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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.

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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.[1,2] However, recent genomic database research clearly indicates an estimated global mutation prevalence of 3.4/1,000, much higher than that estimated previously.[3] 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).[4,5] 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.[6] 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.[7] 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 (0.11%) p.Arg182Cys (0.11%)	p.Arg544Cys (0.90%) p.Arg578Cys (0.10%)

Abbreviation: KRGDB = Korean Reference Genome Database.

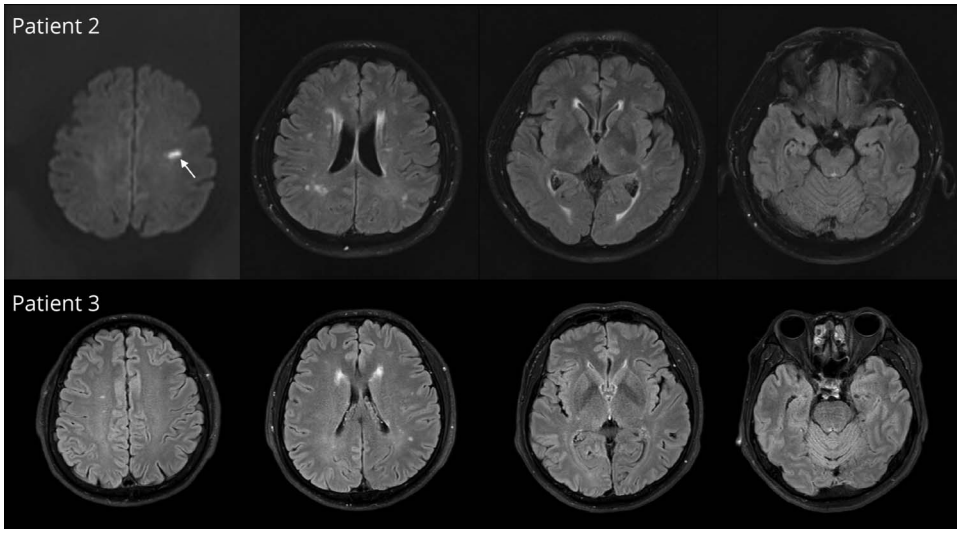
Table 2 Baseline Characteristics of the Jeju Participants

	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/mm^3	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/mm^3	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 m^2	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 χ^2 test, Fisher exact test, Student *t* test, or Wilcoxon rank-sum test according to the characteristics of the variables.

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.

Figure Brain MRI of the 2 Individuals With p.Arg544Cys Variants



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.

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

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Appendix *(continued)*

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Appendix *(continued)*

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Appendix *(continued)*

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