id-design
CREATIVE STUDIO

Vascular Genetic Variants and Ischemic Stroke Susceptibility in Albanians from the Republic of Macedonia

Bajram Kamberi[1]*, Farije Kamberi[2], Mirko Spiroski[3]

[1]Neurological Department, Clinical Hospital, Tetovo, Republic of Macedonia; [2]School Medical Centre, "Nikolla Shtejn" Tetovo, Republic of Macedonia; [3]Institute of Immunobiology and Human Genetics, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Abstract

BACKGROUND: Acute first-ever ischemic stroke (FIS) is a heterogeneous, polygenic disorder. The contribution of vascular genetic variants as inherited causes of ischemic stroke has remained controversial.

AIM: To examine the association of genetic variants in vascular factors with the occurrence of FIS.

MATERIAL AND METHODS: The current research was performed in a group of 39 patients with FIS (study group) and 102 healthy volunteers (control group). We analyzed the prevalence of vascular genetic variants in following genes: *factor V, prothrombin, methylenetetrahydrofolate reductase (MTHFR), factor XIII, plasminogen activator 1, endothelial protein C receptor (EPCR), apolipoprotein B, apolipoprotein E, β-fibrinogen, human platelet antigen 1, angiotensin-converting enzyme (ACE), endothelial nitric oxide synthase (eNOS)* and *lymphotoxin alpha.*

RESULTS: It was found that heterozygous *LTA 804C>A* and *FXIII V34L Leu/Leu* were significantly more frequent in patients with FIS than in control group (p = 0.036 and p = 0.017, respectively). The frequency of *FXIII V34L Val/Val* was significantly lower in patients with FIS than in control group (p = 0.020). Other frequencies of vascular gene variants in patients with FIS and in control group were not significantly different.

CONCLUSIONS: This is the first comprehensive study to present data indicating that polymorphism of vascular genes in the prevalence of acute FIS exists in the Albanian population from the Republic of Macedonia. Variations in these genes have been detected in patients with acute FIS, suggesting that their combination might act in a susceptible or protective manner in this Albanian population.

Introduction

Ischemic stroke is a major public health problem with a heritable component to the risk of disease [1-3]. The last decade has been marked by initiatives aimed at raising awareness of the inherited predisposition to ischemic stroke. There has been an increase in studies that analysed polymorphic genes responsible for arterial and venous ischemic events. An increased understanding of genetics and molecular biology has fuelled this research and shed light on several genetic risk factors that may help to predict arterial events.

Most of the studies related to arterial thrombosis have actually involved patients with coronary syndromes [4], myocardial infarction [5] and events of the arterial circulatory system [6, 7], and they produced conflicting results. To date, most studies on the genetics of ischemic stroke have focused on candidate genes in a control group and unrelated cases. The causes of the majority of ischemic strokes include atherosclerotic, cardiogenic and lacunar (penetrating vessel) mechanisms [8]. Several animal and clinical studies clearly defined genetic predisposition to hereditary thrombophilia or atherosclerosis, in the case of atherosclerosis, and particularly, to the clinical expression of ischemic stroke [9]. Arteriosclerosis of the large vessels is the cause of cerebral infarction in almost half of all

ischemic events [10]. Arteriosclerosis involves numerous genes and some pathways thought to be involved in the development and rupture of atherosclerotic lesions [11].

Based on published data, the most common vascular genetic variations have been extensively studied, particularly in the following genes: _factor V (FV), prothrombin (factor II; PTH), methylenetetrahydrofolate reductase (MTHFR), factor XIII (FXIII)_ [12, 13], _plasminogen activator 1 (PAI-1)_ [14-18], _endothelial protein C receptor (EPCR), apolipoprotein B (APOB)_ [19], _apolipoprotein E (APOE)_ [20], _β-fibrinogen (β-FG, FGB)_ [21], _human platelet antigen 1 (HPA-1)_ [22, 23], _angiotensin-converting enzyme (ACE)_ [24, 25], _endothelial nitric oxide synthase (eNOS)_ [26] and _lymphotoxin alpha_ [27], which have been identified as risk factors for several diseases, such as arterial and/or venous thrombosis. However, with regard to testing for an association with susceptibility to ischemic stroke, study results are conflicting and the association is not always detected. Consequently, routine testing for polymorphisms associated with ischemic stroke is not part of the diagnostic protocol for elderly individuals, but the results are still debatable.

This study is the first comprehensive study of the vascular gene variations associated with first-ever ischemic stroke in the Albanian population from the Republic of Macedonia. The purpose of this research was to examine the association of vascular gene variations in 13 genes and 17 genetic variants in patients with acute FIS and compare the frequencies in the patient and control groups.

Material and Methods

Sampling

This study was a case-control study (comparative analytical study) carried out from September 2008 to August 2010. In total, 231 patients with FIS as a study group and 194 healthy volunteers as a control group were enrolled in the initial study. The inclusion criteria were consecutive patients admitted to the Neurology Department with confirmed diagnosis acute FIS, age ranging from 18 to 90 years, an absence of a history of stroke and completion of interview and analysis. All patients gave informed consent prior to participation in this study. The exclusion criteria were patients younger than 18 years, patients, who had experienced an acute hemorrhagic stroke, a known malignancy, acute or chronic renal or failure or acute myocardial infarction and patients who had recent inflammatory or immunologic disease. Acute FIS was defined as a clinical syndrome on the basis of clinical symptoms and (a) recent infarct(s) in the clinically relevant area of the brain, using brain computed tomography (CT) and/or magnetic resonance imaging (MRI).

Healthy controls that were free from cerebrovascular diseases were selected from among local residents, using the same exclusion criterion as for patients. Informed consent was obtained from all participants prior to conducting the study. The protocol of the study was reviewed and approved by the professional college at Clinical Hospital Tetovo (Nr.02-1280/2, 02.07.2008) [28].

Within a study of the genetics of the Macedonian population, a detailed description of the study subjects and study design were performed as previously described [29, 30]. Briefly, after signed consent was given to obtain and store a DNA sample, 212 subjects were recruited in total (stratified into 58 healthy controls and 12 patients of Macedonian origin and 103 healthy controls and 39 patients of Albanian origin) all residents of Tetovo and its neighbouring region who met the study inclusion criteria to engage in the proposed national project over a 2-year period (2010–2012), examining gene variations associated with diseases of blood vessels in the Republic of Macedonia, which was originally designed as a prospective, nationwide, multicenter case-control study (ID 13-3589/1, 26.07.2010).

Among others, the objectives were to investigate the distribution of candidate genes in healthy Macedonian and Albanian populations and the association of candidate genes with stroke in Macedonians and Albanians from the Republic of Macedonia. Of these, genetic analyses were completed for 141 (66.51%) participants, all of whom were Albanians, while others are in progress. The present study comprises 39 patients with acute FIS treated at the Clinical Hospital Tetovo, Department of Neurology as a study group and 102 healthy volunteers as a control group. Each individual was interviewed on a one-to-one basis, his/her genealogy was recorded for the past three generations, and their Albanian origin was ascertained by making enquiries as far back as their four grandparents. Admixture, if any, was recorded for each individual. Individuals with only one Albanian parent were excluded from the study.

Blood sampling and processing

A sample of peripheral vein whole blood (10 mL) was collected by venipuncture in standard collection tubes containing anticoagulant ethylenediaminetetraacetic acid (EDTA) in the first 24 h of admission and then dispensed into two tubes (one for complete blood picture and the other for extraction of DNA). The first tube was transferred to the biochemical laboratory for routine analysis and the second tube was separated and referred for genetic analysis.

_____

DNA extraction, molecular genetic screening and storage

At the Institute of Immunobiology and Human Genetics, part of the Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia [28-30], genomic DNA was isolated from peripheral blood leukocytes by the phenol-chloroform extraction method [31] or with BioRobot EZ1 workstation (QIAGEN) [32].

Genetic testing of the candidate genes was based on the polymerase chain reaction and reverse hybridization principle using the CVD SripAssay [ViennaLab (Laboradiagnostica GmbH, Austria)]. The DNA samples were subsequently stored in the anthropology project field of the Macedonian Human DNA Bank (hDNAMKD) [33].

Statistical analysis

The population genetics analysis package, PyPop, developed by the Biostatistics Core for the Workshop [34-36] was used for analysis of the genetic data for this report. All analyses were based on available data. The results were collected, tabulated and statistically analysed by personal computer and the commercially available statistical software package SPSS version 17 (SPSS Inc. Chicago, Illinois, USA). The distribution of gene polymorphism frequency was not perfectly normal.

Descriptive statistics were used for the prevalence of genetic polymorphisms. Between-group differences in genotype frequencies were compared using a two-by-two contingency table and analysed by chi-square analysis with Yates' correction test. Pearson P values, crude odds ratios (OR), and Wald's 95% confidence intervals (CI) were calculated to test the associations between mutations/polymorphisms and FIS with free statistical calculators (http://www.quantitativeskills.com/sisa/statistics/two2hlp.htm). The level of statistical significance was set at $p = 0.05$.

Results

We analysed genetic tests for a total of 141 participants, divided into two different groups: data for 39 patients with FIS (mean age: 62.56 years; range: 44–82 years), and the results of 102 healthy subjects (mean age: 48.86 years; range: 27–71 years).

The baseline and demographic characteristics of the study participants are presented in Table 1.

The mean age at first onset FIS was significantly higher in the patient groups older than 60 years than in the age-matched control group, and the difference was statistically significant (t = 7.836; $p = 0.000$).

Table 1: Participants included in the study classified by age group

Age Group	Patients N	%	Controls N	%
18–49	2	5.13	44	43.14
50–59	12	30.77	46	45.10
60–69	16	41.03	11	10.79
>70	9	23.08	1	0.98
Total	39	100	102	100
Mean ± SD (years)	62.56 ± 8.73		48.86 ± 10.60	
Range	44–82		27–71	

t = 7.836; $p = 0.000$

Genotype frequencies were compared separately for all vascular gene variations between cohorts of patients and controls. The genotype distributions in the study and control groups are provided in Table 2.

When genotype frequencies were stratified into distribution frequencies, we observed that in most cases, higher frequencies of gene mutations were detected in the control group than in the patient group. The *APOE E3/E3* polymorphism was present in only one patient, and the *fibrinogen beta (-455G>A)* mutation was not present in any patient, whereas the frequencies of heterozygote carriers of f*actor V (Leiden-G1691A; R2-H1299R), PTH, MTHFR-677, MTHFR-1298, FXIII, LTA, HPA 1, ACE, PAI-1, eNOS-T786C* and *eNOS-G894T* were lower in patients than in control subjects.

Heterozygotes for the *factor V Leiden* mutation were more frequent in control healthy subjects (8.82%) than in the group of patients with FIS (3 or 7.69%).

The prevalence of heterozygote variants in *PTH 20210G>A* was higher among controls than among patients with FIS (6.86% vs. 2.56%, respectively).

Analysis of the study group for the *MTHFR C677T* polymorphism indicated that 15 (38.46%) patients were homozygous for the wild-type (*CC*), 21 (53.84%) were heterozygous (*CT*), and the remaining 3 (7.69%) were homozygous for the mutant allele (*TT*). In the control group of 102 analyzed subjects, the following results were obtained: 27 persons (26.47%) were homozygous for the wild-type (*CC*), 55 individuals (53.92%) were heterozygous for the polymorphism (*CT*), and 20 individuals (19.61%) were homozygous for the variant type (*TT*).

The prevalence of wild-type homozygotes and heterozygotes for the *FXIII V34L Leu34* polymorphism differed significantly between patients with acute FIS and their respective controls.

In the *EPCR* gene, we observed a significant difference in the distribution of the *CC* and *CG/GG* genotype groups between the control and patient groups. The carriers of the *EPCR 4600AG* genotype

were more frequent in patients with FIS (10 or 25.64%) than in control healthy subjects (20 or 19.61%). In contrast, carriers of the *EPCR 4678CG* genotype were more frequent in control healthy subjects (50 or 49.02%) than in patients with FIS (18 or 46.15%), except for carriers of the *EPCR 4678GG* genotype, which were more frequent in patients with FIS (16 or 41.03%) than in healthy subjects (24 or 23.53%).

Table 2: Genotype frequencies, Pearson`s *P*-values, odds ratios and Wald's 95% confidence intervals in patients with FIS and healthy Albanian controls

Gene and genetic variant	Control group (n = 102) (%)	Study group (n = 39) (%)	Statistical analysis	
			OR (95% CI)	Pearson's *P*-values
FV 1691G>A (Leiden)				
N	93 (91.18)	36 (92.31)	0.988 (0.886–1.101)	0.829
HET	9 (8.82)	3 (7.69)	1.147 (0.328–4.018)	0.829
HOM	0 (0)	0 (0)	*	*
FV H1299R				
N	77 (75.49)	32 (82.05)	0.674 (0.265–1.714)	0.405
HET	25 (24.51)	7 (17.95)	1.484 (0.583–3.777)	0.405
HOM	0 (0)	0 (0)	*	*
PTH 20210G>A				
N	95 (93.14)	38 (97.44)	0.357 (0.042–3.002)	0.324
HET	7 (6.86)	1 (2.56)	2.800 (0.333–23.533)	0.324
HOM	0 (0)	0 (0)	*	*
MTHFR 677C>T				
CC	27 (26.47)	15 (38.46)	0.567 (0.264–1.258)	0.164
CT	55 (53.92)	21 (53.84)	0.687 (0.307–1.54)	0.361
TT	20 (19.61)	3 (7.69)	0.270 (0.069–1.06)	0.051
MTHFR 1298A>C				
AA	54 (52.94)	18 (46.15)	1.313 (0.626–2.751)	0.471
AC	44 (43.13)	20 (51.28)	1.388 (0.622–2.909)	0.385
CC	4 (3.92)	1 (2.56)	0.750 (0.079–7.154)	0.802
FXIII V34L				
Val/Val	67 (67.68)	17 (45.94)	2.463 (1.139–5.329)	0.020
Val/Leu	25 (25.25)	13 (35.14)	2.049 (0.871–4.823)	0.097
Leu/Leu	7 (7.07)	7 (18.92)	3.941 (1.217–12.763)	0.017
EPCR 4600A>G				
AA	80 (78.43)	29 (74.36)	1.254 (0.531–2.963)	0.606
AG	20 (19.61)	10 (25.64)	0.798 (0.338–1.884)	0.606
GG	2 (1.96)	0 (0)	*	0.265
EPCR 4678G>C				
CC	28 (27.45)	5 (12.82)	2.573 (0.914–7.24)	0.066
CG	50 (49.02)	18 (46.15)	2.016 (0.675–6.017)	0.203
GG	24 (23.53)	16 (41.03)	3.373 (1.191–11.704)	0.019
eNOS 786T>C				
N	35 (34.31)	11 (28.21)	0.133 (0.592–2.984)	0.489
HET	54 (52.94)	20 (51.28)	1.178 (0.504–2.757)	0.705
HOM	13 (12.75)	8 (20.51)	1.958 (0.645–5.948)	0.232
eNOS 894G>T				
N	41 (40.20)	14 (35.90)	1.200 (0.559–2.578)	0.639
HET	52 (50.98)	24 (61.54)	1.352 (0.626–2.937)	0.446
HOM	9 (8.82)	1 (2.56)	0.325 (0.038–2.803)	0.286
LTA 804C>A				
N	82 (80.39)	22 (56.41)	2.423 (0.144–5.623)	0.036
HET	20 (19.61)	13 (33.33)	0.413 (0.178–0.958)	0.036
HOM	0 (0)	4 (10.26)	*	*
HPA-1 a/b				
1a/1a	75 (73.53)	27 (69.23)	1.235 (0.549–2.775)	0.609
1a/1b	24 (23.53)	11 (28.21)	1.273 (0.551–2.944)	0.572
1b/1b	3 (2.94)	1 (2.56)	0.926 (0.092–9.287)	1.000
ACE I/D				
I/I	0 (0)	1 (2.94)	*	0.079
I/D	49 (61.25)	23 (67.65)	*	0.098
D/D	31 (38.75)	10 (29.41)	*	0.057
APO E ε2/ε3/ε4				
E2/E3	1 (1.25)	0 (0)	*	0.856
E3/E3	60 (75.00)	1	*	0.447
E3/E4	15 (0)	0 (0)	*	0.504
E4/E4	4 (5.0)	0 (0)	*	0.690
PAI-1				
4G/4G	25 (24.51)	11 (28.21)	0.826 (0.36–1.897)	0.653
4G/5G	53 (51.96)	19 (48.72)	0.815 (0.337–1.968)	0.649
5G/5G	24 (23.53)	9 (23.08)	0.852 (0.3–2.421)	0.764

n = absolute number; % = frequency; OR = Odds ratio; CI = Confidence Interval; *, cannot be calculated because expected < 5, χ^2 test.

The *eNOS* mutation frequencies were higher in the patient group. There were no significant differences in genotype distribution between patient and control groups.

Heterozygotes for the *LTA C804A*

polymorphism were more frequent in patients with FIS (13 or 33.33%) than in control healthy subjects (20 or 19.61%).

HPA-1 1a/1b and *ACE I/D* mutation frequencies were higher in the control than in the study group, but the polymorphism frequency was not significantly different between the study and control groups.

No homozygous or heterozygous mutants for *APO B* were detected in any of the groups (in all patients and controls, the *APO-B* genotype distribution was normal; these data are not shown in Table 2).

PAI-1, 4G/5G and *5G/5G* mutation frequencies were higher in the control than in the study group, but the polymorphism frequency was not significantly different between the study and control groups.

The prevalence of all investigated gene variations did not differ significantly between the studied groups (Pearson's P value > 0.05), except for the prevalence of the homozygous wild-type and heterozygous genotypes, *FXIII V34L Leu34* carriers and homozygotes (*GG*) for the factor *EPCR G4678C* polymorphism. There were also no significant differences with regard to ethnicity (all participants were Albanians from the Republic of Macedonia). The frequency of *ACE I/D* carriers was 67.65%, that of *eNOS-G894T* heterozygotes was 61.54% and the *MTHFR C677T CT* genotype was detected in 53.84% of patients.

The most frequent *ACE* genotype in patients with FIS was *I/D*, with an observed frequency of 67.65%; a lower frequency was found for the *D/D* genotype (29.41%) and the lowest frequency was found for the *I/I* genotype (2.94%).

The most frequent *PAI-1* genotype in patients with FIS was *4G/5G*, with an observed frequency of 48.72%; a lower frequency was found for the *4G/4G* genotype (28.21%) and the lowest frequency was found for the *5G/5G* genotype (23.08%). The frequencies of the *PAI-1 4G/4G* and *4G/5G* genotypes were slightly increased in healthy individuals (24.52% and 51.96%, respectively), but that of *PAI-1 5G/5G* was decreased (23.53%).

Discussion

Traditionally, the greatest clinical challenge in the prevention of ischemic stroke is focused on the modification of the main risk factors for atherosclerosis, including symptomatic treatment of hypertension, diabetes, hypercholesterolemia and smoking [10-12].

For the first time in the Republic of

Macedonia, the determination of a heritable component to the risk of acute FIS was carried out in unrelated cohorts from an Albanian population living in the Republic of Macedonia. We performed a genetic analysis of patients presenting with ischemic stroke that clinically was their first stroke episode and community controls who were subsequently included in a national project [28-30]. The purpose of this study was to investigate the role of specific genes and to obtain data that would support an association of gene polymorphisms regarding the aetiology of ischemic stroke.

Excluding *FXIII Leu34* carriers and homozygotes (*GG*) for the factor *EPCR G4678C* polymorphism, these preliminary results of genetic analysis in the present study did not find any significant positive (susceptible) association between the investigated gene polymorphisms and FIS (Pearson's P value greater than 0.05) in this cohort of Albanians from the Republic of Macedonia. These results are of interest because they differ from those of other studies.

Many of the traditional risk factors that increase the risk of ischemic strokes, such as hypertension, atrial fibrillation, diabetes mellitus and cigarette smoking, are well known and are modifiable or avoidable [37]. Genetic risk factors are often considered not to be modifiable; however, knowledge of genetic risk factors can provide insights into pathophysiological pathways and targets for drug therapy [10-12]. In the general population and in patient cohorts, risk factor profiles change with increasing age [38, 39]. Ischemic stroke is the most common cerebrovascular disease, and it is often attributable to the inherited predisposition, especially when apparent at a relatively early age. Approximately 10% of ischemic strokes occur at ages under 45 years [40]. Ischemic stroke is more frequent in women aged 20–30 years and in men older than 35 years [41].

Although many of our patients were middle-aged or elderly (see Table 1), it is clear that in our patient group, the number of elderly participants exceeded the number of middle-aged and younger patients. However, further research would provide clear evidence of the impact of gene polymorphisms and their role in the aetiology of ischemic stroke as the combined effects of multiple susceptibility genes. Heritability seems to have a major role in younger stroke patients and patients with a family history of stroke [42-48].

Inconsistent results obtained from various authors highlight the genetic role among different ethnic groups [49, 50]. In Macedonia [51], *FV Leiden* is more prevalent in Macedonians (6.9%) than in Albanians (2.9%), whereas the prevalence of *FV Leiden* was higher in Kosovo (3.4%) [52] than in Albania (1.8%) [53].

The present study shows that the *PTH* G20210A polymorphism is not important in the case of ischemic stroke, similarly to Erten et al. [54].

Two meta-analyses, one from individuals of European descent [55] and another from those of non-European descent [48] report an association with a modest effect for common variants in the genes for coagulation *factor V, MTHFR, PTH* and *ACE*.

The *MTHFR C677T* mutation has been linked to an increased risk for ischemic medical events in a recent population study [56]. Studies of some populations with certain ethnic backgrounds from Asian countries, North America and Europe [57-60] and from the Eastern Mediterranean region found that the prevalence of the *MTHFR C677T* polymorphism in its homozygous state is variable [61-63].

An elevated total homocysteine (tHcy) level in plasma (hyperhomocysteinemia) is the consequence of decreased activity of *MTHFR* and is associated with a slight increase in the risk of thrombosis, whereas the common *C677T* polymorphism in *MTHFR* has been associated with an increased risk of the development of different cardiovascular diseases [64], including ischemic stroke.

The population frequencies of *MTHFR C677T* homozygotes vary in the United States from 44.6% in Caucasians to 42% of Hispanics and 25.6% African-Americans [65]. The frequency of the *MTHFR C677T* genotype is often reported to be high in European, Asian and Central and South American (10%–32%) populations and low in different African populations (0–3%) and also to show geographical gradients among Chinese Han populations [66]. The frequency of the *MTHFR C677T* genotype is variable in different geographic and ethnic groups: 28%–32.8% in Indian [67-69] and 12.3% in Chinese populations [70].

The prevalence of a homozygous gene mutation in *MTHFR C677T* was high in Turkish stroke patients [71]. In a study in Tamilians [72] the *CT* genotype was seen in 18.1% and the *TT* genotype in 1.38%, whereas the *MTHFR A1298C* polymorphism was more prevalent (47.2%) in a Hungarian study [73].

Previously published studies failed to find an association between *MTHFR* (*MTHFR-677* and *MTHFR-1298*) genotypes and haplotypes and plasma homocysteine levels in patients with occlusive artery disease and deep venous thrombosis [74] or between *MTHFR* (*MTHFR-677* and *MTHFR-1298*) [75] and *PAI-1* polymorphisms [76] and occlusive artery disease or deep venous thrombosis in Macedonians.

Previous studies in Albanians with ischemic stroke also found that elevated plasma homocysteine levels (hyperhomocysteinaemia) were associated with the presence of *MTHFR* (*MTHFR-677* and *MTHFR-1298*) gene polymorphisms [28, 30]. In addition, meat with a high fat content and hyperhomocysteinemia are not the only causes of acute FIS in the Republic of

Macedonia [77]. Moreover, _MTHFR-677_ and _MTHFR-1298_ gene polymorphisms were not associated with occurrence rates of FIS [28, 30], but there is no data on the Albanian population regarding the PAI-1 gene and other susceptibility genes that are involved in the development and progression of ischemic stroke and their possible association with ischemic stroke.

Pezzini et al. [78] showed that the risk of ischemic stroke increased 3-fold with the presence of two or more mutations. Co-occurrence of homozygous _MTHFR TT_ and _ACE D/D_ genotypes yielded a highly significant moderate risk of leukoaraiosis (ischemic white matter demyelination). A synergistic effect of the _MTHFR TT_ and _ACE D/D_ genotypes and drinking or smoking has been found [79].

Our results demonstrate that the _Factor XIII Val34Leu_ polymorphism influences the occurrence of acute FIS, in agreement with one study [12] and in contrast to another [13]. In the recent study was concluded that significant association of _Factor V Leiden (G1691A), Factor R2 (A4070G),_ and _Prothrombin (G20210A)_ genetic polymorphism with occlusive artery disease or deep venous thrombosis in Macedonians was not found [80].

Although previous studies demonstrated that the _PAI-1 4G/4G_ polymorphism might protect against ischemic stroke [14-18], our results show that the _4G/4G_ genotype of the _PAI-1_ gene is less frequent in patients with FIS than in controls, similarly to Endler et al. [17].

We observed a difference in the frequency of the _ACE I/I, I/D_ and _D/D_ polymorphisms between stroke patients and controls, confirming that homozygosity for the _ACE D/D_ polymorphism does not confer a higher risk of ischemic stroke [54] than the _I/D_ and _I/I_ genotypes. Conversely, other studies reported that homozygosity for the _ACE D/D_ polymorphism is a strong risk factor for ischemic stroke [24, 25]. Several studies have reported that the _HPA-1b_ polymorphism is associated with ischemic stroke [22, 23]; however, _GP 1a (HPA5)_ is correlated weakly with ischemic stroke [43]. In the present study, the frequencies of the _1a/1a, 1b/b_ and _1a/1b_ genotypes differed between stroke patients and controls, indicating that the _1b/b_ genotype is not a risk factor for ischemic stroke.

Results of the present study demonstrated that the frequency of the _APOE, ε2/ε3/ε43_ polymorphisms were not significantly different between stroke patients and controls and support the notion that _APOE, E3/E3_ polymorphisms are not associated with ischemic stroke [20, 54]. Excluding _FXIII Leu34_ carriers and homozygotes (_GG_) for the factor _EPCR G4678C_ polymorphism, the preliminary results of genetic analysis in the present study did not find any significant positive (susceptible) association between the investigated gene polymorphisms and FIS (Pearson's P value > 0.05) in this cohort of Albanians from Macedonia.

The small number of participants in each group limited our power of analysis. Consequently, the genotype distribution of the investigated single-susceptibility polymorphisms was under-represented or not present, particularly in the patient group. On the other hand, the very weak statistical findings may suggest that if we had a large number in each group, we could find much stronger associations and differences that we missed in this small study population. However, in the control group, the single susceptibility polymorphisms were present, which means that differences between the tested groups are probably due to ethnic differences rather than recruitment bias. There are possibilities that some negative findings might be a consequence of low statistical power. Such results have previously been reported in studies with certain patient groups that were also without genotype distribution of the investigated single- susceptibility polymorphisms. Larger samples may be needed in order to resolve these limitations, validate our results and confirm our conclusions, which are our next goals. In this case-control cohort with FIS, in which many of our patients were middle-aged or elderly, the single susceptibility polymorphisms with FIS had the lowest prevalence. These data emphasise the need for primary and secondary prevention measures in middle-aged and elderly populations targeting modifiable lifestyle vascular risk factors.

Although previous studies have identified susceptibility genes that are involved in the development and progression of ischemic stroke [6, 41, 48, 55, 78], the present study showed that the examination of individual variants of _FXIII Leu34_ and homozygotes (_GG_) for the factor _EPCR G4678C_ polymorphism could affect the occurrence of ischemic stroke. Other analysed single gene polymorphisms in this study do not represent risk factors in the aetiology of ischemic stroke in patients. However, some results indicate that the _ACE I/D, eNOS-G894T_ heterozygous and _MTHFR C677T CT_ genotype combination may result in a significantly higher risk of FIS in this Albanian population from Macedonia.

This was the first comprehensive study in an Albanian population from the Republic of Macedonia to indicate polymorphic variation in the prevalence of acute FIS. Mutations in these genes in the percentage of patients who have acute FIS have been observed, suggesting that their combination might act in a synergistic and cumulative manner in this Albanian population, the study of which is our next goal and should be tested in groups of different ethnic origin.

In summary, the results of mutant genotype frequency analysis in a sample of Albanians from the Tetovo region can be used for characterization of the current genetic profile of Albanians, anthropological comparisons, and association studies with different diseases.

_____

References

1. Alrajeh SM, Alkali NH. Genetics of ischemic stroke. Neurosciences. 2008; 13(4): 343-349. PMid:21063358

2. Hassan A, Markus HS. Genetics and ischaemic stroke. Brain. 2000; 123(9): 1784-1612. http://dx.doi.org/10.1093/brain/123.9.1784 PMid:10960044

3. Meschia JF, Worral BB, Rich SS. Genetic susceptibility to ischemic stroke. Nat Rev Neurol. 2001; 7(7): 368-378.

4. Himabindu G, Rajasekhar D, Latheef K, Sarma PV, Vanajakshamma V, et al. Factor V Leiden mutation is not a predisposing factor for acute coronary syndromes. Indian Heart J. 2012; 64(6): 570-575. http://dx.doi.org/10.1016/j.ihj.2012.07.006 PMid:23253409 PMCid:PMC3860758

5. Nicolaes GA, Bock PE, Segers K, Wildhagen KC, Dahlback B, Rosing J. Inhibition of thrombin formation by active site mutated (S360A) activated protein. J Biol Chem. 2010; 285(30): 22890-900. http://dx.doi.org/10.1074/jbc.M110.131029 PMid:20484050 PMCid:PMC2906281

6. Kim RJ, Becker RC. Association between factor V Leiden, prothrombin mutation G20210A, and MTHFR C677T mutations and events of the arterial circulatory system: a meta-analysis of published studies. American Heart Journal. 2004; 146(6): 948-957. http://dx.doi.org/10.1016/S0002-8703(03)00519-2

7. Mueller T, Marschon R, Dieplinger B, Haidinger D, Gegenhuber A, Poelz W, et al. Factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations are not associated with chronic limb ischemia: the Linz Peripheral Arterial Disease (LIPAD) study. J Vasc Surg. 2005; 41: 808-815. http://dx.doi.org/10.1016/j.jvs.2005.01.039 PMid:15886665

8. Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. Classification of stroke subtypes. Cerebrovasc Dis. 2009; 27: 493-501. http://dx.doi.org/10.1159/000210432 PMid:19342825

9. Della-Morte D, Pacifici F, Rundek T. Genetic susceptibility to cerebrovascular disease. Curr Opin Lipidol. 2016; 27(2): 185-195. http://dx.doi.org/10.1097/MOL.0000000000000275 PMid:26959706

10. Lusis AJ. Genetic of Atherosclerosis. Trends Genet. 2012; 28(6): 267-275. http://dx.doi.org/10.1016/j.tig.2012.03.001 PMid:22480919 PMCid:PMC3362664

11. Biros E, Karan M, Golledge J. Genetic Variation and Atherosclerosis. Current Genomics. 2008; 9:29-42. http://dx.doi.org/10.2174/138920208783884856 PMid:19424482 PMCid:PMC2674308

12. Hanscombe KB, Traylor M, Hysi PG, Bevan S, Dichgans M, Rothwell PM, Worall BB, Seshadri S, Sudlow C, METASTROKE Consortium; Wellcome Trust Case Control Consortium 2, Williams FM, Markus HS, Lewis CM. Genetic Factors Influencing Coagulation Factor XIII B-Subunit Contribute to Risk of Ischemic Stroke. Stroke. 2015; 46(8): 2069-2074. http://dx.doi.org/10.1161/STROKEAHA.115.009387 PMid:26159793 PMCid:PMC4512747

13. Shemirani AH, Antalfi B, Pongrácz E, Mezei ZA, Bereczky Z, Csiki Z. Factor XIII-A subunit Val34Leu polymorphism in fatal atherothromboric ischemic stroke. Blood Coagul Fibrinolysis. 2014; 25(4): 364-368. http://dx.doi.org/10.1097/MBC.0000000000000055 PMid:24686102

14. Wiklund PG, Nilson L, Ardnor SN, Eriksson P, Johansson L, Stegmayr B, Hamsten A, Holmberg D, Asplund K. Plasminogen activator inhibitor-1 4G/5G polymorphism and risk of stroke: replicated findings in two nested case control studies based on independent cohorts. Stroke. 2005; 36: 1661-1665. http://dx.doi.org/10.1161/01.STR.0000174485.10277.24 PMid:16020771

15. Bang CO, Park HK, Ahn MY, Shin HK, Hwang KY, Hong SY. 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene and insertion/deletion polymorphism of the tissue-type plasminogen activator gene in atherothrombothic stroke. Cerebrovasc Dis. 2001; 11: 294-299. http://dx.doi.org/10.1159/000047656 PMid:11385207

16. de Paula Sabino A, Ribeiro DD, Domingueti CP, Dos Santos MS, Gadelha T, Dusse LM, das Gracas Carvalho M, Fernandes AP. Plasminogen activator inhibitor-1 4G/5G promoter polymorphism and PAI-1 plasma levels in young patients with ischemic stroke. Mol Biol Rep. 2011; 38: 5355-5360. http://dx.doi.org/10.1007/s11033-011-0687-4 PMid:21373825

17. Endler G, Lalouschek W, Exner M, Mitterbauer G, Haring D, Manhalter C. The 4G/4G genotype at nucleotide position -675 in the promoter region of the plasminogen activator inhibitor 1 (PAI-1) gene is less frequent in young patients with minor stroke than in controls. Br J Haematol. 2000; 110: 469-471. http://dx.doi.org/10.1046/j.1365-2141.2000.02164.x PMid:10971410

18. Hoekstra T, Geleijnse JM, Kluft C, Giltay EJ, Kok FJ, Schouten EG. 4G/4G genotype of PAI-1 gene is associated with reduced risk of stroke in elderly. Stroke. 2003; 34: 2822-2828. http://dx.doi.org/10.1161/01.STR.0000098004.26252.EB PMid:14605330

19. Benn M, Nordestgaard BG, Jensen JS, Tybjaerg-Hansen A. Polymorphiss in apoliprotein B and risk of ischemic stroke. J Clin Endocr Metab. 2007; 92: 3611-3617. http://dx.doi.org/10.1210/jc.2007-0221 PMid:17595251

20. Somay G, Misirli H, Guler M, Calişkan N, Erenoğlu YN. Serebrovasculer hastaliklarda apolipoprotein E ve anjiotensin coverting enzim gen polimorfizmi. Turk Beyin Damar Hastaliklari Dergisi. 2002; 8: 113-117.

21. Liu Y, Pan J, Wang S, Li X, Huang Y. Beta-fibrinogen gene -455 A/G polymorphism and plasma fibrinogen level in Chinese stroke patients. Chin Med J (Engl). 2002; 115(2): 214-216.

22. Duan H, Cai Y, Sun X. platelet glycoprotein IIb/IIIa polymorphism HPA-3 b/b is associated with increased risk of ischemic stroke in patients under 60 years of age. Med Sci Monit. 2012; 18: 19-24. http://dx.doi.org/10.12659/MSM.882195 PMCid:PMC3560669

23. Saidi S, Mahjoub T, Slamia LB, Ammou SB, Al-Subaie AM, Almawi WY. Polymorphisms of the human platelet alloantigens HPA-1, HPA-2, HPA-3, and HPA-4 in ischemic stroke. Am J Hematol. 2008; 83: 570-573. http://dx.doi.org/10.1002/ajh.21171 PMid:18383324

24. Zhang Z, Xu G, Liu D, Fan X, Zhu W, Liu X. Angiotensin converting enzyme insertion/deletion polymorphism contributes to ischemic stroke risk: A meta-analysis of 50 case-control studies. Plos One. 2012; 7: 1-9. http://dx.doi.org/10.1371/journal.pone.0046495

25. Kalita J, Somarajan BI, Kumar B, Mittal B, Misra UK. A study of ACE and ADD1 polymorphism in ischemic and hemorrhagic stroke. Clin Chim Acta. 2011; 412: 642-646. http://dx.doi.org/10.1016/j.cca.2010.12.022 PMid:21194526

26. Yao YS, Chang WW, Jin YL, He LP. An updated meta-analysis of endothelial nitric oxide synthase gene: three well-characterized polymorphisms with ischemic stroke. Gene. 2013; 528(2): 84-92. http://dx.doi.org/10.1016/j.gene.2013.06.047 PMid:23845784

_____

27. Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, Sato H, Hori M, Nakamura Y, Tanaka T. Functional SNPSs in the lymphotoksin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet. 2002; 32(4): 650-654. http://dx.doi.org/10.1038/ng1047 PMid:12426569

28. Bajram K. Komparativna analiza na faktorite na rizik i nivnoto vlijanie vrz koncentracijata na plazma homocisteinot vo pojavata na primarniot ishemičen cerebrovaskularen insult vo regionot na Tetovo. [doktorska disertacija]. Skopje: Medicinski fakultet, 2013 [Macedonian].

29. Kamberi B, Kamberi F, Spiroski M. Polymorphisms of Methylenetetrahydrofolate Reductase (MTHFR-677 and MTHFR-1298) Genetic in an Albanian population. European Journal of Neurology. 2014; 21(Suppl. 1):696.

30. Kamberi B, Kamberi F, Spiroski M. Polymorphism in the methylenetetrahydrofolate reductase (C677T) gene and homocysteine levels: a comparison in Albanian patients with acute first-ever ischemic stroke. European Journal of Neurology. 2014; 21(Suppl. 1):535.

31. Towner P. Isolation of DNA by SDS-proteinase K treatment. In: Brown TA, editor. Essential Molecular Biology. New York: Oxford University Press Inc., 1995:52-53.

32. Towner P. Basic of methods for isolation of DNA. In: Brown TA, editor. Essential Molecular Biology. New York: Oxford University Press Inc., 1995:47-49.

33. Spiroski M, Arsov T, Petlichkowski A, Strezova A, Trajkov D, Efinska_Mladenovska O, et al. Case Study: Macedonian human DNA Bank (hDNAMKD) as a source for public health Genetics. in: Georgieva L, Burazeri G, Editors. Health determinants in the scope of new public health. Sofia: Hans Jacobs Company, 2005: 33-44.

34. Lancaster A, Nelson MP, Meyer D, et al. PyPop: a software framework for population genomics: analyzing large-scale multi locus genotype data. Pac Symp Biocomput. 2003; 514-25. PMid:12603054 PMCid:PMC3891851

35. Lancaster AK, Single RM, Solberg OD, et al. PyPop update-a software pipeline large-scale multi locus population genomics. Tissue Antigens. 2007; 69(Suppl. 1): 192-7. http://dx.doi.org/10.1111/j.1399-0039.2006.00769.x PMid:17445199 PMCid:PMC4369784

36. Single RM, Meyer D, Mack SJ, et al. 14th International HLA and Immunogenetics workshop: report of progress in methodology, data collection, and analyses. Tissue Antigens. 2007; 69(Suppl. 1): 185-7. http://dx.doi.org/10.1111/j.1399-0039.2006.00767.x PMid:17445197

37. Hadjiev DI, Mineva PP, Vukov MI. Multiple modifiable risk factors for first ischemic stroke: a population-based epidemiological study. European Journal of Neurology. 2003;10:577-582. http://dx.doi.org/10.1046/j.1468-1331.2003.00651.x PMid:12940842

38. Andersen KK, Andersen ZJ, Olsen TS. Age- and gender-specific prevalence of cardiovascular risk factors in 40,102 patients with first-ever ischemic stroke: a Nationwide Danish Study. Stroke. 2010; 41: 2768-2774. http://dx.doi.org/10.1161/STROKEAHA.110.595785 PMid:20966413

39. Berry JD, Dyer A, Cai X, Garside DB, Ning H, Thomas A, et al. Lifetime risk of cardiovascular disease. N Engl J Med. 2012; 366: 321-329. http://dx.doi.org/10.1056/NEJMoa1012848 PMid:22276822 PMCid:PMC3336876

40. Nedelthchev K, der Maur TA, Georgiadis D, Arnold M, Caso V, Mattle HP, et al. Ischemic stroke in young adults: predictors of outcome and recurrence. J Neurol Neurosurg Psychiatr. 2005; 76: 191-195. http://dx.doi.org/10.1136/jnnp.2004.040543 PMid:15654030 PMCid:PMC1739502

41. Putaala J, Metso AJ, Metso TM et al. Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke: the Helsinki young stroke registry. Stroke. 2009; 40: 1195-1203. http://dx.doi.org/10.1161/STROKEAHA.108.529883 PMid:19246709

42. Seshadri S, et al. Parental occurrence of stroke and risk of stroke in their children: the Framingham study. Circulation. 2010;121:1304-1312. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.854240 PMid:20212282 PMCid:PMC2860311

43. 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: 619-624. http://dx.doi.org/10.1016/j.thromres.2009.07.007 PMid:19660787

44. MacClellan LR, et al. Familial aggregation of ischemic stroke in young women: the Stroke Prevention in Young Women Study. Genet Epidemiol. 2006; 30: 602-608. http://dx.doi.org/10.1002/gepi.20171 PMid:16868965

45. Jerrard-Dunne P, Cloud G, Hassan A, Markus HS. Evaluating the genetic component of ischemic stroke subtypes: a family history study. Stroke. 2003; 34: 1364-1369. http://dx.doi.org/10.1161/01.STR.0000069723.17984.FD PMid:12714707

46. Schulz UG, Flossman E, Rothwell PM. Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. Stroke. 2004; 35: 819-824. http://dx.doi.org/10.1161/01.STR.0000121646.23955.0f PMid:15001788

47. Wang X, et al. A meta-analysis of candidate gene polymorphisms and ischemic stroke in 6 study populations: association of lymphotoxin-alpha in nonhypertensive patients. Stroke. 2009; 40: 683-695. http://dx.doi.org/10.1161/STROKEAHA.108.524587 PMid:19131662 PMCid:PMC2757095

48. Ariyaratnam R, et al. 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. http://dx.doi.org/10.1371/journal.pmed.0040131 PMid:17455988 PMCid:PMC1876409

49. Clark JSC, Adler G, Salkic NN, Ciechanowicz A. Allele frequency distribution of 1691G>A F5 (which confers Factor V Leiden) across Europe, including Slavic populations. J Appl Genet. 2013; 54(4): 441-446. http://dx.doi.org/10.1007/s13353-013-0166-9 PMid:23959593 PMCid:PMC3825156

50. Adler G, Clark JSC, Loniewska B, Czerska E, Salkic NN, Ciechanowicz A. Prevalence of 1691G>A FV mutation in Poland compared with that in other Central, Eastern and South-Eastern European countries. Bosn J Basic Med Sci. 2012; 12(2): 82-87. PMid:22642591 PMCid:PMC4362443

51. Arsov T, Miladinova D, Spiroski M. Factor V Leiden is associated with higher risk of deep venous thrombosis of large blood vessels. Croat Med J. 2006; 47(3): 433-439. PMid:16758522 PMCid:PMC2080416

52. Mekaj Y, Zhubi B, Hoxha H, Belegu R, Mekaj A, Miftari E, Belegu M. Prevalence of resistence to activated protein C (APC-resistence) in blood donors in Kosovo. Bosn J Basic Med Sci. 2009; 9: 329-334. PMid:20002000

53. Atay A, Tekin M, Allajalebeu K, Eğin Y, Akar N. The frequency of FV G1691A and PT G20210A mutations in an Albanian population. Turk J Hematol. 2011; 28: 241-242. http://dx.doi.org/10.5152/tjh.2011.64 PMid:27264377

54. Erten N, Lopaciuk S, Bykowska K, Kwiecinski H, Mickielewicz A, Czlonkowska A, Mendel T, Kuczynska-Zardzewialy A, Szelagowska D, Windyga J, Schroder W, Herrmann FH, Jedrzejowska H. Factor V Leiden, prothrombin gene G20210A variant, and methylenetetrahyfrofolate reductase C677T genotype in young adults with ischemic stroke. Clin Appl Hemost. 2001; 7: 346-350. http://dx.doi.org/10.1177/107602960100700418

55. Casas JP, Hingorani AD, Bautista LE, Sharma P. Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximately 18,000 cases and 58,000 controls. Arch Neurol. 2004; 61:1652-1661. http://dx.doi.org/10.1001/archneur.61.11.1652 PMid:15534175

56. Bhagwat VR, Yadav AS, Rathod IM. Homocysteine, lipid

_____

indices and antioxidants in patients with ischaemic heart disease from Maharashtra, India. Singapore Med J. 2009; 50: 418-24. PMid:19421689

57. Botto L, Yang Q. 5,10-Methyelenetetrahydrofolate reductase gene variants and congenital Anomalies: A HuGE review. Am J Epidemiol. 2000; 151: 862-77. http://dx.doi.org/10.1093/oxfordjournals.aje.a010290 PMid:10791559

58. Ho CH. Prevalence of Prothrombin 20210A and Methylenetetrahydrofolate reductase C677T genetic mutation in the Chinese population. Ann Haematol. 2000; 79: 239-42. http://dx.doi.org/10.1007/s002770050586

59. Radha Rama Devi A, Govindaiah V, Ramakrishna G, Naushad SM. Prevalence of methylene tetrahydrofolate reductase polymorphism in South Indian population. Current Science. 2004; 86: 440-3.

60. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Örning L, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr. 2001; 74: 233-241. PMid:11470726

61. Hermann W, Obeid R, Jouma M. Hyperhomocysteinemia and vitamin B12 deficiency are more striking in Syrians than Germans - causes and implications. Atherosclerosis. 2003; 166: 143-150. http://dx.doi.org/10.1016/S0021-9150(02)00320-9

62. Golbahar J, Fathi Z, Tamadon M. Distribution of 5,10 Methylenetetrahydrofolate reductase (C677T) and its association with red blood cell 5-methyltetrahydrofolate in healthy Iranians. Clin Nutr. 2005; 24: 83-7. http://dx.doi.org/10.1016/j.clnu.2004.07.019 PMid:15681105

63. Eid SS, Rihani GR. Prevalence of factor V Leiden, Prothrombin G20210A and MTHFR C677T mutations in 200 Jordanian Healthy individuals. Clin Lab Sci. 2004; 17: 200-202. PMid:15559724

64. Trimmer EE. Methylene tetrahydrofolate reductase: biochemical characterization and medical significance. Curr Pharm Des. 2013; 19(14): 2574-2593. http://dx.doi.org/10.2174/1381612811319140008 PMid:23116396

65. Den Heijer M, Lewington S, Clarke A. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost. 2005; 3(2): 292-299. http://dx.doi.org/10.1111/j.1538-7836.2005.01141.x PMid:15670035

66. Giovannetti E, Ugrasena DG, Supriyadi E, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T and thymidylate synthase promoter (TSER) polymorphisms in Indonesian children with and without leukemia. Leuk Res. 2008; 32: 19-24. http://dx.doi.org/10.1016/j.leukres.2007.02.011 PMid:17395259

67. Alluri RV, Mohan V, Komandur S, Chawda K, Chaudhuri JR, Hasan Q. MTHFR C677T gene mutation as a risk factor for arterial stroke: A hospital based study. Eur J Neurol. 2005; 12: 40-44. http://dx.doi.org/10.1111/j.1468-1331.2004.00938.x PMid:15613145

68. Panigrahi I, Chatterjee T, Biswas A, Behari M, Choudhry PV, Saxena R. Role of MTHFR C677T polymorphism in ischemic stroke. Neurol India. 2006; 54: 48-50. http://dx.doi.org/10.4103/0028-3886.24703 PMid:16679643

69. Kalita J, Srivastava R, Bansal V, Agarwal S, Misra UK. MTHFR reductase gene polymorphism in Indian stroke patients. Neurology India. 2006; 54(3): 260-263. http://dx.doi.org/10.4103/0028-3886.27148 PMid:16936384

70. Zheng YZ, Tong J, Do XP, Pu XQ, Zhou BT. Prevalence of methylenetetrahydrofolate reductase C677T and its association with arterial and venous thrombosis in the Chines population. Br J Haematol. 2000; 109: 870-874. http://dx.doi.org/10.1046/j.1365-2141.2000.02112.x PMid:10929044

71. Ucar F, Sommez M, Ovali E, Ozmenoglu M, Karti SS, Yilmaz M et al. MTHFR C677T polymorphism and its relation to ischemic stroke in the Black Sea Turkish population. Am J Hematol. 2004;76: 40-43. http://dx.doi.org/10.1002/ajh.20050 PMid:15114595

72. Angeline T, Jeyaraj N, Granito S, Tsongalis GJ. Prevalence of MTHFR gene polymorphisms (C677T and A1298C) among Tamilians. Exp Mol Pathol. 2004; 77: 85-88. http://dx.doi.org/10.1016/j.yexmp.2004.04.006 PMid:15351230

73. Szolnoki Z, Somogyvani F, Kondacs A, Szabo M, Fodor L, Bene J et al. Specific APO E genotypes in combination with the ACE D/D or MTHFR C677T mutation yield an independent genetic risk factor of leukoaraiosis. Acta Neurol Scand. 2004; 109: 222-227. http://dx.doi.org/10.1046/j.1600-0404.2003.00218.x PMid:14763962

74. Spiroski I, Kedev S, Antov S, Arsov T, Krstevska M, Dzhekova-Stojkova S, Bosilkova G, Kostovska S, Trajkov D, Petlichkovski A, Strezova A, Efinska-Mladenovska O, Spiroski M. Methylenetetrahydrofolate reductase (MTHFR-677 and MTHFR-1298) genotypes and haplotypes and plasma homocysteine levels in patients with occlusive artery disease and deep venous thrombosis. Acta Biochim Pol. 2008; 55(3): 587-594. PMid:18800176

75. Spiroski I, Kedev S, Antov S, Arsov T, Krstevska M, Dzhekova-Stojkova S, Kostovska S, Trajkov D, Petlichkovski A, Strezova A, Efinska-Mladenovska O, Spiroski M. Association of methylenetetrahydrofolate reductase (MTHFR-677 and MTHFR-1298) genetic polymorphisms with occlusive artery disease and deep venous thrombosis in Macedonians. Croat Med J. 2008; 49(1): 39-49. http://dx.doi.org/10.3325/cmj.2008.1.39 PMid:18293456 PMCid:PMC2269242

76. Spiroski I, Kedev S, Antov S, Trajkov D, Petlichkovski A, Dzhekova-Stojkova S, Kostovska S, Spiroski M. Investigation of SERPINE 1 genetic polymorphism in Macedonian patients with occlusive artery disease and deep venous thrombosis. Kardiol Pol. 2009; 67: 1088-1094. PMid:20017074

77. Kamberi B, Kamberi G. Traditional Eating Habits and Level of Homocysteine in the Acute First-Ever Ischaemic stroke. Maced J Med Sci. 2012; 5(2): 181-188. http://dx.doi.org/10.3889/MJMS.1957-5773.2012.0231

78. 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 of the risk of ischemic stroke in young adults. Stroke. 2005; 36: 533-539. http://dx.doi.org/10.1161/01.STR.0000155741.31499.c2 PMid:15692115

79. Szolnoki Z, Somogyvani F, Kondacs A, Szabo M, Fodor L, Bene J et al. Evaluation of the modifying effects of unfavorable genotypes on classical clinical risk factors for ischaemic stroke. Neurol Neurosurg Psychiatr. 2003; 74: 1615-1620. http://dx.doi.org/10.1136/jnnp.74.12.1615 PMCid:PMC1757435

80. Spiroski I, Kedev S, Efinska-Mladenovska O. Factor V Leiden (G1691A), Factor V R2 (A4070G), and Prothrombin (G20210A) Genetic Polymorphisms in Macedonian Patients with Occlusive Artery Disease and Deep Vein Thrombosis. SEE J Cardiol. 2015; 2015:30001. http://dx.doi.org/ 10.3889/seejca.2015.30001

_____