Pathogenic NOTCH3 Variants Are Frequent Among the Korean General Population

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Abstract

Objective
This study aimed to determine the frequency of pathogenic NOTCH3 variants among Koreans.

Methods
In this cross-sectional study, we queried for pathogenic NOTCH3 variants in 2 Korean public genome databases: the Korean Reference Genome Database (KRGDB) and the Korean Genome Project (Korea1K). In addition, we screened the 3 most common pathogenic NOTCH3 variants (p.Arg75Pro, p.Arg544Cys, and p.Arg578Cys) for 1,000 individuals on Jeju Island, where the largest number of patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) have been reported in Korea.

Results
The pathogenic NOTCH3 variant (p.Arg544Cys) was found in 0.12% of sequences in the KRGDB, and 3 pathogenic variants (p.Arg75Pro, p.Arg182Cys, and p.Arg544Cys) were present in 0.44% of the Korea1K database. Of the 1,000 individuals on Jeju Island, we found 2 cysteine-altering NOTCH3 variants (p.Arg544Cys variant in 9 and p.Arg578Cys in 1 individual) in 1.00% of the participants (95% confidence interval: 0.48%–1.83%). The presence of cysteine-altering NOTCH3 variants was significantly associated with a history of stroke ($p < 0.001$).

Discussion
Pathogenic NOTCH3 variants are frequently found in the general Korean population. Such a high prevalence of pathogenic variants could threaten the brain health of tens of thousands to hundreds of thousands of older adults in Korea.
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) was known as a rare genetic disorder with an estimated prevalence of 2–5/100,000. However, recent genomic database research clearly indicates an estimated global mutation prevalence of 3.4/1,000, much higher than that estimated previously. In particular, the East or South Asian regions showed the highest frequency of cysteine-altering NOTCH3 mutations with a prevalence of 9.0–11.7/1,000 individuals. This study aimed to determine the frequency of pathogenic NOTCH3 variants among Koreans.

Methods

Korean Public Genome Database Query
We queried for pathogenic NOTCH3 variants associated with CADASIL in 2 publicly available Korean genome databases: the Korean Reference Genome Database (KRGDB) and the Korean Genome Project (Korea1K). The KRGDB was established by the Korean National Institute of Health and contains whole-genome data for 1,722 participants from various cohorts. The Korea1K data set included whole-genome data of 1,094 healthy individuals, mostly from the Ulsan metropolitan region located in the southern part of the Korean peninsula. ANNOVAR bioinformatics software was used for annotating any rare nonsynonymous variants, including missense, inframe insertion/deletion, frameshift, start gain/loss, stop gain/loss, and splice site variants in the NOTCH3 gene. In this study, a minor allele frequency <0.01 was defined as a rare variant. Among the rare variants retrieved from the database, we defined the variant as pathogenic if it was associated with CADASIL and classified as pathogenic or likely pathogenic in ClinVar (ncbi.nlm.nih.gov/clinvar/).

Screening for NOTCH3 Variants Among 1,000 Individuals Living on Jeju Island
Of the 4,350 anonymized genomic DNA samples, a randomly selected 1,000 samples with their health information were provided by the Biobank of Jeju National University Hospital, a member of the Korea Biobank Network. This study was approved by the Institutional Review Board of Jeju National University Hospital (IRB File No. JEJUNUH 2020-09-006). Informed consent was obtained at the time of DNA donation to the Biobank. We screened the 3 most frequently found NOTCH3 mutations on Jeju Island: p.Arg75Pro (c.244G>C), p.Arg544Cys (c.1630>T), and p.Arg578Cys (c.1732C>T). Other detailed information for the PCR, sequencing, and statistical analysis was provided as Supplemental Material, links.lww.com/NXG/A495.

Results
Pathogenic NOTCH3 Variants in 2 Korean Public Genome Databases
Three pathogenic NOTCH3 variants associated with CADASIL, p.Arg75Pro (c.224G>C), p.Arg182Cys (c.544C>T), and p.Arg544Cys (c.1630C>T), were found in 2 Korean public genome databases. In the KRGDB with 1,722 participants, only the p.Arg544Cys variant was identified with a carrier frequency of 0.12%. Three pathogenic variants (p.Arg75Pro, p.Arg182Cys, and p.Arg544Cys) were present in the Korea1K database with 1,094 individuals, and the carrier frequencies were 0.22, 0.11, and 0.11%, respectively. Only the p.Arg544Cys variant was discovered in common (Table 1).

Screening Pathogenic NOTCH3 Variants in 1,000 Individuals on Jeju Island
Of 1,000 individuals on Jeju Island, 3 had a history of stroke, and 6.6% had a family history of stroke. We found 2 cysteine-altering NOTCH3 variants in 1.00% of the participants with a 95% confidence interval of 0.48–1.83% (p.Arg544Cys mutation in 9 individuals and p.Arg578Cys in 1). Compared with individuals without pathogenic NOTCH3 variants, those with the variants were more likely to have a history of stroke (p < 0.001) (Table 2). Of the 10 individuals with pathogenic variants, 2 participants underwent a brain MRI examination (eTable 3, links.lww.com/NXG/A495 and Figure).

Discussion
A recent study from Taiwan reported a very high prevalence of cysteine-altering NOTCH3 variants among Taiwanese (9/1,000), and the study also suggested that the cysteine-altering variant resulted in a 3.40-fold increased risk for stroke and even an 11.05-fold increased risk for small-vessel stroke. In line with the Taiwanese study, this study suggested a high prevalence of pathogenic NOTCH3 variants among the general Korean population, with an estimated number of carriers ranging from 1.2/1,000 to 4.4/1,000. On Jeju Island, located

Table 1 Prevalence of Pathogenic NOTCH3 Variants in 2 Korean Public Genome Databases and 1,000 Individuals Living on Jeju Island

|                      | KRGDB  | Korea 1K | Jeju screening study |
|----------------------|--------|----------|----------------------|
| Number of participants | 1,722  | 1,094    | 1,000                |
| NOTCH3 variant frequency | p.Arg544Cys (0.12%) | p.Arg75Pro (0.22%) | p.Arg544Cys (0.90%) |
|                      |        | p.Arg544Cys (0.11%) | p.Arg578Cys (0.10%) |

Abbreviation: KRGDB = Korean Reference Genome Database.
off the southern coast of the Korean peninsula with a current population of 670,000, the number of mutation carriers could reach 67,000, and they could be at increased risk of stroke or develop other symptoms reported in CADASIL. Long-term impact of harboring pathogenic NOTCH3 variants in seemingly healthy young individuals needs to be investigated further.

Table 2 Baseline Characteristics of the Jeju Participants

|                         | No variants (n = 990) | Variants (n = 10) | Total (N = 1,000) | p Value |
|-------------------------|----------------------|------------------|------------------|---------|
| Demographic             |                      |                  |                  |         |
| Age, y                  | 49.9 ± 11.3          | 51.9 ± 14.5      | 49.9 ± 11.3      | 0.573   |
| Male                    | 454 (45.4)           | 6 (60.0)         | 460 (46.0)       | 0.373   |
| Height, cm              | 164.6 ± 8.5          | 165.8 ± 6.3      | 164.6 ± 8.5      | 0.661   |
| Weight, kg              | 64.1 ± 11.6          | 63.1 ± 8.3       | 64.1 ± 11.6      | 0.771   |
| Vascular risk factors   |                      |                  |                  |         |
| Hypertension            | 4 (0.4)              | 0 (0.0)          | 4 (0.4)          | 0.841   |
| DM                      | 0 (0.0)              | 0 (0.0)          | 0 (0.0)          | —       |
| Hyperlipidemia          | 38 (3.8)             | 0 (0.0)          | 38 (3.8)         | 0.528   |
| Smoking                 | 256 (25.9)           | 2 (20.0)         | 258 (25.8)       | 0.674   |
| Medical history         |                      |                  |                  |         |
| Stroke                  | 0 (0.0)              | 3 (30.0)         | 3 (0.3)          | <0.001  |
| Ischemic heart disease  | 4 (0.4)              | 0 (0.0)          | 4 (0.4)          | 0.841   |
| Family history          |                      |                  |                  |         |
| Stroke                  | 65 (6.6)             | 1 (10.0)         | 66 (6.6)         | 0.664   |
| Laboratory finding      |                      |                  |                  |         |
| Systolic blood pressure, mm Hg | 117.8 ± 11.5   | 118.7 ± 10.2   | 117.8 ± 11.5   | 0.800   |
| Diastolic blood pressure, mm Hg | 74.1 ± 8.2       | 74.9 ± 8.9     | 74.1 ± 8.2     | 0.756   |
| White blood cell, k/mm³ | 5.4 ± 1.5            | 4.5 ± 0.7       | 5.4 ± 1.5       | 0.045   |
| Hemoglobin, g/dL        | 14.0 ± 1.7           | 14.1 ± 1.4      | 14.0 ± 1.7      | 0.943   |
| Platelet count, k/mm³   | 250.3 ± 56.2         | 240.6 ± 33.9    | 250.2 ± 56.1    | 0.586   |
| BUN, mg/dL              | 12.0 ± 3.6           | 12.1 ± 3.2      | 12.0 ± 3.6      | 0.950   |
| Creatinine, mg/dL       | 0.9 ± 0.2            | 1.0 ± 0.2       | 0.9 ± 0.2       | 0.935   |
| Estimated GFR (IDMS-MDRD), mL/min/1.73 m² | 93.1 ± 15.8   | 95.3 ± 15.7    | 93.1 ± 15.8    | 0.812   |
| Fasting glucose, mg/dL  | 89.9 ± 8.8           | 94.1 ± 11.7     | 90.0 ± 8.9      | 0.140   |
| HbA1C, %                | 5.4 ± 0.4            | 5.4 ± 0.4       | 5.4 ± 0.4       | 0.970   |
| CRP, mg/dL              | 0.13 ± 0.43          | 0.03 ± 0.01     | 0.13 ± 0.43     | 0.486   |
| Homocysteine, mg/dL     | 8.5 ± 2.8            | 8.1 ± 1.2       | 8.5 ± 2.8       | 0.765   |
| Total cholesterol, mg/dL| 190.4 ± 26.1         | 172.0 ± 31.3    | 190.2 ± 26.2    | 0.028   |
| LDL cholesterol, mg/dL  | 115.9 ± 24.0         | 110.3 ± 28.7    | 115.9 ± 24.0    | 0.494   |
| HDL cholesterol, mg/dL  | 59.2 ± 14.3          | 48.0 ± 9.5      | 59.0 ± 14.3     | 0.014   |
| Triglyceride, mg/dL     | 82.9 ± 36.8          | 77.0 ± 38.3     | 82.8 ± 36.8     | 0.614   |

Abbreviations: BUN, blood urea nitrogen; DM, diabetes mellitus; GFR, glomerular filtration rate; HDL, high-density lipoprotein; IDMS-MDRD, isotope dilution mass spectrometry—the modification of diet in renal disease; LDL, low-density lipoprotein.

Data are N (%), mean ± SD.

Data were compared using the Pearson χ² test, Fisher exact test, Student t test, or Wilcoxon rank-sum test according to the characteristics of the variables.
This study has several limitations. First, because of the limited sample size of the Korean public genome database and the Jeju screening study, this study was underpowered to prove whether the Korean general population has a high prevalence of pathogenic NOTCH3 variants reported in the previous literature. Second, we screened only the 3 most frequently found pathogenic variants in the Jeju screening study. Therefore, we might miss other rare pathogenic variant associated with CADASIL in these 1,000 individuals.

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Disclosure
Go to Neurology.org/NG for full disclosures.

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Appendix Authors

| Name          | Location                                                                 | Contribution                                                                 |
|---------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
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| Young Mee Kim, PhD | Department of Biochemistry, School of Medicine, Jeju National University, Jeju, Korea | Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data; additional contributions: this author contributed equally to the manuscript as the first author. |
| Yang-Ji Kim, MS | Institute of Medical Science, Jeju National University, Jeju, Korea | Major role in the acquisition of data and analysis or interpretation of data |
| Su-Jeong Hong, MS | Institute of Medical Science, Jeju National University, Jeju, Korea | Major role in the acquisition of data and analysis or interpretation of data |
| Do Yoon Kim, BS | Department of Physiology, Ajou University School of Medicine, Suwon, Republic of Korea; Department of Biomedical Science, Graduate School, Ajou University, Suwon, Republic of Korea | Analysis or interpretation of data |
| Hyun Goo Woo, MD, PhD | Department of Physiology, Ajou University School of Medicine, Suwon, Republic of Korea; Department of Biomedical Science, Graduate School, Ajou University, Suwon, Republic of Korea | Study concept or design |
| Young Ree Kim, MD, PhD | Department of Laboratory Medicine, Jeju National University School of Medicine, Jeju, Korea | Major role in the acquisition of data |
| Joong-Goo Kim, MD | Department of Neurology, Jeju National University School of Medicine, Jeju, Korea | Study concept or design |

Patient 2 is a 58-year-old man who had had an abrupt onset of mild dysarthria at age 54 years. Diffusion MRI at that time showed a small subacute infarction on the left frontal white matter (arrow) and multiple small white matter hyperintensity lesions on fluid attenuated inversion recovery images. Patient 3 is a 50-year-old man and received a brain MRI examination as a screening health checkup and had a few small subcortical white matter hyperintensity lesions and periventricular white matter lesions.
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Appendix (continued)

| Name                   | Location                                                                 | Contribution                                                                 |
|------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Jung Seok Lee, MD      | Department of Neurology, Jeju National University Hospital, Jeju National University School of Medicine, Jeju, Korea | Study concept or design                                                      |
| Mi Hee Kong, MD, PhD   | Department of Family Medicine, Jeju National University Hospital, Jeju, Korea; Department of Family Medicine, Jeju National University School of Medicine, Jeju, Korea | Major role in the acquisition of data                                        |
| Hyeon Ju Kim, MD       | Department of Family Medicine, Jeju National University Hospital, Jeju, Korea; Department of Family Medicine, Jeju National University School of Medicine, Jeju, Korea | Major role in the acquisition of data                                        |

Appendix (continued)

| Name                   | Location                                                                                | Contribution                                                                 |
|------------------------|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Jay Chol Choi, MD, PhD | Department of Neurology, Jeju National University Hospital, Jeju National University School of Medicine, Jeju, Korea; Institute of Medical Science, Jeju National University, Jeju, Korea | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data |
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