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A Novel Mutation (C67Y) in the *NOTCH3* Gene in a Korean CADASIL Patient

We report a 52-yr-old Korean woman with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) whose diagnosis was confirmed by skin biopsy and the presence of a novel mutation in the *NOTCH3* gene. The patient's clinical features were rather unusual in that 1) clinical presentations were only two episodes of stroke and mild dementia unaccompanied by mood disturbances or migraine, and 2) there was no family history. Brain MRI showed T2 hyperintensities in both temporal pole areas in line with the recent suggestion by O'Sullivan et al. that the abnormality could be a radiologic marker of CADASIL. FDG-PET also showed a hypometabolism in the temporal pole areas with an abnormal finding on MRI in addition to the hypometabolism in cortical and subcortical regions. We could learn from this case that CADASIL may be included in the differential diagnoses in patients with vascular dementia associated with a small vessel disease, even in the absence of a family history, especially when there are no known stroke risk factors and when the MRI shows T2 hyperintensity in the temporal pole regions.

Key Words : *CADASIL; Dementia, Multi-infarct; Tomography, Emission-Computed; The NOTCH3 Gene; Polymorphism (Genetics)*

So-Young Moon, Hahn-Young Kim, Jung-Im Seok, Jae-Chul Kwon, Chang-Seok Ki*, Jong-Won Kim*, Yeon-Lim Suh†, Duk L. Na

Departments of Neurology, Clinical Pathology,* and Diagnostic Pathology†, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea

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Address for correspondence
Duk L. Na, M.D.
Department of Neurology, Samsung Medical Center, Sungkyunkwan University, School of Medicine, 50 Ilwon-dong, Kangnam-gu, Seoul 135-710, Korea
Tel: +82-2-3410-3591, 3599, Fax: +82-2-3410-0052
E-mail: dukna@smc.samsung.co.kr

INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominantly inherited condition characterized by migraine, recurrent strokes, mood disturbance, and progressive cognitive impairment. Since the defective gene associated with CADASIL was discovered in *N0TCH3* in 1996 (1), at least 80 CADASIL patients with *N0TCH3* mutations have been reported in different ethnic groups (2-6). Among Asian population, to our knowledge, two Japanese families (7) and one Korean family (8) with mutations already identified in Caucasian's have been reported. We report a Korean patient with CADASIL who carries a novel mutation in the *N0TCH3* gene without a known family history.

CASE REPORT

A 52-yr-old woman with a formal education for 9 yrs suddenly developed a left hemiplegia one day before admission. Five years previously, although the patient denied, the patient's family had noticed her subtly dragging the left leg and thereafter her left shoe would get worn-out more rapidly than the right shoe. One year before admission, she developed a memory disturbance insidiously. Past medical history was not remarkable for stroke risk factors such as hypertension, diabetes mellitus, heart disease, or hyperlipidemia. She denied of mood disturbances or attacks of migraine. Family history was also negative for stroke, migraine, and dementia.

On examination, blood pressure was 117/70 mmHg. She was alert but showed mild abulia and occasional inappropriate laughing. She scored 23 of 30 on Mini-mental State Examination. Sensory extinction was noted in both visual and tactile modalities. Other neurologic examinations were significant for mild dysarthria, left central facial palsy, left spastic hemiplegia (grade 0 in upper limb and grade 2 in lower limb), bilateral hyperreflexia, and extensor plantar responses with the left side being more prominent. Sensory and neurovascular examinations including neck bruit were normal. The results of neuropsychological tests are presented in Table. In summary, the patient was mildly impaired at naming, verbal and visual memories. Comparatively, she spent 18 min on copying the Rey-Osterrieth Complex figure, showing a left hemispatial neglect and moderate visuoconstructive disability (18/36). The patient's frontal/executive functions were also impaired; she had a defective response inhibition on go-no-go test, impaired motor set-shifting, decreased performances in stroop and controlled oral association.

Laboratory studies included the following tests yielded normal results: antinuclear antibody, anti-ds-DNA antibody, anticardiolipin antibody (IgM and IgG), antiplatelet antibody,

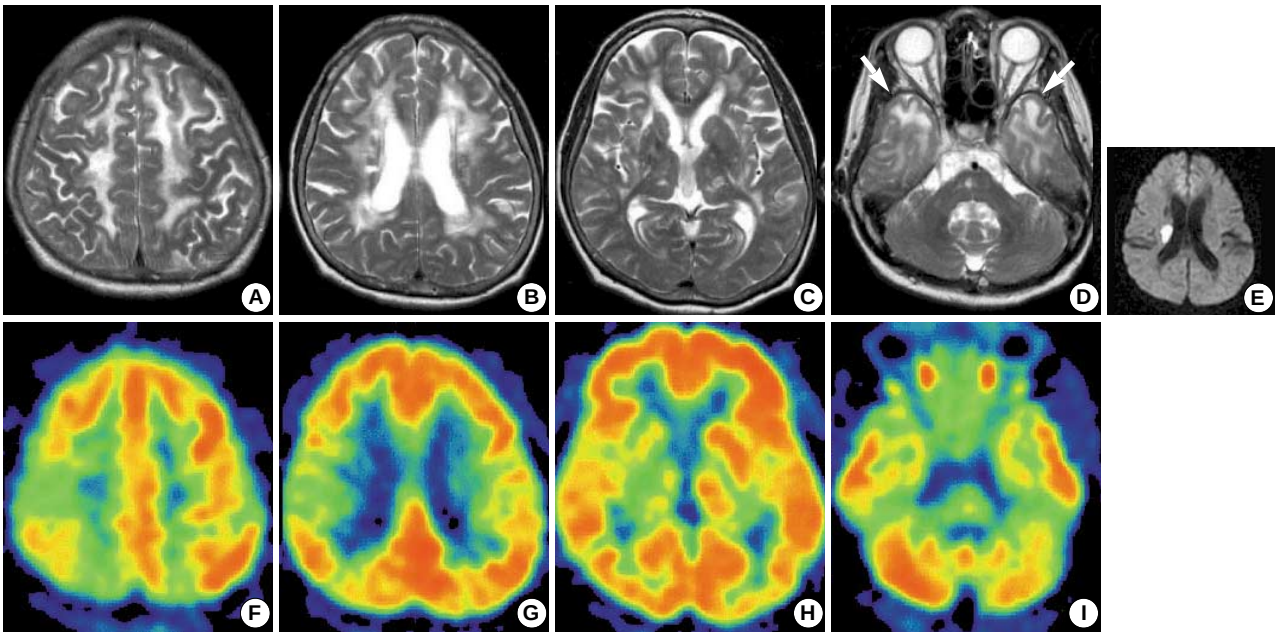

Fig. 1. Neuroimaging studies. Axial T2-weighted MR images (A, B, C and D) show 1) diffuse confluent ischemic changes in periventricular and subcortical white matter, 2) lacunes in the basal ganglia, thalamus, and brainstem, and 3) abnormal white matter hyperintensities in both temporal pole areas (arrow). A diffusion-weighted MR images (E) shows high signal intensity, suggestive of recent infarction, in the right corona radiata. FDG-PET scans (F, G, H, and I) obtained with the same angle and slices as in MRI show an abnormally decreased glucose metabolism bilaterally in fronto-parieto-temporal cortices, basal ganglia, and thalamus, more markedly in the right hemisphere than in the left.

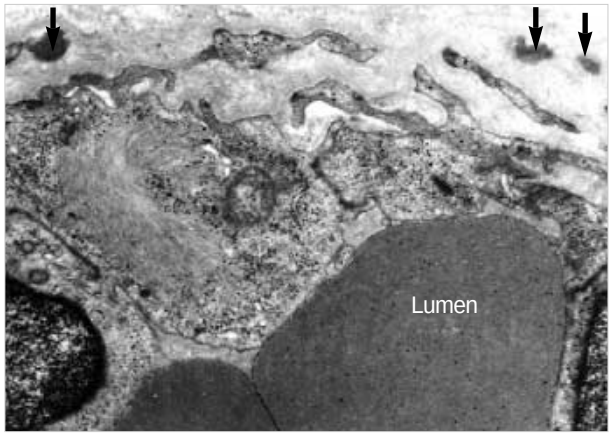

Fig. 2. Electron microscopy analysis of a skin biopsy sample showing an arteriole with fragmented vascular smooth muscle cells and a thickened basal lamina distorted by irregular deposits of granular osmiophilic material (arrow) ($\times$10,000).

antithrombin III, protein C and S, homocysteine, and lipoprotein(a). Electrocardiogram, transthoracic echocardiogram, and ultrasonographic evaluation for intra- and extra-cerebral vessels were normal. T2-weighted brain magnetic resonance (MR) images showed diffuse confluent ischemic changes in periventricular and subcortical white matter or lacunes in the basal ganglia, thalamus, and brainstem (Fig. 1). On diffusion-weighted images, a high signal intensity suggestive of recent infarction was observed in the right corona radiata (Fig. 1E). Gradient echo images did not show any evidence of large or small hemorrhages. MR angiography was normal. FDG-PET showed an abnormally decreased uptake bilaterally in the fronto-temporo-parietal cortex, basal ganglia, and thalamus (Fig. 1). The hypometabolism was more prominent in the right hemisphere than in the left.

Ultrastructural examination of the skin biopsy, with special attention to the dermal arteries, revealed vascular smooth muscle cells with a thickened basal lamina distorted by irregular deposits of granular osmiophilic material, a finding consistent with CADASIL (Fig. 2).

With an informed consent, mutational analysis of the *NOTCH3* gene was performed as previously described (2). Genomic DNA was extracted from peripheral blood leukocytes of the patient and both exon 3 and 4 regions of the *NOTCH3* gene was amplified by polymerase chain reaction (PCR) (primer sequences were by courtesy of Dr. E. Tournier-Lasserve, Genetique des Maladies Vasculaires, Inserm, Paris, France) and directly sequenced on an ABI Prism 377 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). We found a heterozygous G-to-A transition of the third nucleotide in exon 3 of the *NOTCH3* gene, resulting in a Cys67Tyr substitution within the fourth epidermal growth factor-like repeat domain of the Notch3 receptor (Fig. 3A). The G-to-A transition creates a novel *Rsa*I

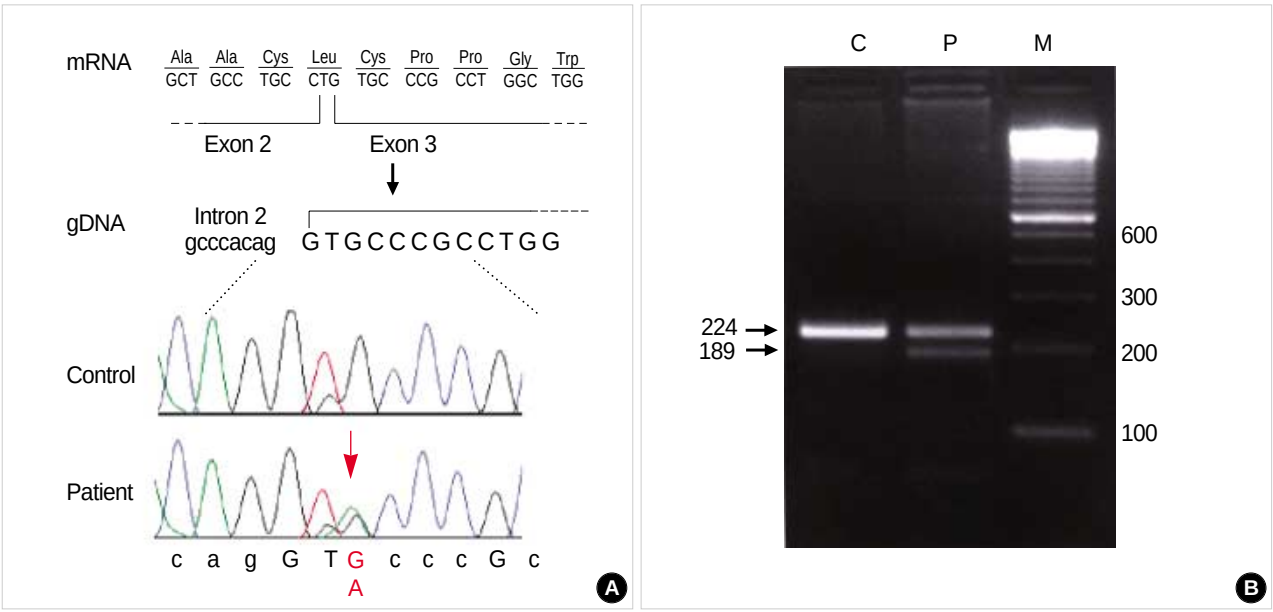

Fig. 3. Mutation analysis of the *Notch3* gene. (A) Sequence analysis shows a G to A transition of the third nucleotide of exon 3 (arrow), which results in a C67Y mutation. (B) Confirmation of the C67Y mutation with the PCR-RFLP method. The mutant allele created a *Rsa*I restriction site that a 224 bp PCR amplicon was digested into 189 and 35 bp fragments. mRNA, messenger RNA; gDNA, genomic DNA; RNA M, 100 bp size marker; P, patient; C, control.

Table 1. Results of neuropsychological tests in the patient

Cognitive domain/neuropsychologic tests	Results
Attention	
Digit span: forward/backward	6/3
Language and related functions	
Fluency	NL
Auditory comprehension	NL
Repetition	NL
Naming (K-BNT)	45/60 (48%ile)
Reading	NL
Writing	NL
Calculation	NL
Right-left orientation	NL
Body part identification	NL
Limb praxis	NL
Visuospatial functions	
Interlocking pentagon	NL
Rey-Osterrieth Complex Figure Test (Rey CFT)	18/36 (<1%ile)
Memory	
Orientation: time; place	4/5; 5/5
Remote memory	4/5
3 words registration; recall	3/3; 1/3

Cognitive domain/neuropsychologic tests	Results
Memory	
HVLT: Free recall (1st; 2nd; 3rd; total)	3/12; 8/12; 7/12; 18/36 (1.5%ile)
20-min delayed recall	3/12
Recognition (true positive-false positive)	8-1=7
Rey CFT:	
Immediate recall; 20-min delayed recall	4/36 (<1%ile); 4/36 (<1%ile)
Recognition (true positive-false positive)	6-1=5 (2%ile)
Frontal/Executive Function	
Contrasting program	NL
Go-no-go test	AB
Fist-edge-palm	AB
Alternating hand movement	AB
Alternating square and triangle	NL
Luria loop	NL
Semantic word fluency: animal; supermarket items	9; 8 (6%ile)
Phonemic word fluency (sum of three consonants)	9 (9%ile)
Stroop test: word reading: correct/incorrect	98/0
color naming: correct/incorrect	47/0
General Index	
MMSE	23/30

K-BNT: The Korean version of the Boston Naming Test, HVLT: Hopkins Verbal Learning Test (Korean version), MMSE: Mini-Mental State Examination (Korean version), NL= within normal limit, AB= abnormal.

recognition site, which was confirmed by the PCR-RFLP method (Fig. 3B). The mutation was not observed in 60 healthy Koreans.

Since the patient's parents were all dead and her siblings (one stepbrother, one stepsister, and one brother) refused to be tested, we could not confirm whether the mutation is de novo or not.

DISCUSSION

The diagnosis of CADASIL in our patient was confirmed by skin biopsy and the presence of the *N0TCH3* gene mutation. However, our patient may differ from "typical" CADASIL patients in that 1) there was no family history, although the patient's parents lived up to 60 and 65 yr of age, respectively, and her 4 siblings were alive and aged more than 55 yr and 2) our patient had no history of mood disorders or attacks of migraine which have been reported to be the frequent early symptoms in the Caucasian CADASIL patients (4). Rather, our patient presented only with two episodes of strokes and mild dementia. Despite severe leukoaraiosis on MRI, absence of known risk factors for stroke in detailed laboratory tests motivated us to consider CADASIL. A similar case without a family history has been reported, but the patient had a history of migraine (6).

Regarding the neuroimaging findings of CADASIL, MRI abnormalities have not been specific for the disease and therefore are not sufficient to establish the diagnosis. Recently, however, MRI of genetically confirmed CADASIL patients showed an abnormally increased signal on T2-weighted images in both temporal poles, which was never observed in non-CADASIL stroke patients (9). T2-weighted and FLAIR MRI of our patient showed the same finding (Fig. 1D), thereby supporting for the notion that a temporal pole hyperintensity may be a radiologic marker of CADASIL. To our knowledge, little has been reported about FDG-PET findings of CADASIL (10). In our patient cerebral glucose metabolism was decreased not only in the subcortical regions (basal ganglia and thalamus) but also in the cortex, more markedly on the right side. These findings were consistent with neuropsychological findings that showed a left hemispatial neglect and visuospatial dysfunction in the presence of general cognitive decline. The FDG-PET also showed a hypometabolism in both temporal pole areas that corresponded to the regions with an MRI abnormality.

Our patient had a Cys67Tyr substitution within the *N0TCH3* gene, a mutation that has not been identified in the previous patients with CADASIL. Considering the absence of a family history, it may be a de novo mutation, although genetic studies of the family were not performed. Among the Asian population, two families with CADASIL from Japan have been reported so far. However, given that the incidence of subcortical vascular dementia is higher than that of Alzheimer's disease in Asian countries compared to Europe and United States (11), and that, as a cause of stroke, intracranial arteriopathy is more frequent than extracranial arteriopathy (12), it is possible that cases of CADASIL might have been overlooked.

In conclusion, we could learn from this case that CADASIL may be included in the differential diagnoses in patients with vascular dementia associated with a small vessel disease, even in the absence of a family history, especially when there are no known stroke risk factors and when the MRI shows a T2 hyperintensity in the temporal pole regions.

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