Significant Association between *OPG/TNFRSF11B* Variant and Common Complex Ischemic Stroke

Xin Xiong, PhD,[*,1] Duraid Hamied Naji, PhD,[*,1] Binbin Wang, PhD,[†,1] Yuanyuan Zhao, PhD,[*] Junhan Wang, MD,[‡] Dan Wang, PhD,[*] Yuting Zhang, MS,[*] Sisi Li, MS,[*] Shanshan Chen, PhD,[§] Yufeng Huang, PhD,[‖] Qin Yang, PhD,[*] Xiaojing Wang, PhD,[*] Dan Yin, PhD,[*] Xin Tu, PhD,[*] Qiuyun Chen, PhD,[¶,#] Xu Ma, PhD,[†] Chengqi Xu, PhD,[*] and Qing K. Wang, PhD[*,¶,#,**,††]

Background: The serum level of osteoprotegerin (encoded by *OPG* or *TNFRSF11B*) was previously shown to be increased in patients with ischemic stroke. A single nucleotide polymorphism rs3134069 in the *TNFRSF11B* gene was previously associated with ischemic stroke in a population of diabetic patients in Italy. It remains to be determined whether rs3134069 is associated with ischemic stroke in the general population or populations without diabetes. *Materials and Methods:* We genotyped rs3134069 and performed a case-control association study to test whether rs3134069 is associated with ischemic stroke in 2 independent Chinese Han populations, including a China-Central population with 1629 cases and 1504 controls and a China-Northern population with 1206 cases and 720 controls. *Results:* rs3134069 showed significant association with ischemic stroke in the China-Central population ($P = 9.24 \times 10^{-3}$, odds ratio [OR] = 1.50). The association was replicated in the independent China-Northern population ($P = 2.45 \times 10^{-4}$, OR = 1.53). The association became more significant in the combined population ($P = 7.09 \times 10^{-6}$, OR = 1.41). The associations remained significant in the male population, female population, and population without type 2 diabetes. Our expression quantitative trait loci analysis found that the minor allele C of rs3134069 was significantly associated with

From the *Key Laboratory of Molecular Biophysics of the Ministry of Education, Cardio-X Institute, College of Life Science and Technology, Center for Human Genome Research, Huazhong University of Science and Technology, Wuhan, China; †National Research Institute for Family Planning, Beijing, China; ‡Department of Clinical Laboratory of University Hospital; §Key Laboratory for Molecular Diagnosis of Hubei Province, The Central Hospital of Wuhan, Tongji Medical College; ‖Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ¶Center for Cardiovascular Genetics; #Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; **Department of Molecular Medicine, Case Western Reserve University, Cleveland, Ohio; and ††Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, Ohio.

Received October 25, 2017; revision received January 2, 2018; accepted January 28, 2018.

Grant support: This study was supported by the China National Natural Science Foundation Program (31430047, 31671302), Hubei Province's Outstanding Medical Academic Leader Program, Hubei Province Natural Science Key Program (2014CFA074), the China National Natural Science Foundation grant (91439129, NSFC-J1103514), NIH/NHLBI grants R01 HL121358 and R01 HL126729, Fundamental Research Funds for the Central Universities (HUST: 2016YXMS254).

Address correspondence to Chengqi Xu, PhD or Qing K. Wang, PhD, College of Life Science and Technology and Center for Human Genome Research, Huazhong University of Science and Technology, Luoyu Road 1037, Wuhan, 430074, China. E-mails: cqxu@hust.edu.cn, qkwang@hust.edu.cn, wangq2@ccf.org.

[1] These authors contributed equally to this work.

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.01.029

a decreasedexpression level of *TNFRSF11B* ($P = .002$). *Conclusions:* This study demonstrates that rs3134069 in *TNFRSF11B* increases risk of ischemic stroke by decreasing *TNFRSF11B* expression. **Key Words:** *TNFRSF11B*—ischemic stroke—genetics—single nucleotide polymorphism.

Introduction

Stroke is a common cause of death worldwide,[1] and accounts for over 20% of total deaths in China.[2] Ischemia is responsible for about 87% of stroke cases.[3] Atherosclerosis in the brain is the major cause for formation of thrombi, and is estimated to cause 70%-80% of ischemic strokes.[4] Ischemic stroke is known to be affected by genetic risk factors, environmental factors, and their interactions. Genome-wide association studies have identified some loci conferring risk to ischemic stroke, including 12p13 (*NINJ2*), 12q24 (*ALDH2*), 7p21 (*HDAC9*), 9p21, 4q25 (*PITX2*), 16q22 (*ZFHX3*), 9q34 (*ABO*), and 1p13.2 (*TSPAN2*).[5-10] Candidate gene approaches also identified *ALOX5AP*, *NOS3*, *PCSK9*, *PDE4D*, *SGK1*, *VKORC1*, and other genes confer risk to ischemic stroke.[11-16] However, all these genomic variants in aggregate explain only a small proportion of heritability of ischemic stroke, a phenomenon referring to as "missing heritability".[17] Therefore, many more genomic variants associated with ischemic stroke need to be identified to fully elucidate the genetic architecture of this important disease.

Osteoprotegerin (OPG), which belongs to the tumor necrosis factor receptor superfamily, is encoded by the *TNFRSF11B* gene on chromosome 8q23.[18] Several studies have demonstrated that OPG could promote endothelial cell survival.[19-21] Moreover, several studies have shown a correlation between increased endogenous serum OPG levels and the presence and severity of clinical coronary artery disease (CAD),[22,23] stroke,[24-26] cardiovascular morbidity and mortality[27] as well as the progression of atherosclerosis in humans.[28,29] Further evidence suggests that circulating OPG levels also are associated with the extent of vascular calcification.[30-32] Moreover, increased OPG serum levels were found in *Ldlr*$^{-/-}$ knockout (KO) mice on a Western diet.[33] Recently, a variant in the *TNFRSF11B* gene was found to be associated with coronary atherosclerosis in patients with rheumatoid arthritis.[34] Moreover, Biscetti et al reported that in a small population of 364 diabetic patients with a history of ischemic stroke and 492 diabetic subjects without history of ischemic stroke, single-nucleotide polymorphism (SNP) rs3134069 showed a significant genotypic association with ischemic stroke under the background of diabetes.[34] However, it is unknown whether SNP rs3134069 is associated with ischemic stroke in subjects without type 2 diabetes (T2D). Khouloud et al found that PPARγ variant C161T was associated with risk to ischemic stroke in populations with T2D, but not in populations without T2D.[34] Moreover, only 15% of patients with ischemic stroke have a history of T2D and remaining 85% do not have a history of T2D.[35] Therefore, it is necessary to determine whether *TNFRSF11B* variants are associated with ischemic stroke in a more complex population, especially in populations without T2D.

In this study, we analyzed the association between *TNFRSF11B* SNP rs3134069 and ischemic stroke in 2 independent case-control ischemic stroke populations with a total of 5059 Chinese Han subjects. Moreover, we studied whether rs3134069 was a functional variant associated with the expression level of *TNFRSF11B* mRNA by eQTL analysis.

Materials and Methods

Study Subjects

Samples enrolled in this study were selected from the GeneID database, which is an ongoing study in the Chinese Han population and has collected more than 80,000 DNA samples and also all available clinical data. The goal of the GeneID database is to identify susceptibility genes or other risk factors of cardiovascular and cerebrovascular diseases in the Chinese Han population.[36-43]

In this study, we enrolled a case-control population with a total of 5059 samples, including 2835 patients with ischemic stroke and 2224 comparable controls. The study contained 2 independent populations matched by geographical areas they were enrolled. The China-Central population consisted of 1629 patients with ischemic stroke and 1504 controls, and the cases enrolled were from the patients who were under treatment of ischemic stroke in the hospitals of Wuhan city, whereas the controls of the China-Central population were enrolled from people who subjected physical examinations from the same hospitals of Wuhan city. The China-Northern population included 1206 patients with ischemic stroke enrolled from the patients who were under treatment of ischemic stroke in the hospitals of Beijing, and 720 controls enrolled from other patients who showed no sign of ischemic stroke by related medical examinations in the hospitals of Beijing. All subjects were self-reported to be of Chinese Han origin.

The diagnosis of ischemic stroke was made based on the standard World Health Organization criteria.[44] The clinical diagnosis was made carefully by at least 2 independent neurologists based on a medical history of stroke, stroke signs by neurological examinations, and cerebral ischemia by computed tomography (CT) or magnetic