



Research article

Connection between small vessel disease related stroke and the MTHFR C677T polymorphism in a Hungarian population



Ádám Annus^a, Lilla Ágnes Juhász^a, Elza Szabó^a, Ferenc Rárosi^b, László Szpisjak^a,
László Vécsei^{a,c}, Péter Klivényi^{a,*}

^a University of Szeged, Albert Szent-Györgyi Health Centre, Department of Neurology, H-6725, Szeged, Semmelweis u. 6, Hungary

^b University of Szeged, Department of Medical Physics and Informatics, H-6720, Szeged, Korányi fasor 9, Hungary

^c MTA-SZTE Neuroscience Research Group, H-6725, Szeged, Semmelweis u. 6, Hungary

ARTICLE INFO

Keywords:

Circulatory system
Nervous system
Medical imaging
Radiology
Genetics
Clinical genetics
Neurology
Clinical research

ABSTRACT

Introduction: There are conflicting results in the literature regarding the connection between thrombophilias and ischaemic stroke. However, most of the clinical studies have not differentiated between various ischaemic stroke subtypes. Our aim was to investigate whether there is an association between the methylene tetrahydrofolate reductase (MTHFR) C677T polymorphism and ischaemic stroke due to small vessel disease (SVD) in patients ≤ 50 years of age.

Patients and methods: We performed a retrospective search in the database used at our Health Centre. Our study population consisted of 100 ischaemic stroke patients. 65 patients had MTHFR C677T variants: 21 were homozygous (TT allele), 45 were heterozygous (CT). 35 stroke patients did not carry MTHFR C677T polymorphism (wild genotype, CC). Stroke subtypes were determined according to the TOAST classification. Pearson's chi-squared test of independence was used to evaluate differences between subgroups and multivariate logistic regression was also performed.

Results: More than half of our study population (52.00%) had lacunar strokes. The ratio of SVD in patients ≤ 50 years of age with TT homozygous variant was significantly higher compared to heterozygous and wild type subjects ($p = 0.032$ and $p = 0.03$ respectively). Multivariate logistic regression also showed, that apart from hypertension, only TT homozygosity was a predictive factor for SVD related stroke ($p = 0.014$, OR 1.619, 95% CI 1.390–18.338).

Conclusion: Our results demonstrate that in a Hungarian population of ischaemic stroke patients ≤ 50 years of age, SVD is the most common stroke subtype. In addition, we found association of SVD stroke with hypertension and MTHFR 677TT homozygous polymorphism.

1. Introduction

Thrombophilias are acquired or inherited hypercoagulable states, which predispose patients to arterio-venous thrombotic events [1, 2]. It would seem logical to assume that thrombophilias play a role in the development of ischaemic stroke. However, for the time being, there is inconclusive evidence in the literature regarding the connection between thrombophilias and ischaemic stroke, even in young patients [3]. For instance, the AHA/ASA guideline for secondary ischaemic stroke or transient ischaemic attack (TIA) prevention published in 2014, determines the usefulness of searching for thrombophilias as “unknown” [4]. Consequently, therapeutic recommendations are uncertain in such

conditions. However, it should be kept in mind that the majority of the studies that investigated the association between ischaemic stroke and thrombophilias did not differentiate between different stroke subtypes [5, 6, 7, 8].

Hyperhomocysteinaemia (hHcy) is considered as a risk factor for cardiovascular diseases [8, 9, 10]. A strong predisposing factor to hHcy is the polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene at position 677. CC genotype is the normal variant, and T is the pathologic allele [8, 10]. This genetic variant is one of the commonest forms of inherited thrombophilias [11]. Based on the gnomAD database (<https://gnomad.broadinstitute.org>), T allele frequency in all populations is 0.3085. There is growing evidence in the literature, that hHcy

* Corresponding author.

E-mail address: klivenyi.peter@med.u-szeged.hu (P. Klivényi).

<https://doi.org/10.1016/j.heliyon.2020.e05305>

Received 11 June 2020; Received in revised form 6 August 2020; Accepted 15 October 2020

2405-8440/© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

and MTHFR C677T polymorphism are mainly associated with cerebral small vessel disease (SVD) [8, 12, 13].

The aim of our study was to evaluate whether SVD related stroke is more frequent compared to other subtypes in patients with MTHFR C677T polymorphism in Hungarian ischaemic stroke patients ≤ 50 years of age. We aimed to investigate a young stroke population to minimize the chronic atherothrombotic effects of traditional vascular risk factors (e.g.: hypertension, hyperlipidaemia, diabetes mellitus, etc.), which are more pronounced in the elderly and would allow for confounding.

2. Patients and methods

2.1. Study population

Our work is a single centre, retrospective, observational study. We did a retrospective search in the e-Medsolution system used at our Health Centre to look for patients ≤ 50 years of age who had a definite ischaemic stroke and underwent MTHFR genotyping since 2007. We used 50 years of age as cut-off value since stroke in young adults is usually defined accordingly in most epidemiological studies [14, 15, 16]. During the study period, there was no institutional protocol for the indication of MTHFR genotyping. It was left to the discretion of the treating physician and was not guided by aetiology. We found 100 patients who fit the inclusion criteria. MTHFR C677T polymorphism was found in 65 out of 100 patients (65.00%). 21 had homozygous (TT, 21.00%) and 41 had heterozygous (CT, 41.00%) variants. 35 patients did not have MTHFR C677T polymorphism (CC, wild-type subjects, 35.00%). The diagnosis of ischaemic stroke was set up by an expert in neurology based on clinical presentation and radiological findings. To determine the stroke subtype, we applied the Trial of Org 10172 in Acute Stroke Treatment – TOAST classification [17]. 85 patients (85.00%) had MRI and 15 participants (15.00%) had CT imaging (with or without angiography). Electrocardiography, carotid ultrasonography and transthoracic echocardiography were performed in all patients. The diagnosis of lacunar stroke was made if CT or MRI imaging showed an acute or subacute small vessel territory ischaemia less than 1.5 cm in diameter which correlated with the symptoms of the patient. Where the infarct was not clearly visible on brain imaging, lacunar stroke was diagnosed if there was no evidence of large-artery atherosclerosis (LAA) or other determined aetiology (e.g.: dissection), no potential source of cardioembolic stroke (CE), the clinical symptoms correlated with one of the typical lacunar syndromes and leukoaraiosis was seen in cases where MRI scans were available [18]. Peripheral blood samples were obtained from all participants to determine the MTHFR genotype after informed consent was provided. The study was conducted in accordance with ethical regulations disclosed in the GINOP (2.3.2-15- 2016-00048) project and approved by Medical Research Council of Hungarian Ministry of Human Resources (35403-2/2017/EKU). Informed consent was obtained from all patients participated in this study.

Hypertension was defined as systolic and/or diastolic blood pressure ≥ 140 and 90 mmHg respectively (based on 3 independent measures). Patients taking anti-hypertensive medications before stroke were considered hypertensive. Hyperlipidaemia was diagnosed if: a) the patient had already taken statins or fenofibrate, b) serum cholesterol was ≥ 5.20 mmol/l, c) triglyceride level was ≥ 1.70 mmol/l or, d) low-density lipoprotein (LDL) level was ≥ 3.00 mmol/l. Diabetes mellitus was diagnosed if fasting plasma glucose was ≥ 7.00 mmol/l or haemoglobin A1c level was $\geq 6.50\%$. Patients taking antidiabetic medications (oral or injective) were also included. History of continuous smoking in the past or tobacco use at the time of stroke presentation was also addressed.

2.2. Laboratory analysis

Peripheral venous blood samples were taken into Vacutainer™ tubes containing EDTA. Following genomic DNA isolation, MTHFR C677T

genotyping was performed using real-time PCR with LightCycler probe system (Roche).

2.3. Statistical analysis

Continuous variables were expressed as mean \pm SD. Mean of continuous variable (age) was compared within the MTHFR C677T genotype and TOAST aetiology subgroups with Welch's ANOVA. For categorical variables Pearson's chi-squared test of independence was applied. Where sample sizes were small (i.e. equal to, or less than 5) we used Fisher's exact test. Further analysis was carried out with multivariate logistic regression. All vascular risk factors (male sex, age, hypertension, diabetes mellitus, hyperlipidaemia, smoking and MTHFR polymorphism) were taken into account as candidate predictors of SVD. Backward likelihood ratio model selection method was applied. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Statistical significance was met when p-value was < 0.05 . For statistical analysis, IBM SPSS version 25 statistical software was used.

3. Results

Table 1 summarizes the baseline characteristics of our study population. We believe that the groups are well matched, although more hyperlipidaemic patients were included in the wild-type group. Age distribution of the study population is shown in Figure 1.

An interesting finding was that more than half of our study population (52.00%) had SVD related stroke. The second most common aetiology was cryptogenic stroke (24.00%). Table 2 highlights the distribution of stroke subtypes in homozygous, heterozygous and wild type patients. Table 3 demonstrates the risk factor profile of patients with SVD and non-SVD related stroke.

We found that TT homozygous patients had significantly higher proportion of strokes due to SVD compared to heterozygous and wild-type subjects ($p = 0.032$ and $p = 0.03$ respectively). Heterozygous patients did not have more SVD related strokes compared to wild-type subjects ($p = 0.581$). Multivariate regression analysis demonstrated the association of SVD related stroke with hypertension ($p = 0.002$, OR 1.378, 95% CI 1.624–9.683) and MTHFR TT homozygosity ($p = 0.014$, OR 1.619, 95% CI 1.390–18.338).

The frequency of T allele in our study population was 0.4300. When we examined the allele frequency of T in patients with SVD (0.5000), compared to patients with non-SVD related strokes (0.3542), the difference was statistically significant ($p = 0.037$).

4. Discussion

Polymorphism in the MTHFR gene at position 677 leads to reduced enzymatic activity of the MTHFR enzyme [19]. Consequently, there is a reduced conversion of N [5],N¹⁰-methylene tetrahydrofolate to N [5]-methyl tetrahydrofolate. The latter is required for homocysteine (Hcy) to be metabolised to L-methionine (Met) by methionine-synthase [20]. Therefore, it is believed that MTHFR C677T polymorphism is a strong predisposing factor for hHcy [8, 10]. The conversion of Hcy to Met also requires folic acid and vitamin B12 as cofactors, explaining why diet can have an impact on Hcy concentrations. A limitations of our study is that we did not measure Hcy, folic acid or vitamin B12 levels. It is important to note however, that serum Hcy levels are also influenced by a variety of comorbidities (e.g.: hyperlipidaemia, renal and hepatic insufficiency) and medications (metformin, fenofibrate, thiazide diuretics) [21]. Nonetheless, a mendelian randomisation analysis of European-descent ischaemic stroke patients found that genetically higher blood Hcy levels are associated with SVD related stroke, but not with large artery stroke or cardioembolic stroke [22].

An unexpected finding of our study was the high percentage (52.00%) of SVD related stroke. Yesilot et al. reported that among European patients with first-ever ischaemic stroke aged between 42 to 49 years, SVD

Table 1. Demographic data of our study population.

	Homozygous (n = 21)	Heterozygous (n = 44)	Wild type (n = 35)	p value
Mean age \pm SD (years)	37.81 \pm 9.92	41.68 \pm 6.95	42.63 \pm 5.85	0.143
Male	14 (66.67%)	22 (50.00%)	25 (71.43%)	0.139
HT	10 (47.62%)	26 (59.09%)	20 (57.14%)	0.692
DM	1 (4.76%)	5 (11.36%)	5 (14.29%)	0.421
HL	8 (38.09%)	15 (34.09%)	24 (68.57%)	0.007
Smoking	10 (47.62%)	20 (45.45%)	20 (57.14%)	0.581

Abbreviations: SD: standard deviation, HT: hypertension, DM: diabetes mellitus, HL: hyperlipidaemia.

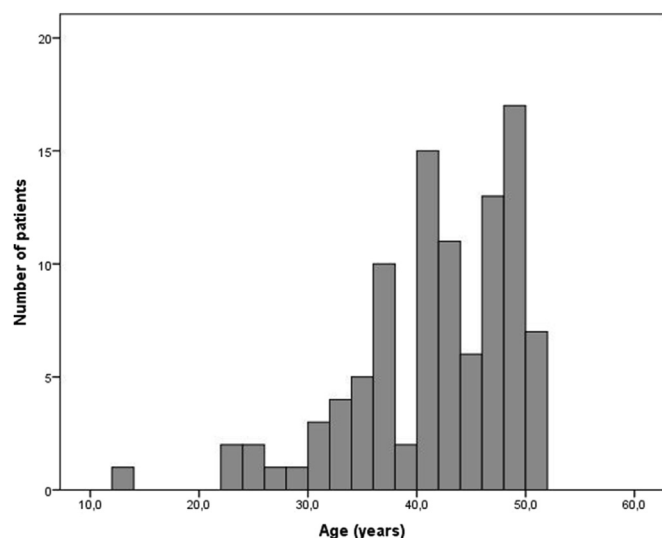


Figure 1. Age distribution of our study population.

was responsible for 17% of all strokes [23]. Aetiology remained undetermined in 38% in the same cohort, compared to our 24.00%. One possible explanation for this finding may be the high prevalence of hypertension in our study (56.00%), since hypertension is the most

important risk factor for SVD related stroke. Our regression analysis also demonstrated this association. Putaala et al. published that in a European population of ischaemic stroke patients under 50 years of age, the prevalence of hypertension was 35.9% [24]. Therefore, we can conclude that hypertension was more common in our cohort. The reason behind this finding however, is unclear. Bias in patient selection due to the retrospective nature of our study cannot be ruled out. The lower socio-economic status of the population of South Eastern Hungary compared to most of the regions that participated in the study by Putaala et al. could also be a factor in the higher prevalence of hypertension [24, 25].

A novelty of our study is that we have examined the connection between the MTHFR C677T genotype and stroke subtypes in a young ischaemic stroke population. Both the chi-squared test and logistic regression analysis demonstrated association of SVD with MTHFR homozygous polymorphism. MTHFR C677T heterozygosity was not a risk factor for SVD.

An important limitation of our study is that genetic testing was left to the discretion of the treating physician. Consequently, it is possible that not all patients who fit our inclusion criteria underwent MTHFR C677T genotyping at our Health Centre since 2007. This, and the retrospective design of our study could contribute to a potential bias in patient selection and therefore interpretation of the prevalence of SVD related stroke.

Another shortcoming of our paper is that not all patients had MRI scans, although a fairly high percent underwent MRI imaging (85.00%). In patients who were diagnosed as having SVD related stroke, 90.38%

Table 2. Distribution of stroke subtypes.

TOAST classification	Homozygous (n = 21)	Heterozygous (n = 44)	Wild type (n = 35)	Total (n = 100)
LAA	2 (9.52%)	3 (6.82%)	2 (5.71%)	7 (7.00%)
CE	3 (14.29%)	2 (4.55%)	1 (2.86%)	6 (6.00%)
SVD	16 (76.19%)	20 (45.4549.33%)	16 (45.71%)	52 (52.00%)
O	0 (0.00%)	7 (15.91%)	4 (11.43%)	11 (11.00%)
Cr	0 (0.00%)	12 (27.27%)	12 (34.29%)	24 (24.00%)

Abbreviations: LAA: large-artery atherosclerosis, CE: cardioembolic, SVD: small vessel disease, O: other determined aetiology, Cr: cryptogenic.

Table 3. Risk factor profile of patients with SVD and non-SVD related stroke.

	SVD (n = 52)	non-SVD (n = 48)	p value
Mean age \pm SD (years)	42.31 \pm 7.94	40.00 \pm 6.82	0.121
Male	33 (63.46%)	28 (58.33%)	0.599
HT	36 (69.23%)	20 (41.67%)	0.006
DM	7 (13.46%)	4 (8.33%)	0.529
HL	28 (53.85%)	19 (39.58%)	0.153
Smoking	25 (48.08%)	25 (52.08%)	0.689
MTHFR C677T homozygosity	16 (30.77%)	5 (10.42%)	0.015
MTHFR C677T heterozygosity	20 (38.46%)	24 (50.00%)	0.246
MTHFR C677T wild type	16 (30.77%)	19 (39.58%)	0.356

Abbreviations: SD: standard deviation, HT: hypertension, DM: diabetes mellitus, HL: hyperlipidaemia, MTHFR: methylene tetrahydrofolate reductase, SVD: small vessel disease.

had MRI scans. We believe that this high percentage reduces the risk of a potential bias in the determination of SVD, since lacunes and white-matter hyperintensities can be well visualized on DWI and FLAIR images. The allele frequency of T was higher in our study population (0.4300) compared to the allele frequency reported in the general non-Finnish European population (0.3380, based on gnomAD). This difference was statistically significant ($p = 0.006$), and implies that the T allele is more frequent in ischaemic stroke patients. In a meta-analysis, MTHFR C677T polymorphism was associated with a graded, dose-dependent increase in ischaemic stroke risk, and the pooled risk estimate in TT homozygotes was twice compared to CT heterozygotes [26]. Our results also demonstrate that allele frequency of T is significantly higher among ischaemic stroke patient due to SVD compared to non-SVD.

A few other studies have investigated the connection between MTHFR C677T polymorphism and ischaemic stroke subtypes. Choi et al. demonstrated an association between hHcy, TT homozygous variant and small artery occlusion [8]. The study population only included Korean subjects, and the mean age of patients in the stroke arm were higher compared to our patients (61.4 years). Interestingly, a more significant connection was found in patients who suffered multiple lacunar strokes compared to those with single artery occlusion. Due to the small sample size of our study, we did not perform subgroup analysis to test this hypothesis.

Another study group found that the MTHFR C677T genotype is significantly associated with lacunar stroke and white matter hyperintensity volume in hypertensive individuals, but not in normotensive subjects [13]. As expected, the association was more prominent in TT homozygous patients. This meta-analysis included stroke or TIA patients in all age groups. As mentioned before, the high percentage of SVD related stroke in our population might be due to the high prevalence of hypertension.

Hassan and colleagues were able to demonstrate a connection between MTHFR C677T variant and ischaemic leukoaraiosis, but not with isolated lacunar stroke. Mean age of the patients with cerebral small vessel disease was 67.1 years [27]. Eikelboom et al. did not find a connection between MTHFR genotype and ischaemic stroke subtypes [28]. Instead, they demonstrated that hHcy was significantly associated with large-artery atherosclerosis, and to a lesser extent, with cerebral small vessel disease.

In conclusion, we believe that our study further supports a role for MTHFR C677T polymorphism in the development of cerebral small vessel disease. To our knowledge, we are the first to report this association in young ischaemic stroke patient, i.e. ≤ 50 years of age. Furthermore, in a subset of Hungarian patients, the frequency of SVD related stroke was much higher than expected.

The VITATOPS (VITamins TO Prevent Stroke) trial demonstrated a modest benefit of vitamin supplementation (daily administration of 2 mg folic acid, 25 mg vitamin B6, 0.5 mg vitamin B12) in reducing the risk of stroke, myocardial infarction and vascular death in patients who previously had symptomatic cerebral small vessel disease (RR 0.80, 95% CI 0.67–0.96) [29]. Therefore, in our opinion, it is reasonable to check the MTHFR C677T genotype, Hcy, folic acid and vitamin B12 levels in young ischaemic stroke patients with SVD since vitamin supplementation as part of secondary stroke prevention therapy might reduce the risk of stroke recurrence.

Declarations

Author contribution statement

Á. Annus: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

L. Juhász and E. Szabó: Performed the experiments.

F. Rárosi and L. Szpisjak: Analyzed and interpreted the data.

L. Vécsei: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

P. Klivényi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the project GINOP (2.3.2-15- 2016-00048), and the MTA-SZTE Neuroscience Research Group of the Hungarian Academy of Sciences and the University of Szeged.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- [1] D. Green, Thrombophilia and stroke, *Top. Stroke Rehabil.* 10 (2003) 21–33.
- [2] K.W. Ng, P.K. Loh, V.K. Sharma, Role of investigating thrombophilic disorders in young stroke, *Stroke Res. Treat.* 2011 (2011) 670138.
- [3] J.G. Morris, S. Singh, M. Fisher, Testing for inherited thrombophilias in arterial stroke: can it cause more harm than good? *Stroke* 41 (2010) 2985–2990.
- [4] W.N. Kernan, B. Ovbiagele, H.R. Black, et al., Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association, *Stroke* 45 (2014) 2160–2236.
- [5] S. Sastry, G. Riding, J. Morris, et al., Young adult myocardial infarction and ischemic stroke: the role of paradoxical embolism and thrombophilia (the YAMIS study), *J. Am. Coll. Cardiol.* 48 (2006) 686–691.
- [6] W. Lalouschek, M. Schillinger, K. Hsieh, et al., Matched case-control study on factor V Leiden and the prothrombin G20210A mutation in patients with ischemic stroke/transient ischemic attack up to the age of 60 years, *Stroke* 36 (2005) 1405–1409.
- [7] M. Margaglione, G. D'Andrea, N. Giuliani, et al., Inherited prothrombotic conditions and premature ischemic stroke: sex difference in the association with factor V Leiden, *Arterioscler. Thromb. Vasc. Biol.* 19 (1999) 1751–1756.
- [8] B.O. Choi, N.K. Kim, S.H. Kim, et al., Homozygous C677T mutation in the MTHFR gene as an independent risk factor for multiple small-artery occlusions, *Thromb. Res.* 111 (2003) 39–44.
- [9] R. Clarke, L. Daly, K. Robinson, et al., Hyperhomocysteinemia: an independent risk factor for vascular disease, *N. Engl. J. Med.* 324 (1991) 1149–1155.
- [10] C.J. Boushey, S.A. Beresford, G.S. Omenn, A.G. Motulsky, A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes, *Jama* 274 (1995) 1049–1057.
- [11] N. Graham, H. Rashid, B.J. Hunt, Testing for thrombophilia: clinical update, *Br. J. Gen. Pract. : J. Roy. Coll. Gen. Pract.* 64 (2014) e120–e122.
- [12] K. Fassbender, O. Mielke, T. Bertsch, B. Nafe, S. Froschen, M. Hennerici, Homocysteine in cerebral macroangiopathy and microangiopathy, *Lancet* 353 (1999) 1586–1587.
- [13] L.C. Rutten-Jacobs, M. Traylor, P. Adib-Samii, et al., Association of MTHFR C677T genotype with ischemic stroke is confined to cerebral small vessel disease subtype, *Stroke* 47 (2016) 646–651.
- [14] M.S. Ekker, E.M. Boot, A.B. Singhal, et al., Epidemiology, aetiology, and management of ischaemic stroke in young adults, *Lancet Neurol.* 17 (2018) 790–801.
- [15] C.A. Stack, J.W. Cole, A diagnostic approach to stroke in young adults, *Curr. Treat. Options Cardiovasc. Med.* 19 (2017) 84.
- [16] E. Boot, M.S. Ekker, J. Putaala, S. Kittner, F.E. De Leeuw, A.M. Tuladhar, Ischaemic stroke in young adults: a global perspective, *J. Neurol. Neurosurg. Psychiatr.* 91 (2020) 411–417.
- [17] H.P. Adams Jr., B.H. Bendixen, L.J. Kappelle, et al., Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment, *Stroke* 24 (1993) 35–41.
- [18] C. Loeb, The lacunar syndromes, *Eur. Neurol.* 29 (Suppl 2) (1989) 2–7.
- [19] B.I. Somarajan, J. Kalita, B. Mittal, U.K. Misra, Evaluation of MTHFR C677T polymorphism in ischemic and hemorrhagic stroke patients. A case-control study in a Northern Indian population, *J. Neurol. Sci.* 304 (2011) 67–70.
- [20] B.N. Manolescu, E. Oprea, I.C. Farcasanu, M. Berteanu, C. Cercasov, Homocysteine and vitamin therapy in stroke prevention and treatment: a review, *Acta Biochim. Pol.* 57 (2010) 467–477.
- [21] A.S. Wierzbicki, Homocysteine and cardiovascular disease: a review of the evidence, *Diabetes Vasc. Dis. Res.* 4 (2007) 143–150.
- [22] S.C. Larsson, M. Traylor, H.S. Markus, Homocysteine and small vessel stroke: a mendelian randomization analysis, *Ann. Neurol.* 85 (2019) 495–501.

- [23] N. Yesilot Barlas, J. Putaala, U. Waje-Andreassen, et al., Etiology of first-ever ischaemic stroke in European young adults: the 15 cities young stroke study, *Eur. J. Neurol.* 20 (2013) 1431–1439.
- [24] J. Putaala, N. Yesilot, U. Waje-Andreassen, et al., Demographic and geographic vascular risk factor differences in European young adults with ischemic stroke: the 15 cities young stroke study, *Stroke* 43 (2012) 2624–2630.
- [25] B. Leng, Y. Jin, G. Li, L. Chen, N. Jin, Socioeconomic status and hypertension: a meta-analysis, *J. Hypertens.* 33 (2015) 221–229.
- [26] S. Cronin, K.L. Furie, P.J. Kelly, Dose-related association of MTHFR 677T allele with risk of ischemic stroke: evidence from a cumulative meta-analysis, *Stroke* 36 (2005) 1581–1587.
- [27] A. Hassan, B.J. Hunt, M. O'Sullivan, et al., Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction, *Brain : J. Neurol.* 127 (2004) 212–219.
- [28] J.W. Eikelboom, G.J. Hankey, S.S. Anand, E. Lofthouse, N. Staples, R.I. Baker, Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke, *Stroke* 31 (2000) 1069–1075.
- [29] V.T.S. Group, B vitamins in patients with recent transient ischaemic attack or stroke in the VITamins TO Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial, *Lancet Neurol.* 9 (2010) 855–865.