Neurological Sciences (2019) 40:2565–2572
https://doi.org/10.1007/s10072-019-04006-y

ORIGINAL ARTICLE

Genetic and lifestyle predictors of ischemic stroke severity and outcome

Ivana Celap[1] · Nora Nikolac Gabaj[1] · Vida Demarin[2] · Vanja Basic Kes[2] · Ana-Maria Simundic[3,4]

Received: 11 March 2019 / Accepted: 8 July 2019 / Published online: 20 July 2019

Abstract

Background Different models that include clinical variables and blood markers have been investigated to predict acute ischemic stroke treatment course and recovery.

Aim The aim of the study was to investigate associations between lipid levels, lifestyle factors, hemostatic (F5, F2, SERPINE1, F13A1, and FGB), and atherogenic (APOA5 and ACE) gene variants and acute ischemic stroke (AIS) severity.

Materials and methods This study included 250 patients with AIS in which F5, F2, SERPINE1, F13A1, FGB, APOA5, and ACE genotypes were determined. Total cholesterol (TC), high-density cholesterol, low-density cholesterol, and triglycerides concentrations were measured within 24 h of the AIS onset. Examination of the neurological deficit was done using National Institutes of Health Stroke Scale/Score (NIHSS).

Results APOA5 genotype [TC + CC] was more frequent ($P = 0.026$) in patients with the NIHSS score ≥ 21. Univariate regression analysis has shown that triglycerides (OR 0.55, 95% CI 0.34–0.91; $P = 0.019$), obesity (0.28, 95% CI 0.10–0.73; $P = 0.010$), age (OR 1.08, 95% CI 1.04–1.13; $P < 0.001$), and APOA5 genotype (TC + CC) (OR 2.40, 95% CI 1.10–5.25; $P = 0.034$) are significantly associated with a severe stroke. When all variables were included in model age (OR 1.06, 95% CI 1.01–1.11; $P = 0.018$), obesity (OR 0.25, 95% CI 0.08–0.77; $P = 0.016$) and APOA5 genotype (TC + CC) (OR 3.26, 95% CI 1.29–8.23; $P = 0.012$) remained significant for the risk of severe AIS.

Conclusion APOA5 genotype (TC + CC), age, and obesity could be used as prognostic risk factors for a very severe stroke (NIHSS ≥ 21).

Keywords APOA5 variant · Stroke severity · Risk factors · Triglycerides

Background

In the past decades, treatment of patients with an acute ischemic stroke (AIS) has been improved resulting with a decreased number of deaths and an increased number of patients with a long-term post-stroke disability. Accordingly, many researchers have attempted to find a predictive model for AIS outcome. Ideally, the model should include reliable variables that will be measured early after AIS onset directing the course of treatment, and consequently, reducing the time of recovery and healthcare costs. Numerous clinical variables have been employed in the prediction of short- and long-term outcomes, mostly using different scoring systems based on physical examination and radiological imaging [1–3].

Age, time from stroke onset to treatment, severity of neurological deficit, infarct size, and localization have been

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-019-04006-y) contains supplementary material, which is available to authorized users.

✉ Ivana Celap
ivana.celap@gmail.com

[1] Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center, Zagreb, Croatia

[2] Department of Neurology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia

[3] Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, Croatia

[4] Department of Medical Biochemistry and Hematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

repeatedly reported as the most powerful predictors for stroke outcome [4–6]. Besides, different simple blood tests, such as glucose and lipid levels, that would help in AIS prognosis and outcome have been investigated for years but findings are inconsistent. In recent meta-analyses, increased risk of unfavorable AIS outcome in prediabetic and diabetic patients has been shown [7, 8]. On the other hand, studies that investigated the prognostic value of lipid levels have shown that short- and long-term outcomes of the AIS could be associated with triglycerides levels measured within 24 h of the AIS onset. However, results are inconsistent in terms of whether lower or higher triglycerides levels contribute to better outcomes [9, 10]. Similarly, contradictory findings have been reported for other lipid parameters, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) [11].

Genome-wide association studies have revealed a certain level of inheritability for AIS depending on stroke subtype and cardiovascular phenotype [12]. Since AIS is a complex disease, many studies have investigated combined effects of hemostatic genetic variants such as F5 (rs6025), F2 (rs1799963), SERPINE1 (rs1799768), F13A1 (rs5985), and FGB (rs1800790) as well as other variants involved in atherogenesis (i.e., in lipid metabolism APOA5 (rs662779)) and blood pressure ACE (rs4340) and the AIS [13–18]. Mostly due to negative associations between genetic variants and AIS onset, there are a limited number of studies that explored relationships of candidate gene variants and stroke outcome or severity [19–21].

Considering all of the above, we hypothesized that gene variants that alter hemostatic, metabolic, and vascular homeostasis could be associated with AIS severity and outcome. To test the hypothesis, we investigated the association of genetic variants involved in hemostatic (F5 (rs6025), F2 (rs1799963), SERPINE1 (rs1799768), F13A1 (rs5985), and FGB (rs1800790)) and atherogenic (APOA5 (rs662779) and ACE (rs4340)) pathways as well as blood lipid levels with acute ischemic stroke severity.

Materials and methods

Subjects

This cross-sectional observational study was conducted in the University Hospital Centre Sestre milosrdnice, Zagreb, Croatia, from 2009 to 2011. The study included 250 consecutive patients who were presented to the emergency unit of the Neurology Department within 24 h of the onset of the acute ischemic stroke. All of the patients were Caucasians of the Croatian origin. A consultant neurologist made the diagnosis of the acute ischemic stroke based on neurological examination, computed tomography, and medical history. Risk factors, specified according to National Stroke Association, were collected from the medical record of each patient. Risk factors data included lifestyle (smoking status, alcohol use, and obesity), medical (atrial fibrillation, hypertension, and diabetes mellitus) and uncontrollable factors (previous cerebrovascular and cardiovascular events, age, and sex).

Stroke type was determined according to Oxfordshire Community Stroke Project clinical classification (OCSP) and Trial of ORG 10172 in Acute Stroke Treatment (TOAST). According to OCSP, subjects were grouped in four categories: total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI), and posterior circulation infarct (POCI). Based on TOAST classification, patients were divided into five categories: large-artery disease (LAD), cardioembolic stroke, small-artery disease (SAD), stroke of other determined etiology, or stroke of undetermined etiology. At the admission to the emergency unit, the neurological deficit in each patient was obtained using National Institutes of Health Stroke Scale/Score (NIHSS).

Exclusion criteria were hemorrhagic stroke and transient ischemic attack as well as AIS with symptoms lasting longer than 24 h.

Sestre milosrdnice University Hospital Center ethics committee approved the study and all participants signed informed consent.

Samples

Blood samples for serum and DNA analysis were collected by venepunction in tubes without anticoagulant and with K_2EDTA (BD, Franklin Lake, NJ, USA), respectively. All samples were taken the next morning after admission to neurology intensive care unit (within 24 h of admission). After spontaneous blood clotting, serum samples were centrifuged at 1500*g* for 10 min and immediately analyzed. Isolated DNA samples were stored at + 4 °C until genotyping.

Methods

Determination of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (Tg), and glucose was done on biochemistry analyzer AU2700 (Beckman Coulter, Brea, CA, USA) using original reagents and manufacturer protocols.

DNA was extracted from EDTA blood samples using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). F5, F2, SERPINE1, F13A1, APOA5, and FGB genotypes were determined by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method, whereas ACE genotypes were done by PCR described by Saracevic et al. [22]. PCR and RFLP conditions are available in supplementary material. Within each run of samples, three

controls (wt/wt; wt/mt; mt/mt) and one blank sample were tested. Accuracy of F5, F2, SERPINE1, F13A1, and ACE methods was determined by participation in external quality assurance provided by DGKL (now Reference Institute for Bioanalytics), while for APOA5 and FGB methods, 10% of all samples were retested.

Statistical analysis

Kolmogorov–Smirnov test was used to determine the normality of distribution. Age is presented as median and range (min–max). Other results are presented as mean and standard deviation, median and interquartile range, or as counts and proportions where appropriate. The difference between the observed and expected genotype frequencies for Hardy–Weinberg equilibrium calculation and the distribution of genotypes according to TOAST, OCSP, and NIHSS were tested using chi-squared test or Fisher exact test depending on the number of genotypes. Univariate logistic regression analysis was used to identify those variables (selection criterion of $P < 0.05$) that were subjected to forward stepwise multivariate modeling. Values < 0.05 were considered significant. Statistical analysis was performed using the MedCalc® statistical software version 17.9.1 (MedCalc, Ostend, Belgium).

Results

Demographic data and biochemistry parameters in stroke patients are presented in Table 1. According to OCSP, majority of patients had LACI (34.8%) and TACI (37.6%) types of stroke. TOAST classification revealed that 34.7% of patients had SVD type of stroke. The most common risk factor in the studied group was hypertension (80.1%) followed by FA (27.9%), DM type 2 (27.0%), and obesity (25.8%).

Distribution of the studied genetic variants has shown no deviation from Hardy–Weinberg equation.

To assess the differences according to stroke severity, subjects were grouped using NIHSS as follows: in the first subgroup were subjects with a minor to moderately severe/severe stroke (NIHSS score 0–20), and in the second subgroup were subjects with a very severe stroke (NIHSS score 21–42) [23, 24]. Results revealed a statistically significant difference in the distribution of the APOA5 -1131T>C genotypes between subgroups ($P = 0.026$). Distribution of wildtype (T) and mutant (C) alleles of APOA5 variant between studied subgroups was not statistically significant ($P = 0.051$, data not presented). There were no significant differences in genotype distribution between subgroups for the rest of the studied variants (Table 2).

Univariate logistic regression analysis is done to identify predictors for very severe stroke (NIHSS ≥ 21). Results have shown that APOA5 genotype (TC + CC) [OR 2.40 (95% CI 1.10–5.25); $P = 0.034$], age [OR 1.08 (95% CI 1.04–1.13); $P < 0.001$], and CRP concentrations [OR 1.01 (95% CI 1.00–1.02); $P = 0.006$] are associated with a higher risk for a very severe stroke. Further, results revealed that F5 genotype [OR 2.6 × 10–9 (95% CI 0.00–0.00); $P = 0.047$], triglycerides level [OR 0.55 (95% CI 0.34–0.91); $P = 0.019$], and obesity [OR 0.28 (95% CI 0.10–0.73); $P = 0.010$] are associated with a lower risk for a very severe stroke. However, in multivariate logistic regression analysis adjusted for age and sex, only APOA5 genotype (TC + CC) [OR 3.26 (95% CI 1.29–8.23); $P = 0.012$], age [OR 1.06 (95% CI 1.01–1.11); $P = 0.018$], and

Table 1 Demographic data, types of stroke, and biochemistry parameters in the stroke patients ($N = 250$)

Variable		Stroke patients ($N = 250$)
Age		74 (35–92)
Male gender (*N*, %)		132 (52.8)
OCSP ($N = 242$)	LACI (*N*, %)	87 (34.8)
	TACI (*N*, %)	94 (37.6)
	PACI (*N*, %)	22 (8.8)
	POCI (*N*, %)	39 (15.6)
TOAST ($N = 245$)	LAD (*N*, %)	67 (27.3)
	Cardioembolic (*N*, %)	64 (26.1)
	SVD (*N*, %)	85 (34.7)
	Other determined etiology (*N*, %)	11 (4.5)
	Undetermined etiology (*N*, %)	18 (7.3)
NIHSS		15 (7–27)
History of FA (*N*, %)		67 (27.9)
History of hypertension (*N*, %)		202 (80.1)
Current smoking status (*N*, %)		31 (12.0)
BMI > 30 kg/m^2 (*N*, %)		63 (25.8)
History of AMI (*N*, %)		29 (11.8)
History of CVI (*N*, %)		48 (19.4)
History of DM type 2 (*N*, %)		67 (27.0)
History of alcohol use (*N*,%)		47 (17.0)
CRP (mg/L)		8.5 (7.4–11.9)
TC (mmol/L)		5.5 ± 1.3
Tg (mmol/L)		1.5 (1.4–1.6)
HDL-C (mmol/L)		1.3 ± 0.8
LDL-C (mmol/L)		3.4 (3.3–3.5)
Glucose (mmol/L)		7.0 (6.6–7.3)

OCSP, Oxfordshire Community Stroke Project; *LACI*, lacunar infarct; *TACI*, total anterior circulation infarct; *PACI*, partial anterior circulation infarct; *POCI*, posterior circulation infarct; *TOAST*, Trial of Org 10172 in Acute Stroke Treatment; *LAD*, large-artery disease; *SVD*, small vessel disease; *NIHSS*, National Institutes of Health Stroke Scale; *FA*, atrial fibrillation; *AIM*, acute myocardial infarction; *DM type 2*, diabetes mellitus type 2; *CRP*, C-reactive protein; *TC*, total cholesterol; *Tg*, triglycerides; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol

Table 2 Distribution of genotypes according to NIHSS subgroups

	Genotype	NIHSS < 21 ($N = 201$)	NIHSS ≥ 21 ($N = 48$)	P
F2	GG	196	47	1.000
	GA	5	1	
F5	GG	192	48	0.213
	GA	9	0	
F13A1	VV	106	31	0.319
	VL	66	13	
	LL	19	4	
FGB	GG	100	28	0.452
	GA	82	17	
	AA	13	3	
SERPINE1	5G5G	44	14	0.870
	5G4G	91	17	
	4G4G	60	17	
ACE	II	41	6	0.341
	ID	98	27	
	DD	57	15	
APOA5	TT	168	35	*0.026*
	TC+CC*	24	12	

NIHSS, National Institutes Health Standard Score; *F2*, factor 2; *F5*, factor 5; *FGB*, β-fibrinogen; *SERPINE1*, serine protease inhibitor E1; *F13A1*, factor 13 subunit A1; *ACE*, angiotensin-converting enzyme; *APOA5*, apolipoprotein A5

*Since only one mutant homozygous genotype (CC) was obtained, to test the genotype distribution between subgroups dominant model was used (TT vs TC + CC)

obesity [OR 0.25 (95% CI 0.08–0.77); $P = 0.016$] remained associated with a stroke severity (Table 3).

Further, we have tested the distribution of the studied genetic variants according to OCSP and TOAST classifications. Results have shown a statistically significant difference in the distribution of F5 Leiden genotypes between OCSP classification groups ($P = 0.025$), while the difference was not found for other studied genotypes (Table 4). However, we assume that this difference is a result of no GA genotypes in 2 groups. Thus, we have not performed further analysis. Similarly, there was no statistically significant difference in genotype distribution between groups according to TOAST classification (Table 5).

Discussion

In this study, we investigated the association of the AIS severity and conventional risk factors, such as age, sex, FA, DM, hypertension and use of tobacco, lipid parameters, CRP and F5, F2, F13A1, FGB, SERPINE1, ACE, and APOA5 variants.

The major finding in our study is that C allele carriers (TC+CC genotype) of rs662779 (APOA5 -1131T>C) variant have 3.26 times higher odds for a very severe stroke than non-carriers (TT genotype) [OR 3.26 (95 CI% 1.29–8.23); $P = 0.012$]. APOA5 -1131T>C variant has been associated with higher lipid levels, particularly triglycerides, that are shown to be a risk factor for atherosclerosis and stroke [25–27]. In fact, it has been reported that APOA5 -1131T>C decreases apolipoprotein A5 levels resulting in decreased triglycerides catabolism and consequent hypertriglyceridemia. Patients in our study had lipid parameters within levels recommended by National Cholesterol Education Program Adult Treatment Panel III and that is in accordance with the study of Can Demirdöğen et al. [28] but in contrary to the study of Maasz et al. [29]. More conflicting results revealed the studies that investigated the association between lipid levels and stroke severity. Simundic et al. have reported that there are higher serum lipid levels at admission associated with the higher AIS severity ($P = 0.030$) [30] while Jain et al. have found an association between lower triglycerides levels and worse NIHSS score ($P = 0.004$) [10]. Similar evidence has been published by Weir et al. [31]. Furthermore, the association between higher triglycerides levels and less severe clinical symptoms at admission ($P = 0.014$) has been found by Pikija et al. [32]. All of these studies measured lipid levels within the first 24 h of admission, as it was the case in our study. In a case–control study, Simundic et al. also reported that stroke patients overall had lower lipid levels than the healthy subjects [28]. Several other authors have described similar results. It could be explained with disturbances in lipid metabolism during the early hours of the stroke onset due to acute inflammation response and cerebral ischemia. Thus, the investigators questioned the prognostic value of lipid parameters determined within 48 h of the stroke onset. Regarding that, Perovic et al. have shown that triglycerides levels at admission are significantly lower than at discharge ($P < 0.001$). The authors also reported the poor functional outcome in patients that had decreased HDL-C levels measured at 48 h after the AIS onset and at the discharge [33]. Present study results relate lower triglycerides with a very severe AIS in univariate regression analysis [OR 0.55 (0.34–0.91); $P = 0.019$], but that association disappeared when multiple variables were entered in the regression model ($P = 0.306$). Still, it remains unclear why APOA5 -1131T>C variant and triglycerides have a reciprocal role in the risk of stroke severity in our study. A partial explanation could be found in previously mentioned studies and the inability to measure baseline (prior to stroke) triglycerides levels. Secondly, altered concentrations of apolipoprotein A5 may exert its role more extensively on some metabolic pathway in lipid metabolism other than that of triglycerides. Further studies are needed on a larger number of subjects (including

Table 3 Multivariate model adjusted for age and sex

	Univariate logistic regression			Multivariate logistic regression		
	OR	95% CI	P	OR	95% CI	*P*
ACE	1.25	0.79–1.99	0.342	/	/	/
APOA5	2.40	1.10–5.25	*0.034*	3.26	1.29–8.23	*0.012*
F2	0.83	0.10–7.31	0.870	/	/	/
F5*	0.00	0.00–0.00	*0.047*	0.00	0.00–0.00	0.995
F13A1	0.78	0.47–1.28	0.311	/	/	/
FGB	0.82	0.48–1.39	0.448	/	/	/
SERPINE1	0.97	0.63–1.48	0.870	/	/	/
TC	0.85	0.65–1.10	0.212	/	/	/
CRP	1.01	1.00–1.02	*0.006*	1.01	1.00–1.01	0.164
Glucose	1.04	0.97–1.12	0.276	/	/	/
HDL-C	1.05	0.73–1.52	0.790	/	/	/
LDL-C	0.82	0.60–1.14	0.246	/	/	/
TG	0.55	0.34–0.91	*0.019*	0.75	0.43–1.30	0.306
Age	1.08	1.04–1.13	*< 0.001*	1.06	1.01–1.11	*0.018*
Sex	0.78	0.41–1.46	0.432	/	/	/
FA	1.66	0.83–3.31	0.153	/	/	/
DM type 2	1.15	0.57–2.31	0.695	/	/	/
AMI	1.73	0.71–4.19	0.225	/	/	/
Alcohol use	0.49	0.18–1.33	0.163	/	/	/
Hypertension	1.04	0.47–2.33	0.918	/	/	/
BMI > 30 kg/m^2	0.28	0.10–0.73	*0.010*	0.25	0.08–0.77	*0.016*
Smoking	0.26	0.06–1.12	0.071	/	/	/

F2, factor 2; *F5*, factor 5; *FGB*, β-fibrinogen; *SERPINE1*, serine protease inhibitor E1; *F13A1*, factor 13 subunit A1; *ACE*, angiotensin-converting enzyme; *APOA5*, apolipoprotein A5; *FA*, atrial fibrillation; *AIM*, acute myocardial infarction; *DM type 2*, diabetes mellitus type 2; *CRP*, C-reactive protein; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol

*No case of F5 variant in a group of patients with a very severe stroke

lager groups of stroke subtypes) and different study design to resolve this question.

Well-known risk factor for AIS, obesity, has been recorded in one-quarter of all studied patients and multivariate regression analysis revealed that obese patients had a lower risk for severe stroke ($P = 0.016$). Recent studies showed that obese stroke patients had better long-term outcomes than non-obese patients, so-called obesity paradox in stroke. The Framingham Heart Study in a 10-year follow-up study on 782 stroke patients (87% ischemic stroke) has shown that moderately increased weight was protective for AIS when compared to normal weight subjects [HR = 0.72 (95%CI 0.53–0.99); $P = 0.041$] and that overweight and mildly obese patients had better 10-year outcome [34]. Further, a 30-month follow-up study from TEMPiS trial has found that overweight and obese patients have better survival and better combined outcomes of survival and non-functional status than patients with normal weight. Indeed, the authors reported that underweight patients have the higher mortality risk than the very obese patients [HR 2.42 (95% CI 1.55–3.76); $P < 0.001$] vs [HR 0.36 (95% CI 0.20–0.66); $P < 0.001$] [35]. Similar results have been shown in Yang et al. on Korean patients with AIS during 6-month follow-up [36]. On the other hand, an investigation which was done on the large scale of patients (71,617) has shown that the risk for death by stroke in obese did not significantly differ from the risk in normal weight patients, measuring death by stroke as the most severe outcome within 1 week post-stroke [37]. The results of our study suggest that obesity is favorable for non-severe stroke and probably/possibly better short-term outcome in patients with AIS. The mechanism supporting that observation has yet to be explained. Possible reasons why obesity could be protective for severe stroke could be found in a pro-inflammatory context of stroke. Firstly, TNF-α receptors from adipose tissue may bind TNF-α thus decreasing pro-inflammatory processes. Furthermore, increased lipid levels in obese

Table 4 Distribution of genotypes according to OCSP classification ($N = 242$)

	Genotype	LACI ($N = 87$)	PACI ($N = 94$)	POCI ($N = 22$)	TACI ($N = 39$)	P
F2	GG	84	94	21	37	0.236
	GA	3	0	1	2	
F5	GG	87	88	20	39	*0.025*
	GA	0	6	2	0	
F13A1	VV	46	54	12	25	0.772
	VL	28	30	8	10	
	LL	10	5	2	3	
FGB	GG	42	49	13	21	0.657
	GA	38	34	9	16	
	AA	6	8	0	1	
SERPINE1	5G5G	20	21	8	8	0.582
	5G4G	43	38	10	16	
	4G4G	23	31	4	14	
ACE	II	22	16	5	4	0.139
	ID	38	48	7	25	
	DD	26	27	10	9	
APOA5	TT	70	75	22	30	0.189
	TC + CC	15	13	0	7	

OCSP, Oxfordshire Community Stroke Project; *LACI*, lacunar infarct; *TACI*, total anterior circulation infarct; *PACI*, partial anterior circulation infarct; *POCI*, posterior circulation infarct; *F2*, factor 2; *F5*, factor 5; *FGB*, β-fibrinogen; *SERPINE1*, serine protease inhibitor E1; *F13A1*, factor 13 subunit A1; *ACE*, angiotensin-converting enzyme; *APOA5*, apolipoprotein A5

patients could play a role in binding endotoxin lipoproteins decreasing inflammatory cytokines [38].

As expected, our results have shown that the older age is associated with a higher risk for severe stroke ($P = 0.018$).

Table 5 Distribution of studied genotypes according to TOAST classification ($N = 245$)

	Genotype	LAD	CE	SVD	ODE	UE	P
F2	GG	66	61	83	11	18	0.675
	GA	1	3	2	0	0	
F5	GG	50	54	75	10	13	0.108
	GA	13	5	9	1	5	
F13A1	VV	37	36	45	7	12	0.985
	VL	22	19	29	3	4	
	LL	5	6	8	1	1	
FGB	GG	31	34	48	5	8	0.920
	GA	27	24	32	6	8	
	AA	6	4	5	0	1	
SERPINE1	5G5G	15	11	22	4	6	0.831
	5G4G	29	28	39	4	7	
	4G4G	20	23	24	3	4	
ACE	II	16	9	17	3	1	0.313
	ID	35	29	39	5	12	
	DD	14	25	28	3	3	
APOA5	TT	50	54	75	10	13	0.108
	TC + CC	13	5	9	1	5	

TOAST, Trial of ORG 10172 in Acute Stroke Treatment; *LAD*, large-artery disease; *CE*, cardioembolism; *SVD*, small vessel disease; *ODE*, other determined etiology; *UE*, undetermined etiology; *F2*, factor 2; *F5*, factor 5; *FGB*, β-fibrinogen; *SERPINE1*, serine protease inhibitor E1; *F13A1*, factor 13 subunit A1; *ACE*, angiotensin-converting enzyme; *APOA5*, apolipoprotein A5

Age has been established as an unmodifiable stroke risk factor for decades and our results just underpin the fact that the older patients would more likely suffer from the serious neurological deficits and death [39–41].

Our study revealed a significant difference in the distribution of F5 Leiden genotypes between groups according to OCSP classification ($P = 0.025$). Since OCSP classification is based on clinical syndromes and is not specific for the stroke etiology, and due to the low number of F5 Leiden heterozygous subjects in OCSP classification groups, we may only speculate about F5 Leiden role.

Finally, we have failed to demonstrate the association of other studied gene variants (F5, F2, ACE, SERPINE1, F13A1, and FGB) and AIS severity. Frequency of the variants did not differ from other Caucasian studies nor between studies subgroups. We may speculate that that may be in a line with published meta-analyses suggesting that there are no independent associations between these variants and ischemic stroke despite the synergistic effect which has been found by some researchers [16, 42–44].

A major limitation of our study is that the stroke subtypes were unevenly distributed and thus we have not investigated the effect of APOA5 -1131T>C variant on the severity of the stroke subtypes. Besides, due to inconsistently collected data for modified Rankin score, we have not tested the association of APOA5 -1131T>C variant and short-term outcome.

The results of the presented study have brought the conclusion that C allele carriers of APOA5 -1131T>C variant are more frequent in the group of AIS patients with a very severe

stroke. Furthermore, older AIS patients have more odds to suffer from a severe stroke while, conversely, obese patients would have less severe AIS.

Compliance with ethical standards Sestre milosrdnice University Hospital Center ethics committee approved the study and all participants signed informed consent.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Harvey RL (2015) Predictors of functional outcome following stroke. Phys Med Rehabil Clin N Am 26:583–598
2. Veerbeek JM, Kwakkel G, Van Wegen EEH, Ket JCF, Heymans MW (2011) Early prediction of outcome of activities of daily living after stroke: a systematic review. Stroke 42:1482–1488
3. Woldag H, Gerhold LL, de Groot M, Wohlfart K, Wagner A, Hummelsheim H (2006) Early prediction of functional outcome after stroke. Brain Inj 20:1047–1052
4. Bentsen L, Christensen L, Christensen A, Christensen H (2014) Outcome and risk factors presented in old patients above 80 years of age versus younger patients after ischemic stroke. J Stroke Cerebrovasc Dis 23:1944–1948
5. Rojas JI, Zurrú MC, Romano M, Patrucco L, Cristiano E (2007) Acute ischemic stroke and transient ischemic attack in the very old-risk factor profile and stroke subtype between patients older than 80 years and patients aged less than 80 years. Eur J Neurol 14:895–899
6. Sato S, Toyoda K, Uehara T, Toratani N, Yokota C, Moriwaki H, Naritomi H, Minematsu K (2008) Baseline NIH Stroke Scale Score predicting outcome in anterior and posterior circulation strokes. Neurology. 70:2371–2377
7. Osei E, Fonville S, Zandbergen AAM, Koudstaal PJ, Dippel DWJ, den Hertog HM (2017) Glucose in prediabetic and diabetic range and outcome after stroke. Acta Neurol Scand 135:170–175
8. Tanaka R, Ueno Y, Miyamoto N, Yamashiro K, Tanaka Y, Shimura H, Hattori N, Urabe T (2013) Impact of diabetes and prediabetes on the short-term prognosis in patients with acute ischemic stroke. J Neurol Sci 332:45–50
9. Tziomalos K, Giampatzis V, Bouziana SD, Spanou M, Kostaki S, Papadopoulou M, Angelopoulou SM, Tsopozidi M, Savopoulos C, Hatzitolios AI (2017) Prognostic significance of major lipids in patients with acute ischemic stroke. Metab Brain Dis 32:395–400
10. Jain M, Jain A, Yerragondu N, Brown RD, Rabinstein A, Jahromi BS et al (2013) The triglyceride paradox in stroke survivors: a prospective study. Neurosci J 2013:870608
11. Deng Q, Li S, Zhang H, Wang H, Gu Z, Zuo L, Wang L, Yan F (2019) Association of serum lipids with clinical outcome in acute ischaemic stroke: a systematic review and meta-analysis. J Clin Neurosci 59:236–244
12. Bevan S, Traylor M, Adib-Samii P, Malik R, Paul NLM, Jackson C, Farrall M, Rothwell PM, Sudlow C, Dichgans M, Markus HS (2012) Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genomewide associations. Stroke. 43:3161–3167
13. Babu MS, Prabha TS, Kaul S, Al-Hazzani A, Shafi G, Roy S et al (2012) Association of genetic variants of fibrinolytic system with stroke and stroke subtypes. Gene. 495:76–80
14. Krajcoviechova A, Wohlfahrt P, Mayer O, Vanek J, Hajkova J, Hlinovsky D et al (2015) Tobacco smoking strongly modifies the association of prothrombin G20210A with undetermined stroke: consecutive survivors and population-based controls. Atherosclerosis 240:446–452
15. Tasdemir S, Erdem HB, Sahin I, Ozel L, Ozdemir G, Eroz R, Tatar A (2016) Correlation with platelet parameters and genetic markers of thrombophilia panel (factor II g.20210G>A, factor V Leiden, MTHFR (C677T, A1298C), PAI-1, β-fibrinogen, factor XIIIA (V34L), glycoprotein IIIa (L33P)) in ischemic strokes. NeuroMolecular Med 18:170–176
16. They-They TP, Battas O, Nadifi S (2013) Synergistic effect of MTHFR C677T and F2 G20210A polymorphisms on ischemic stroke. Neurosci Bull 29:725–730
17. Atadzhanov M, Mwaba MH, Mukomena PN, Lakhi S, Rayaprolu S, Ross OA et al (2013) Association of the APOE, MTHFR and ACE genes polymorphisms and stroke in Zambian patients. Neurol Int 5:69–72
18. Markoula S, Giannopoulos S, Kostoulas C, Tatsioni A, Bouba I, Maranis S, Georgiou I, Kyritsis AP (2011) Gender association of the angiotensin-converting enzyme gene with ischaemic stroke. J Renin-Angiotensin-Aldosterone Syst 12:510–515
19. Malueka RG, Dwianingsih EK, Sutarni S, Bawono RG, Bayuangga HF, Gofir A et al (2017) The D allele of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is associated with worse functional outcome of ischemic stroke. Int J Neurosci 7454:1–21
20. Martiskainen M, Oksala N, Pohjasvaara T, Kaste M, Oksala A, Karhunen PJ, Erkinjuntti T (2014) Beta-fibrinogen gene promoter A -455 allele associated with poor longterm survival among 55-71 years old Caucasian women in Finnish stroke cohort. BMC Neurol 14:137
21. Shemirani AH, Antalfi B, Pongrácz E, Mezei ZA, Bereczky Z, Csiki Z (2014) Factor XIII-A subunit Val34Leu polymorphism in fatal atherothrombotic ischemic stroke. Blood Coagul Fibrinolysis 25:364–368
22. Saracevic A, Simundic AM, Celap I, Luzanic V (2013) Angiotensin-converting enzyme insertion/deletion polymorphism genotyping error: the cause and a possible solution to the problem. Mol Biol Rep 40:4459–4463
23. Fonarow GC, Saver JL, Smith EE, Broderick JP, Kleindorfer DO, Sacco RL, Pan W, Olson DM, Hernandez AF, Peterson ED, Schwamm LH (2012) Relationship of National Institutes of Health Stroke scale to 30-day mortality in Medicare beneficiaries with acute ischemic Stroke. J Am Heart Assoc 1:42–50
24. Ver HA (2011) The NIH stroke scale: a window into neurological status. Nursing Spectrum (Greater Chicago) 24:44–49
25. Kim M, Kim M, Yoo HJ, Lee E, Chae JS, Lee S-H et al (2017) A promoter variant of the APOA5 gene increases atherogenic LDL levels and arterial stiffness in hypertriglyceridemic patients. PLoS ONE 12:e0186693
26. Mahrooz A, Zargari M, Ansari V, Makhlough A, Hashemi-Sooteh MB (2016) Association of APOA5 gene promoter region -1131T>C polymorphism (rs662799) to plasma triglyceride level in patients with type 2 diabetic nephropathy. J Clin Diagn Res 10: BC09–BC13
27. Guardiola M, Cofán M, de Castro-Oros I, Cenarro A, Plana N, Talmud PJ, Masana L, Ros E, Civeira F, Ribalta J (2015) APOA5 variants predispose hyperlipidemic patients to atherogenic dyslipidemia and subclinical atherosclerosis. Atherosclerosis 240:98–104
28. Can Demirdöğen B, Şahin E, Türkanoğlu Özçelik A, Bek S, Demirkaya Ş, Adali O (2012) Apolipoprotein A5 polymorphisms in Turkish population: association with serum lipid profile and risk of ischemic stroke. Mol Biol Rep 39:10459–10468
29. Maasz A, Kisfali P, Jaromi L, Horvatovich K, Szolnoki Z, Csongei V, Safrany E, Sipeky C, Hadarits F, Melegh B (2008) Apolipoprotein A5 gene IVS3+G476A allelic variant confers susceptibility for development of ischemic stroke. Circ J 72:1065–1070

30. Simundic AM, Nikolac N, Topic E, Basic-Kes V, Demarin V (2008) Are serum lipids measured on stroke admission prognostic? Clin Chem Lab Med 46(8):1163–1167
31. Weir CJ, Sattar N, Walters MR, Lees KR (2003) Low triglyceride, not low cholesterol concentration, independently predicts poor outcome following acute stroke. Cerebrovasc Dis 16:76–82
32. Pikija S, Milevčić D, Trkulja V, Kidemet-Piskač S, Pavliček I, Sokol N (2006) Higher serum triglyceride level in patients with acute ischemic stroke is associated with lower infarct volume on CT brain scans. Eur Neurol 55:89–92
33. Perovic E, Mrdjen A, Harapin M, Simundic AM (2016) Short term changes of serum lipids in acute ischemic stroke. Clin Lab 62: 2107–2113
34. Aparicio HJ, Himali JJ, Beiser AS, Davis-Plourde KL, Vasan RS, Kase CS et al (2017) Overweight, obesity, and survival after stroke in the Framingham heart study. J Am Heart Assoc 6:e004721
35. Doehner W, Schenkel J, Anker SD, Springer J, Audebert H (2013) Overweight and obesity are associated with improved survival, functional outcome, and stroke recurrence after acute stroke or transient ischaemic attack: observations from the tempis trial. Eur Heart J 34:268–277
36. Jang SY, Shin Y II, Kim DY, Sohn MK, Lee J, Lee SG et al (2015) Effect of obesity on functional outcomes at 6 months post-stroke among elderly Koreans: a prospective multicentre study. BMJ Open 5:e008712
37. Dehlendorff C, Andersen KK, Olsen TS (2014) Body mass index and death by stroke no obesity paradox. JAMA Neurol 71:978–984
38. Oesch L, Tatlisumak T, Arnold M, Sarikaya H (2017) Obesity paradox in stroke - myth or reality? A systematic review. PLoS ONE 12:e0171334
39. Kissela BM, Khoury JC, Alwell K, Moomaw CJ, Woo D, Adeoye O, Flaherty ML, Khatri P, Ferioli S, de Los Rios la Rosa F, Broderick JP, Kleindorfer DO (2012) Age at stroke: temporal trends in stroke incidence in a large, biracial population. Neurology 79: 1781–1787
40. Kelly-Hayes M (2010) Influence of age and health behaviors on stroke risk: lessons from longitudinal studies. J Am Geriatr Soc 58: S325–S328
41. Wang Y, Rudd AG, Wolfe CDA (2013) Age and ethnic disparities in incidence of stroke over time: the South London stroke register. Stroke 44:3298–3304
42. Roach REJ, Roshani S, Meijer K, Hamulyák K, Lijfering WM, Prins MH, Büller HR, Middeldorp S (2011) Risk of cardiovascular disease in double heterozygous carriers and homozygous carriers of F5 R506Q (factor V Leiden) and F2 (prothrombin) G20210A: a retrospective family cohort study. Br J Haem 153:134–136
43. Beye A, Pindur G (2017) Clinical significance of factor V Leiden and prothrombin G20210A-mutations in cerebral venous thrombosis - comparison with arterial ischemic stroke. Clin Hemorheol Microcirc 67:261–266
44. Herm J, Hoppe B, Siegerink B, Nolte CH, Koscielny J, Haeusler KG (2017) A prothrombotic score based on genetic polymorphisms of the hemostatic system differs in patients with ischemic stroke, myocardial infarction, or peripheral arterial occlusive disease. Front Cardiovasc Med 4:39