Supplemental methods

Screening for NOTCH3 variants among 1,000 individuals living on Jeju Island

The three mutant sites were divided into target1 (p.Arg75Pro) and target2 (p.Arg544Cys and p.Arg578Cys) and screened by Sanger sequencing (Supplemental Table 1). The targets were amplified with oligonucleotide or specific primers designed by the Bioneer Corporation (Bioneer, Daejeon, Republic of Korea) (Supplemental Table 2). The *polymerase chain reaction (PCR)* was performed with the AllInOneCycler™ 96 well PCR system (Bioneer, Daejeon, Republic of Korea) as follows: 30 cycles of denaturation at 95 °C for 30 s, annealing at 70 °C for 30 s, and extension at 72 °C for 30 s. The amplified products were purified with an AccuPrep® PCR/Gel Purification Kit (Bioneer, Daejeon, Republic of Korea). Sequencing analysis was performed with ABI3730XL (ThermoFisher Scientific, Waltham, Massachusetts, USA) using a BigDye Terminator v3.1 sequencing kit (ThermoFisher Scientific, Waltham, Massachusetts, USA). ChromasPro Version 2.0.1.(Technelysium Pty Ltd, South Brisbane, Australia) was used to identify the mutations.

Statistical analysis

The characteristics of 1,000 individuals were summarized as means±standard deviation (SD) or medians (interquartile range [IQR]) for continuous variables and as numbers (%) for categorical variables. The characteristics of the individuals with and without pathogenic variants were compared using Pearson's chi-square test, Fisher's exact test, Student's t-test, or the Wilcoxon rank-sum test according to the variables' characteristics. All statistical analyses were performed using Stata data analysis software version 15.1. (StataCorp, College Station, Texas, USA). In all analyses, a P-value < 0.05 was considered statistically significant.

eTable 1. Target

	Target	Size	Forward Primer	Reverse Primer
1	p.Arg75Pro	717 bp	NOTCH3-F1-1	NOTCH3-R1-1
2	p.Arg544Cys, p.Arg578Cys	754 bp	NOTCH3-F2-1	NOTCH3-R2-1

eTable 2. Primer sequences

Classification	Primer Name	Sequence
PCR amplify	NOTCH3-F1-1	CTTTGAGACCGAGTCTTACTCTGTCAC
	NOTCH3-R1-1	CAGGAGCAGAGGAAGCGTCCATC
	NOTCH3-F2-1	CAATACCTTCCCCTGGCACCTGC
	NOTCH3-R2-1	CAGTCGTAGCGGTTGATGCCATCAC
Sequencing analysis	NOTCH3-R1	AGGGATCTGGCAGGGAGCAGTC
	NOTCH3-F2	GAGAGTCTTGGCTAGGGACAAA

e Table 3. The characteristics of individuals with pathogenic *NOTCH3* variants

No	Age, y	Sex	Clinical presentation	*NOTCH3* variant	HTN	DM	HL	Smoking	History of stroke	Family history of stroke
1	40	F	None	p.Arg544Cys	N	N	Y	N	N	N
2	58	M	Dysarthria	p.Arg544Cys	N	N	N	Y	Y	Y
3	50	M	None	p.Arg544Cys	N	N	Y	Y	N	N
4	53	F	None	p.Arg544Cys	N	N	N	N	N	N
5	23	M	None	p.Arg544Cys	N	N	N	Y	N	N
6	46	M	Headache	p.Arg544Cys	N	N	N	Y	Y	N
7	23	F	None	p.Arg544Cys	N	N	N	N	N	N
8	68	M	None	p.Arg544Cys	N	N	N	Y	Y	N
9	44	M	None	p.Arg544Cys	N	N	N	Y	N	N
10	67	F	None	p.Arg578Cys	N	N	Y	N	N	N

HTN, hypertension; DM, diabetes mellitus; HL, hyperlipidemia