Screening for *NOTCH3* Gene Mutations Among 151 Consecutive Korean Patients with Acute Ischemic Stroke

Jay Chol Choi, MD, PhD,* Keun-Hwa Lee, PhD,*† Sook-Keun Song, MD,* Jung Seok Lee, MD,* Sa-Yoon Kang, MD,* and Ji-Hoon Kang, MD*

Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a single-gene disorder of cerebral small blood vessels caused by mutations in the *NOTCH3* gene. The initial detection of CADASIL may be more difficult among Asian populations because common clinical phenotypes and neuroimaging findings are not frequently found in these populations. The purpose of this study was to screen the *NOTCH3* gene for mutations among consecutive patients with acute ischemic stroke from our region in Korea. *Methods:* Between April 2008 and March 2009, 151 consecutive patients with acute ischemic stroke were screened for *NOTCH3* mutations. All patients underwent a detailed clinical examination and structured interview for clinical symptoms and family history. We reviewed brain magnetic resonance imaging data from stroke patients to assess the severity of white-matter hyperintensity lesions, the number of cerebral microbleeds, and the number of lacunar infarctions. Polymerase chain reaction was used to screen exons 3, 4, 6, 11, and 18 of the *NOTCH3* gene. *Results:* Among 151 consecutive patients with acute ischemic stroke, 6 patients (4.0%; 95% confidence interval [CI] 0.9-7.1) possessed a *NOTCH3* gene mutation. All patients exhibited the same R544C mutation in exon 11. Four of these 6 patients presented with large artery atherosclerosis. The prevalence of CADASIL in patients with neuroimaging features consistent with advanced small-vessel disease was 36.0% (95% CI 8.0-64.8). *Conclusions:* In this region, *NOTCH3* gene mutations are frequently found in acute stroke patients who present with neuroimaging features consistent with advanced small-vessel disease. **Key Words:** Ischemic stroke—*NOTCH3*—screening.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a single-gene disorder of the cerebral small blood vessels that is caused by mutations in the *NOTCH3* gene.[1] The main clinical manifestations are recurrent stroke, cognitive deficits, migraines, and psychiatric disturbances.[2,3] More than 170 different mutations have been identified in CADASIL patients, and more than 95% of these are missense point mutations.[4] The reported distribution of these mutations has varied according to world region.[5]

Although certain clinical and neuroimaging features are more frequent among CADASIL patients than in *NOTCH3*– patients, none are pathognomonic.[6] In addition, the initial detection of CADASIL may be even more difficult among Asian populations for several reasons. First, Asians exhibit a lower prevalence of migraines (0-26.9%).[7-9] Second, studies from Asian regions have described infrequent involvement (22-43%) of the anterior temporal lobe upon examination with brain magnetic resonance imaging (MRI).[9,10] Third, some

From the *Departments of Neurology; and †Microbiology and Immunology, Jeju National University, Jeju, Korea.

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Drs Choi and Lee contributed equally to the manuscript.

Address correspondence to Jay Chol Choi, MD, PhD, Department of Neurology, Jeju National University, 1753-3 Ara 1-dong, Jeju-si, Jeju-do, 690-767, Korea. E-mail: strokedoc.choi@gmail.com.

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Asians present with atypical stroke manifestations, including intracranial large artery disease and intracerebral hemorrhages.[11,12] Finally, the high prevalence of vascular risk factors for small-vessel diseases, such as hypertension, among Asian patients with CADASIL increases the difficulty of differential diagnoses.[9,11]

Ischemic stroke is the most common clinical presentation in patients with CADASIL.[3] The purpose of this study was to screen for *NOTCH3* mutations in consecutive patients who presented with acute ischemic stroke (AIS) in our region, where the clinical differentiation of CADASIL from common ischemic stroke is more difficult.

Methods

Patients

Jeju is an island situated off the southern tip of the Korean Peninsula with a population of about 550,000. The subjects in this study were patients with AIS who were admitted between April 2008 and March 2009. All patients were admitted to the Jeju National University Hospital within 7 days of symptom onset and were diagnosed with AIS after neurologic examination, cranial computed tomography, or MRI. Patients who were <20 years old, refused to undergo genetic testing, or did not want to participate for other reasons were excluded from the study. We received written informed consent from all participants, and this study was approved by the local ethics committee.

Clinical Assessment

We used a structured questionnaire to determine symptoms other than stroke index and classified them as previous stroke, dementia, psychiatric illness, headache, or epileptic seizure. A first-degree family history (father, mother, and siblings) was also obtained. Fasting venous samples were drawn for laboratory evaluation of complete blood count, electrolytes, glucose, liver battery, hemoglobin A1c (HbA1c), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglyceride levels. Hypertension was defined as a blood pressure of at least 140/90 mm Hg on 2 separate measurements or the use of an antihypertensive agent. Diabetes mellitus was defined as a fasting blood glucose level of at least 126 mg/dL or the use of antidiabetic medications. Smoking status was determined by self-reporting as either a smoker (current or ex-smoker) or a nonsmoker. We used the Korean version of the Mini-Mental Status Examination to screen diagnose cognitive deficits among the stroke patients, except when the patients were unable to complete the test because of aphasia or altered mental status.

The diagnosis of vascular dementia was made using National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences criteria.[13] Headache was diagnosed and classified according to the International Classification of Headache Disorders (ICHD)-II.[14] According to the Trial of Org 10172 in Acute Stroke Treatment criteria,[15] ischemic stroke was classified as large-artery atherosclerosis, small-vessel occlusion, cardioembolism, ischemic stroke of undetermined etiology, and ischemic stroke of other determined etiologies.

Neuroimaging Analysis

All patient underwent brain MRI at our center (1.5 Tesla system; Siemens, Sonata, Germany). Detailed MRI protocols at our center and diagnostic criteria of each lesion were published previously.[12] In brief, we measured the severity of white-matter hyperintensity lesions (WMHLs),[16] the number of cerebral microbleeds (CMBs),[17] and the number of lacunar infarctions (LIs).[18] Arterial stenosis was assessed on magnetic resonance angiography (MRA). Severe arterial stenosis was defined as >50% stenosis when compared to the proximal lumen of a normal artery.[19]

Genetic Testing

DNA was extracted from whole blood samples from stroke patients using the bead beater-phenol extraction method. Primers for *NOTCH3* were kindly supplied by Professor Yoo Han Wook (Asan Medical Center, Seoul, Korea). We screened exons 3, 4, 6, 11, and 18 of the *NOTCH3* gene, and these exons have been shown to cover most of the *NOTCH3* gene mutations found in Korea.[7, 10] A GeneAmp polymerase chain reaction system 9600 (PerkinElmer, Foster City, CA) was used for target amplification with the following parameters: 5 minutes at 95°C, followed by 40 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 60 seconds at 72°C, with termination using a final extension step at 72°C for 10 minutes. The polymerase chain reaction products were purified with the QIAEX II Gel Extraction Kit (QIAGEN, Germany) according to the manufacturer's instructions before a direct sequencing reaction using a BigDye Terminator cycle sequencing kit with AmpliTaq DNA polymerase. Nucleotide sequences were analyzed using BioEdit software (v 5.0.9.1; T. A. Hall Software, Ibis Biosciences, Carlsbad, CA), CHROMAS (v 2.33), and BLAST (Basic Local Alignment Search Tool).

Statistical Analysis

We used the Mann–Whitney *U* or Fisher exact tests according to the types of variables examined to compare the characteristics between patients with and without *NOTCH3* mutations. All analyses were performed using SPSS (v 12.0; SPSS Inc, Chicago, IL). $P < .05$ was considered statistically significant.