Polymorphisms of *MTHFR*, *eNOS*, *ACE*, *AGT*, *ApoE*, *PON1*, *PDE4D*, and Ischemic Stroke: Meta-Analysis

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Introduction: The association between ischemic stroke and genetic polymorphisms of methylenetetrahydrofolate reductase (*MTHFR*; 677C>T and 1298A>C), endothelial nitric oxide synthase (*eNOS*; −786T>C, +894G>T, and variable number tandem repeat [VNTR]), phosphodiesterase 4D (*PDE4D*; SNPs 83 and 87), angiotensin-converting enzyme (*ACE*) I/D, angiotensinogen (*AGT*) 235M>T, paraoxonase 1 (*PON1*) 192Q>R, and apolipoprotein E (*ApoE*) ε2ε3ε4 remains inconclusive. Therefore, this updated meta-analysis aimed to clarify the presumed influence of genetic polymorphisms on ischemic stroke by meta-analyzing the comprehensive coverage of all individual association studies. *Methods:* All case-control studies published in different languages such as English, Japanese, Korean, Spanish, Chinese, Hungarian, Ukrainian, or Russian were identified from databases. The pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated via fixed- and random-effect models. Sensitivity analysis, heterogeneity test, Hardy Weinberg Equilibrium, and Egger's regression analyses were performed in this study. *Results:* A total of 490 case-control studies with 138,592 cases and 159,314 controls were included in this meta-analysis. Pooled ORs from all the genetic models indicated that *MTHFR* 677TT and 1298CC, *eNOS* +894TT and VNTR, *PDE4D* SNP 83, *ACE* DD, *AGT* 235TT, *PON1* 192RR, and *ApoE* ε4 polymorphisms were increasing the risks of ischemic stroke. Nevertheless, *PDE4D* SNP 87 and *eNOS* −786T>C polymorphisms are not associated with ischemic stroke risks. *Conclusions:* Hence, the evidence from this

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Received May 1, 2017; revision received May 12, 2017; accepted May 17, 2017.

Grant support: We acknowledge the support from the Ministry of Higher Education Fundamental Research Grant Scheme (FRGS/1/2015/SKK08/UTAR/02/3) and Universiti Tunku Abdul Rahman under the UTAR Research Fund 6200/LF3.

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1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2017.05.048

meta-analysis concluded that *MTHFR* (677C>T and 1298A>C), *eNOS* (+894G>T and VNTR), *PDE4D* SNP 83, *ACE* I/D, *AGT* 235M>T, *PON1* 192Q>R, and *ApoE* ε2ε3ε4 polymorphisms predispose individuals to ischemic stroke. **Key Words:** Ischemic stroke—*MTHFR*—*eNOS*—*ACE*—*AGT*—*ApoE*—*PON1*—*PDE4D*.

Introduction

Ischemic stroke is the most common form of cerebrovascular disease that contributes to morbidity and mortality cases around the world.[1] Nonmodifiable risk factors for ischemic stroke are age, race, gender, and family history. Other modifiable risk factors that can be treated are atherosclerosis, dyslipidemia, hypertension, diabetes, and cardiovascular diseases.[2] Ischemic stroke pathophysiology involves low production of cyclic nucleotide second messengers, hyperhomocysteinemia, oxidation event, accumulation of low-density lipoprotein (LDL), and dyslipidemia.[3-5] Decreased cyclic nucleotide second messengers' production, in response to low nitric oxide and high angiotensin II levels, is promoting LDL oxidation.[3,6] An excessive LDL oxidation promotes atherothrombosis and subsequently contributes to ischemic stroke.[3,6] Hence, it is suggesting that candidate gene polymorphisms involved in the ischemic stroke pathophysiology such as methylenetetrahydrofolate reductase (*MTHFR*), endothelial nitric oxide (*eNOS*), phosphodiesterase 4D (*PDE4D*), angiotensin-converting enzyme (*ACE*), angiotensinogen (*AGT*), apolipoprotein E (*ApoE*), and paraoxonase 1 (*PON1*), are associated with the risk of ischemic stroke.[5,7,8]

Despite the fact that a number of meta-analyses were examining the association between polymorphisms of *MTHFR* (677C>T [Ala222Val or rs1801133] and 1298A>C [Glu429Ala or rs1801131]), *eNOS* (–786C>T [rs2070744], +894G>T [298Asp>Glu or rs1799983] and variable number tandem repeat [VNTR]), *PDE4D* (SNP 83 [rs966221 or –89-21006T>C] and SNP 87 [rs2910829 or 42+11485C>T]), *ACE* I/D (rs4646994), *AGT* 235M>T (rs699 or 803T>C), *ApoE* (388T>C [rs429358 or Cys156Arg] and 526C>T [rs7412 or Arg202Cys]), and *PON1* 192Q>R (rs662 or 575A>G) and ischemic stroke risks, however, these associations remain inconclusive.[9-15] Hence, we aimed to investigate the causal relationship between these polymorphisms and ischemic stroke risk by conducting a meta-analysis using big data.

Methods

Search Strategy

This meta-analysis followed the Cochrane Collaboration definition and Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 guidelines. Articles reported on the association between *MTHFR*, *eNOS*, *PDE4D*, *ACE*, *AGT*, *ApoE*, and *PON1* genes and ischemic stroke were retrieved by performing an extensive search on Pubmed, Scopus, Web of Science, Virtual Library of Health, Jstage, KoreaMed, Korea Science Citation Index, Indian Citation Index, Index Medicus for South-East Asia Region, Mycite, Western Pacific Region Index Medicus, and China National Knowledge Infrastructure databases without any language restriction.[4] Medical subject heading of "*MTHFR*," "methylenetetrahydrofolate reductase," "*eNOS*," "endothelial nitric oxide synthase," "*NOS3*," "*ACE*," "angiotensin-converting enzyme," "*AGT*," "angiotensinogen," "ischemic stroke," "cerebrovascular disease," "cerebrovascular accident," "brain infarction," "brain ischemia," "cerebral ischemia," "polymorphism," "variant," "gene mutation," "SNP," "gene variation," and the equivalent Chinese terms were used. All potential studies were manually identified from the references of all the retrieved articles, which include abstracts and proceedings from conferences as well as international meetings. In the case of missing data, the corresponding or first author was contacted via e-mail. The last date of literature search was March 18, 2017.

Selection Criteria

All included studies were written in English, Japanese, Korean, Spanish, Chinese, Hungarian, Ukrainian, or Russian. Studies were included if cases were being confirmed by neuroimaging (computed tomography scan or magnetic resonance imaging); otherwise, the assessment of cases was done by neurologists, geriatrician stroke specialists, or physicians following the international standard or equivalent criteria. The control subjects were healthy and unrelated individuals without any symptomatic vascular diseases proven by physicians. Only studies that provide sufficient genotypic data for the effect sizes calculation were included. For exclusion criteria, studies with a sample size less than 50 cases and controls, and study subjects younger than 18 years old were excluded. If more than one article was published by the same group of authors or consisted overlapping data, only the latest study or the study with the largest sample size was included in the final analysis model. In case an individual study reported more than one ethnicity, it was analyzed as two separate studies.

Data Extraction

Titles, abstracts, and full texts of all the retrieved articles were screened independently by four investigators (L.K.W., L.X.Y., K.W.K., A.A.) by following the inclusion