency was significantly higher in the small vessel occlusion group compared with controls (0.7 *vs* 0.45; P = 0.03). Although patients with stroke of undetermined aetiology had higher frequency of the 4G4G genotype compared with controls, the difference did not reach statistical significance (P = 0.057).

The −675 ID, 4G/5G *PAI-1* polymorphism leading to a single insertion/deletion of a guanine base at position 675 in the promoter region of *PAl-1* gene results in two alleles, 4G and 5G, which impact the concentration of plasminogen activator inhibitor-1 (PAl-1). It is suggested that individuals who are homozygous for 4G4G have higher concentrations of PAI-1, with consequent increase in thrombotic susceptibility comparing with homo- and heterozygotes (5G5G and 4G5G, respectively) (Dawson et al., 1991; Francis, 2002; Sartori et al., 2003).

Several studies conducted in populations of non-European origin observed an association of the 4G4G genotype of the *PAI-1* gene and ischaemic stroke (Bang et al., 2001; Cao et al., 2014). Akhter et al. (2017) conducted a study on 100 young Indians with IS. They have confirmed that the 4G4G genotype is linked to higher levels of PAI-1 also in the population they have studied. Additionally, the 4G variant was significantly associated with IS in young Indians (Akhter et al., 2017). In our study, the relationship between 4G homozygotes and stroke was similar; unfortunately, we did not examine PAI-1 plasma levels. On the other hand, the relationship between *PAI-1* variants and PAI-1 levels in European populations has been confirmed.

Studies on the genetic factors of IS in European populations are scarce, but we found several publications. Swedish authors observed significantly higher frequency of the 4G4G genotype in elderly population with ischaemic stroke compared to controls (Wiklund et al., 2005). Furthermore, these authors proposed that the *PAI-1* 4G allele, in the presence of elevated serum triglycerides, may be associated with increased ischaemic stroke risk (Wiklund et al., 2005). On the other hand, Tasdemir et al. (2016) reported lack of association of the *PAI-1* gene polymorphism and ischaemic stroke in young Turkey population. Greek researchers carried out their study on *PAI-1*, *FV*, *FII*, *FXIII*, and *MTHFR* variants in 51 subjects with first-ever IS. Authors found that only the prevalence of 5G/5G homozygotes was higher in the control group. Therefore, they concluded that future studies to investigate the possible protective role of the 5G5G homozygotes are needed (Ranellou et al., 2015). Hungarian researchers performed trombophilia examination in 867 stroke patients and 743 healthy controls; only *APOE4* polymorphism (P < 0.0005) was more specifically associated with increased risk of ischaemic stroke (Szolnoki et al., 2003). Croatian authors explored genetic variants in young adults with ischaemic stroke similar to our research, and they observed lower prevalence of the 4G4G polymorphism compared to our findings, and no association of the 4G4G polymorphism or 4G allele with IS was found (Supanc et al., 2014). Serbian authors reported results similar to Croatians, but they pointed out that carriers of the 4G4G genotype were younger at the stroke occurrence compared with 4G5G and 5G5G genotypes, 67.38 ± 13.83 *vs* 70.85 ± 8.67 (P = 0.021) (Dusanović Pjevic et al., 2019). Unfortunately, we failed to examine the cumulative effect of our studied SNPs as it was performed by Croatian and Serbian researchers (Stankovic et al., 2010; Supanc et al., 2014).

In a large meta-analysis, the 4G4G polymorphism was more frequently observed in the group of patients with large-artery disease and small-artery disease than those with other types of ischaemic stroke (Attia et al., 2007). In a meta-analysis by Bentley et al. (2010), 187 studies including 37,481 stroke subjects were tested, and only *ACE* variants were more specifically associated with increased risk of lacunar stroke than with other types of IS.

In our study, no significant differences in the baseline and clinical characteristics of the stroke subjects according to different *PAI-1* genotypes were found.

Conclusions

To the best of our knowledge, this is the first study on the association between genetic risk factors of thrombophilia SNPs: rs1799752, rs1799889, rs1412, rs429358, rs6025, rs1800595, rs62623459 and IS in young Bosnian adults. Overall, our results confirm a significantly higher frequency of hypertension and dyslipidaemia in young adults with stroke, and contribution of 4G *PAI-1* homozygotes to increased risk of IS. A major limitation of the study is the small size of the investigated group. Therefore, these results are to be re-evaluated in subsequent studies with larger sample sizes to validate the results and test their clinical utility.

Disclosure of conflict of interest

The authors declare no conflict of interests.

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