JNN

ORIGINAL ARTICLE

pISSN 2635-425X eISSN 2635-4357
https://doi.org/10.31728/jnn.2021.00106

Relationship between Methylenetetrahydrofolate Reductase C677T Homozygous Mutation and Cerebral Small Vessel Disease Subtypes

Jung Hoon Han, MD; Han Jun Kim, MD; Kyungmi Oh, MD, PhD; Chi Kyung Kim, MD, PhD

Department of Neurology, Korea University Guro Hospital, Korea University College of Medicine, Seoul, Korea

Background: Neuroimaging detects cerebral small vessel disease (CSVD) subtypes, including infarction, asymptomatic lacunes, cerebral microbleeds, white matter hyperintensities (WMHs), and enlarged perivascular space. Methylenetetrahydrofolate reductase (MTHFR) plays an essential role in the metabolism of folic acid and homocysteine. The purpose of this study was to investigate the relationship between the MTHFR C677T mutation and CSVD subtypes.

Methods: A total of 144 patients with acute ischemic stroke who visited the Korea University Guro Hospital between April 2020 and August 2020 were retrospectively reviewed. After excluding 24 patients, due to missing laboratory, clinical, or imaging information, a total of 120 patients were analyzed.

Results: Among the 120 participants, 25% were included in the MTHFR C677T homozygous mutation group, which had significantly lower folic acid levels (6.24±4.21 ng/mL vs. 8.24±4.21 ng/mL, p=0.03) and higher total homocysteine levels (17.09±14.07 μmol/L vs. 9.65±3.19 μmol/L, p<0.01). Using multiple logistic regression analysis, the homozygous mutation (adjusted odds ratio [aOR]=4.29; 95% confidence interval [CI]=1.16–15.90) and age (aOR=1.06; 95% CI=1.01–1.11) were independently associated with moderate to severe WMHs. Additionally, moderate to severe WMHs were more frequent in the homozygous mutation group (86.7% vs. 66.7%, p=0.01). In a detailed analysis, the homozygous mutation group showed a significantly higher rate of moderate to severe periventricular WMH (PWMH) (86.7% vs. 65.6%, p<0.01).

Conclusion: The MTHFR C677T homozygous mutation was positively correlated with moderate to severe PWMH subtypes of CSVD.

J Neurosonol Neuroimag 2021;13(2):64-70

Key Words: Methylenetetrahydrofolate reductase; Polymorphism, single nucleotide; Cerebral small vessel diseases

Received: November 8, 2021
Revised: December 13, 2021
Accepted: December 13, 2021

Correspondence:
Chi Kyung Kim, MD

Department of Neurology, Korea University Guro Hospital, Korea University College of Medicine, 148 Gurodong-ro, Guro-gu, Seoul 08308, Korea
Tel: +82-2-2626-1250
Fax: +82-2-2626-2249
E-mail: ckkim7@korea.ac.kr

INTRODUCTION

Cerebral small vessel disease (CSVD) describes a pathological process that occurs in small arteries, arterioles, capillaries, and small veins of the brain.[1] CSVD encompasses recent infarction, asymptomatic lacunes, cerebral microbleeds (CMBs), white matter hyperintensities (WMHs), and enlarged perivascular space that are detected used neuroimaging with sharing of the risk factors.[2] CSVD is associated with various neurological conditions such as dementia, cognitive decline, gait disturbance, mood disorder, and stroke, thus emphasizing its clinical importance.[3]

Homocysteine is a sulfur-containing amino acid, which is required for methionine and cysteine biosynthesis.[4] Appropriate levels of homocysteine are utilized as important precursors for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis. Homocysteine also participates in other crucial functions, such as controlling cell cycles and producing proteins by

contributing to methylation at a cellular level.[5] Several studies[6-8] have determined the relationship between elevated homocysteine and arterial steno-occlusion, including studies on homozygous homocystinuria children.[9] Elevated homocysteine is an independent cerebrovascular risk factor, particularly of CSVD.[10] Major clinical trials have investigated whether lowering homocysteine levels can promote favorable cardiovascular events; however, most of these trials were unsuccessful.[11-13]

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that plays an essential role in the metabolism of folic acid and homocysteine. Its encoding gene is known to have 12 exons and is found on chromosome p36.3, with 14 single nucleotide polymorphisms related to its enzyme activity. Among them, the MTHFR C677T mutation is a single nucleotide substitution that occurs in exon 4. It is the most widely studied mutation to date. The heterozygous and homozygous mutations at this position lead to a reduction of enzyme activity to 67% and 25%, respectively, compared to the wild type.[14] The MTHFR C667T mutation has been detected in approximately 60% of a healthy population and in 70% of an ischemic stroke population in South Korea.[15] Regarding ischemic stroke, it has been reported that the MTHFR gene mutation is associated with cerebral infarction, especially small vessel occlusion.[16] Therefore, the purpose of this study is to determine whether there is an association between the MTHFR C677T gene mutation and various CSVD subtypes in acute cerebral ischemic stroke patients.

SUBJECTS AND METHODS

1. Patient collection

Data of 144 patients who were diagnosed with acute ischemic stroke (AIS) and visited the Korea University Guro Hospital between April 2020 and August 2020 were retrospectively collected. All patients underwent diffusion-weighted imaging (DWI) within 12 h from entering our center. Of these 144 subjects, 24 patients that did not have either laboratory, clinical, or imaging information were excluded, and a total of 120 cases were subsequently analyzed.

2. Definitions

AIS was specified for cases that showed hyperintensity lesions (*b* value=1,000) along with correlating low signal intensities on the apparent diffusion coefficient map on DWI,[17] as well as if the patient visited our center within seven days following the onset of stroke-related symptoms.[18] The researchers classified AIS according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST)[19] and four groups of large artery atherosclerosis, cardio-embolism, small vessel occlusion, and other or undetermined etiology were classified for analysis. WMHs were defined as lesions with hyperintense signals detected in T2 or fluid-attenuated inversion recovery (FLAIR) of magnetic resonance images in the subcortical area without T1 signal change. The severity of WMHs was categorized into mild, moderate, and severe groups representing 1, 2, and 3 of the Fazeka scale, respectively.[20,21] The severity of WMHs was also analyzed using Fazeka's scale for periventricular WMH (PWMH) and deep WMH (DWMH).[20] CMBs were specified as a low signal intensity lesion of 5–10 mm in size with a round shape on gradient echo (GRE) or susceptibility-weighted imaging (SWI).[22] Its presence was further evaluated according to subcortical and cortical areas. Asymptomatic chronic lacunar infarction was defined as a lesion <20 mm in size and was located in the subcortical area showing hyperintensity on T2 or FLAIR images along with a low signal intensity on T1, which should be a distinct lesion from the index ischemic stroke area on DWI.[23]

3. Data collection and interpretation

For all patients, DWI, GRE, and cerebral computed tomography angiography were taken within 12 hours. FLAIR, SWI, and cerebral magnetic resonance angiography were taken within 36–48 hours. Transthoracic echocardiography and Holter-EKG were performed to screen for possible cardioembolic sources. Image interpretation was performed by two physicians (J.H., H.J.) with over 7 years of experience in the Department of Neurology, resulting in strong interrater reliability (κ=0.81). Age, female sex, history of diabetes mellitus, hypertension, and smoking information were collected as baseline characteristics. Low-density lipopro-

tein-cholesterol (LDL-C), erythrocyte sedimentation rate, C-reactive protein, cystatin C, creatinine, folic acid, vitamin B12, and total homocysteine data were collected as baseline laboratory test results.

4. MTHFR C677T single nucleotide polymorphism analysis

Real-time PCR (RT-qPCR) was used for MTHFR C677T gene analysis. DNA was extracted from whole blood samples that were stored in a vacutainer with ethylenediaminetetraacetic acid. RT-qPCR was conducted with Real-Q MTHFR (BioSewoom, Seoul, Korea) Kit. This kit contains FAM at the 5' end for the wild-type amplification and VIC at the 5' end for mutant type amplification as reporter dyes. In both cases, a minor groove binder (MGB) was included as a quencher at the 3' end. After each DNA sample was added to the 677 master mixture, PCR was performed using Rotor-gene Q (QIAGEN) and the following program: 50℃ for 2 min, 95℃ for 10 min, followed by 40 cycles of 15 s at 95℃, and 45 s at 62℃.

5. Statistical analysis

We investigated the differences between baseline characteristics, laboratory data, and clinical data between the MTHFR C677T homozygous mutation group and the non-homozygous group (wild-type or heterozygous mutation). To identify independent risk factors

Table 1. Baseline characteristics of patients

	MTHFR C677T mutation status		*p*-value
	Non-homozygote mutation (n=90, 75%)	Homozygote mutation (n=30, 25%)	
Age (years)	73 (64–78)	72 (60–78)	0.84
Sex, female	35 (38.9)	11 (36.7)	0.83
Diabetes, yes	31 (34.4)	11 (36.7)	0.83
Hypertension, yes	55 (61.1)	23 (76.7)	0.12
Ever-smoker, yes	14 (15.6)	4 (13.3)	0.77
LDL-cholesterol (mg/dL)	95.2±37.5	103.1±30.7	0.30
HbA1c (%)	6.34±1.33	6.21±1.43	0.66
Erythrocyte sedimentation rate (mm/hour)	21.9±19.1	27.4±24.9	0.21
C-reactive protein (mg/L)	12.3±29.8	6.3±8.1	0.08
Cystatin C (mg/L)	1.05±0.36	1.18±0.71	0.19
Creatinine (mg/dL)	0.76±0.33	0.92±0.54	0.06
Folic acid (ng/mL)	8.24±4.21	6.24±4.21	0.03
Vitamin B12 (pg/mL)	666.4±425.3	569.4±365.4	0.27
Total homocysteine (μmol/L)	9.65±3.19	17.09±14.07	<0.01
NIHSS	2 (1–6)	3 (1–5)	0.85
Acute ischemic stroke TOAST classification			0.79
Large artery disease	36 (40.0)	7 (23.3)	
Cardioembolism	12 (13.3)	2 (6.7)	
Small vessel occ	21 (23.3)	14 (46.7)	
Others and undetermined	21 (23.3)	7 (23.3)	
Moderate to severe WMHs	60 (66.7)	26 (86.7)	0.01

Values are presented as number (%), median (interquartile ranges), or mean±standard deviation. Non-homozygote mutation includes wild type and heterozygote mutation. Moderate to severe WMHs refers to score 2 or 3 measured by Fazeka's score.
MTHFR; methylenetetrahydrofolate reductase, IQR; interquartile range, LDL; low-density lipoprotein, NIHSS; National Institutes of Health Stroke Scale, TOAST; trial of ORG 10172 in acute stroke treatment, WMH; white matter hyperintensity.

related to moderate or severe WMHs, multivariable logistic regression analysis was performed using possible confounders of homozygous mutation status, female sex, age, diabetes mellitus, hypertension, smoking status, and LDL-C. To determine the relationship between MTHFR mutation status and CSVD in addition to acute lacunar infarction, WMHs, CMBs by location, and asymptomatic chronic lacunar infarction were analyzed. Student's *t*-test or Mann–Whitney test was used for continuous values, while the χ^2 test or Fisher's exact test was used for categorical values. Statistical significance was defined as a *p*-value less than 0.05. SPSS for Windows (version 20.0; SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

RESULTS

The median age of the patients was 72 years (interquartile range [IQR]=63–78) years. There were 46 men (38.3%). The median NIHSS score was 2 (IQR 1–5). Among the 120 participants, 30 (25.0%) were included in the MTHFR C677T homozygous mutation group. There was no significant difference in the demographic or clinical data between the MTHFR C677T homozygous mutation group and the non-homozygous group. Among laboratory tests, a significantly lower level of folic acid (6.24±4.21 ng/mL vs. 8.24±4.21 ng/mL, *p*=0.03) and a higher level of total homocysteine (17.09±14.07 μmol/L vs. 9.65±3.19 μmol/L, *p*<0.01) were found in the homozygous mutation group compared to the non-homozygous group. Although there was no significant difference between the stroke subtypes, acute lacunar infarction was approximately twice as frequent in the homozygous mutation group (46.7% vs. 23.3%, *p*=0.79) (Table 1).

1. Independent variables for moderate to severe white matter hyperintensity (Table 2)

We investigated variables that may be related to moderate-to-severe WMH severity using multivariable logistic regression analyses. In univariate analysis, the presence of homozygous mutation (odds ratio [OR]=3.22; 95% confidence interval [CI]=1.04–10.17), female sex (OR=3.20; 95% CI=1.26–8.14), and age (OR=1.08; 95% CI=1.04–1.12) were significant factors associated with moderate to severe WMH. After adjusting for possible confounders, the presence of homozygous mutations (adjusted odds ratio [aOR]=4.29; 95% CI=1.16–15.90), age (aOR=1.06; 95% CI=1.01–1.11) were independently associated with moderate to severe WMHs.

2. Comparison of subtypes of cerebral small vessel disease according to homozygous mutation status (Table 3)

We analyzed whether the presence of various types of CSVD differed according to the MTHFR C677T homozygous mutation. There was no difference in CMB occurrence according to homozygous mutation. As for the rate of overall moderate to severe WMHs, predefined as 2 or 3 of Fazeka's score, the rate of moderate to severe WMHs was higher in the homozygous mutation group (86.7% vs. 66.7%, *p*=0.01). In a detailed analysis, the rate of moderate to severe DWMH did not differ significantly between the two groups. However, the homozy-

Table 2. Multivariable logistic regression analysis for moderate to severe WMHs

	Crude OR	Adjusted OR
MTHFR C677T homozygote mutation, yes	3.22 (1.04–10.17)	4.29 (1.16–15.90)
Sex, female	3.20 (1.26–8.14)	1.43 (0.45–4.57)
Age	1.08 (1.04–1.12)	1.06 (1.01–1.11)
Diabetes mellitus, yes	1.18 (0.51–2.74)	1.26 (0.46–3.46)
Hypertension, yes	1.45 (0.64–3.29)	1.15 (0.44–3.03)
Ever-smoker, yes	0.19 (0.06–0.53)	0.31 (0.09–1.09)
LDL-cholesterol	1.00 (0.99–1.01)	1.00 (0.99–1.01)

Moderate to severe WMHs refers to score 2 or 3 measured by Fazeka's score.
WMH; white matter hyperintensity, OR; odd ratio, MTHFR; methylenetetrahydrofolate reductase, LDL; low-density lipoprotein.

gous mutated group showed a significantly higher rate of moderate to severe PWMH (86.7% vs. 65.6%, $p<0.01$). Asymptomatic chronic lacunar infarction was also more frequently observed in the homozygous mutation group (63.3% vs. 34.4%, $p<0.01$).

DISCUSSION

This study analyzed whether MTHFR C677T homozygous mutation status may differ according to the AIS subtype. This study also examined whether MTHFR C677T homozygous mutation could serve as an independent factor for moderate to severe WMHs and demonstrated a distinction in various CSVDs in addition to acute lacunar infarction. Although there was no significant difference in the overall AIS subtypes, acute lacunar infarction was twice as frequent in the homozygous mutation group. Homozygous mutation of MTHFR C677T appeared to be an independent factor for moderate to severe WMHs. The frequency of asymptomatic chronic lacunar infarction increased in the MTHFR C677T homozygous mutation group.

WMHs can be separated into DWMHs and PWMHs. Arteriopathies such as thickened arterioles are found in DWMH, whereas demyelination, loosening of the ependymal lining, and elevated intraventricular pressure are pathologically observed in PWMH. They are also clinically distinct in that DWMH is associated with depression whereas PWMH is related to cognition.[24] Folic acid interacts with the complex MTHFR-homocysteine cycle which is necessary for various methylation. Once it is absorbed in the gastrointestinal tract, it is delivered to the brain by a variety of folic acid transporters such as folate receptors, reduced folate carriers, and proton-coupled folate transporters, many of which are located in epithelial cells of the ependymal choroidal plexus.[25] The finding from our analysis showing that MTHFR C667T homozygous mutation was related to moderate to severe WMHs, particularly in the periventricular area, could be explained by these previous studies.

This study had a few limitations. First, our study could be considered as research with a relatively small sample size compared to epidemiological studies. However, the results of this study were not inadequate, as this study analyzed detailed single-nucleotide polymorphisms among genetic mutations.[26] Second, our study was single-centered. Nonetheless, this study is based on the Korea University Stroke Group registry, which has been used as a dataset for several articles published in several renowned journals.[27,28] Third, parts of our analysis investigated the relationship between MTHFR mutations and chronic CSVD variables in an acute ischemic stroke population, which can be regarded as a bias. However, although various types of CSVDs share vascular risk factors and pathogenesis, the results of previous studies regarding the subtypes are not always

Table 3. Comparison of subtypes of cerebral small vessel disease according to homozygote mutation status

	MTHFR C677T mutation status		*p*-value
	Non-homozygote mutation (n=90, 75%)	Homozygote mutation (n=30, 25%)	
Moderate to severe WMHs, overall	60 (66.7)	26 (86.7)	0.01
Moderate to severe DWMH	27 (30.0)	14 (46.7)	0.10
Moderate to severe PWMH	59 (65.6)	26 (86.7)	0.03
Cerebral Microbleeds, overall	29 (32.2)	12 (40.0)	0.44
Cerebral Microbleeds, subcortical	22 (24.4)	12 (40.0)	0.10
Cerebral Microbleeds, cortical	15 (16.7)	4 (13.3)	0.70
Asymptomatic chronic lacunar infarction	31 (34.4)	19 (63.3)	<0.01

Values are presented as number (%). Non-homozygote mutation includes wild type and heterozygote mutation. Moderate to severe WMH, DWMH and PWMH refers to score 2 or 3 measured by Fazeka's score.
MTHFR; methylenetetrahydrofolate reductase, WMH; white matter hyperintensity, DWMH; deep white matter hyperintensity, PWMH; periventricular white matter hyperintensity.

consistent according to the population. Thus, the investigators assumed that chronic asymptomatic CSVDs may have different effects on patients with acute symptoms. Despite these limitations, our study investigated the relationship between C677T, a major MTHFR homozygous mutation, with CSVD, particularly PWMH, among the various types of CSVD. In most of the large-sized cohort studies that investigated the relationship between MTHFR mutations and CSVD, the study population was subjected to a community-based non-acute period cohort. The novelty of this study is that we included patients with AIS, unlike previous studies. Future prospective, multi-center prospective studies are needed to verify our findings, and it is believed that materials related to the complex MTHFR metabolism pathways can help ischemic stroke patients in the acute period.

Ethics Statement

The study protocol was approved by the Institutional Review Board (IRB) of the Korea University Medical Center, Guro Hospital (IRB No. 2011GR0218). The requirement for informed consent was waived.

Availability of Data and Material

Data are available from the authors with reasonable request to HJH.

Acknowledgments

None.

Sources of Funding

This research was supported by R&D support grant (K2108031) of Korea University Guro Hospital, Republic of Korea.

Conflicts of Interest

No potential conflicts of interest relevant to this article was reported.

REFERENCES

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol.* 2010;9:689-701.
2. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* 2013;12:822-838.
3. Ter Telgte A, van Leijsen EMC, Wiegertjes K, Klijn CJM, Tuladhar AM, de Leeuw FE. Cerebral small vessel disease: from a focal to a global perspective. *Nat Rev Neurol.* 2018;14:387-398.
4. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation.* 1999;99:178-182.
5. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. *Nutr J.* 2015;14:6.
6. Wilcken DE, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. *J Clin Invest.* 1976;57:1079-1082.
7. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med.* 1991;324:1149-1155.
8. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA.* 1995;274:1049-1057.
9. Fazekas F, Schmidt R, Scheltens P. Pathophysiologic mechanisms in the development of age-related white matter changes of the brain. *Dement Geriatr Cogn Disord.* 1998;9 Suppl 1:2-5.
10. Hassan A, Hunt BJ, O'Sullivan M, Bell R, D'Souza R, Jeffery S, et al. Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. *Brain.* 2004;127:212-219.
11. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA.* 2004;291:565-575.
12. McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI, Williams SM. A controlled trial of homocysteine lowering and cognitive performance. *N Engl J Med.* 2006;354:2764-2772.
13. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med.* 2006;354:1567-1577.

14. Wan L, Li Y, Zhang Z, Sun Z, He Y, Li R. Methylenetetrahydrofolate reductase and psychiatric diseases. *Transl Psychiatry*. 2018;8:242.
15. Choi BO, Kim HJ, Cho HJ, Kim JY, Kim SH, Lee JH, et al. Association between MTHFR C677T polymorphism and ischemic stroke. *J Korean Neurol Assoc*. 2005;23:607-613.
16. Rutten-Jacobs LC, Traylor M, Adib-Samii P, Thijs V, Sudlow C, Rothwell PM, et al. Association of MTHFR C677T genotype with ischemic stroke is confined to cerebral small vessel disease subtype. *Stroke*. 2016;47:646-651.
17. Marks MP, de Crespigny A, Lentz D, Enzmann DR, Albers GW, Moseley ME. Acute and chronic stroke: navigated spin-echo diffusion-weighted MR imaging. *Radiology*. 1996;199:403-408.
18. Bernhardt J, Hayward KS, Kwakkel G, Ward NS, Wolf SL, Borschmann K, et al. Agreed definitions and a shared vision for new standards in stroke recovery research: the stroke recovery and rehabilitation roundtable taskforce. *Int J Stroke*. 2017;12:444-450.
19. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, 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*. 1993;24:35-41.
20. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*. 1987;149:351-356.
21. Leonards CO, Ipsen N, Malzahn U, Fiebach JB, Endres M, Ebinger M. White matter lesion severity in mild acute ischemic stroke patients and functional outcome after 1 year. *Stroke*. 2012;43:3046-3051.
22. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, et al. Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol*. 2009;8:165-174.
23. Longstreth WT Jr, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. *Arch Neurol*. 1998;55:1217-1225.
24. De Groot JC, De Leeuw FE, Oudkerk M, Van Gijn J, Hofman A, Jolles J, et al. Periventricular cerebral white matter lesions predict rate of cognitive decline. *Ann Neurol*. 2002;52:335-341.
25. Alam C, Kondo M, O'Connor DL, Bendayan R. Clinical implications of folate transport in the central nervous system. *Trends Pharmacol Sci*. 2020;41:349-361.
26. Li H, Qu W, Obrycki JJ, Meng L, Zhou X, Chu D, et al. Optimizing sample size for population genomic study in a global invasive lady beetle, Harmonia axyridis. *Insects*. 2020;11:290.
27. Seo WK, Jung JM, Kim JH, Koh SB, Bang OY, Oh K. Free fatty acid is associated with thrombogenicity in cardioembolic stroke. *Cerebrovasc Dis*. 2017;44:160-168.
28. Eun MY, Jung JM, Choi KH, Seo WK. Statin effects in atrial fibrillation-related stroke: a systematic review and meta-analysis. *Front Neurol*. 2020;11:589684.