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ORIGINAL RESEARCH

Association of Rare *NOTCH3* Variants With Prevalent and Incident Stroke and Dementia in the General Population

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BACKGROUND: It is uncertain whether rare *NOTCH3* variants are associated with stroke and dementia in the general population and whether they lead to alterations in cognitive function. This study aims to determine the associations of rare *NOTCH3* variants with prevalent and incident stroke and dementia, as well as cognitive function changes.

METHODS AND RESULTS: In the prospective community-based Shunyi Study, a total of 1007 participants were included in the baseline analysis. For the follow-up analysis, 1007 participants were included in the stroke analysis, and 870 participants in the dementia analysis. All participants underwent baseline brain magnetic resonance imaging, carotid ultrasound, and whole exome sequencing. Rare *NOTCH3* variants were defined as variants with minor allele frequency <1%. A total of 137 rare *NOTCH3* carriers were enrolled in the baseline study. At baseline, rare *NOTCH3* variant carriers had higher rates of stroke (8.8% versus 5.6%) and dementia (2.9% versus 0.8%) compared with noncarriers. After adjustment for associated risk factors, the epidermal growth factor-like repeats (EGFr)-involving rare *NOTCH3* variants were associated with a higher risk of prevalent stroke (odds ratio [OR], 2.697 [95% CI, 1.266–5.745]; *P*=0.040) and dementia (OR, 8.498 [95% CI, 1.727–41.812]; *P*=0.032). After 5 years of follow-up, we did not find that the rare *NOTCH3* variants increased the risk of incident stroke and dementia. There was no statistical difference in the change in longitudinal cognitive scale scores.

CONCLUSIONS: Rare *NOTCH3* EGFr-involving variants are genetic risk factors for stroke and dementia in the general Chinese population.

Key Words: cognitive function ■ dementia ■ *NOTCH3* ■ rare variants ■ stroke

Several monogenic diseases are associated with stroke and vascular dementia. The most common is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL),[1] which is caused by *NOTCH3* variants and is clinically characterized by migraine with aura, subcortical ischemic events, mood disturbances, apathy, and cognitive impairment.[2]

The *NOTCH3* gene is highly variable in community-dwelling elderly, with both common and rare single-nucleotide polymorphisms distributed throughout the gene.[3] Rare *NOTCH3* variants have been identified in a considerable proportion of the general population and are associated with imaging changes related to cerebral small vessel disease. Thirty-three and 31 rare *NOTCH3* single-nucleotide polymorphisms with minor

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CLINICAL PERSPECTIVE

What Is New?

- This Chinese population-based cohort study of 1007 participants indicated that carriers of the epidermal growth factor-like repeats (EGFr)-involving rare *NOTCH3* variant have a higher prevalence of stroke and dementia.
- This study adds to our understanding of the clinical features and impact of rare *NOTCH3* variants.

What Are the Clinical Implications?

- Rare *NOTCH3* variant carriers not only have more severe imaging features of cerebral small vessel disease but also present with clinical symptoms.
- They should be regarded as a predisposed population for stroke and dementia.

Nonstandard Abbreviations and Acronyms

CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
EGFr	epidermal growth factor-like repeats
ICAS	intracranial atherosclerotic stenosis
WES	whole exome sequencing

allele frequency <5% were identified in the Austrian Stroke Prevention Study[3] and the 3C-Dijon Study,[4] respectively. In addition, several studies have found that rare *NOTCH3* variants are significantly enriched in patients with Alzheimer disease (AD) compared with controls.[5,6] In our team's previous study,[7] we found that the carrying rate of rare *NOTCH3* variants was 13.8% in the general population of the Chinese community. We also found that rare *NOTCH3* variants were associated with a higher volume of white matter hyperintensities and a greater burden of cerebral small vessel disease. Therefore, we hypothesized that individuals with rare *NOTCH3* variants would also have an increased risk of stroke or dementia.

To explore this further, we conducted a 5-year follow-up study in a community-based population cohort. The rare *NOTCH3* variant carriers were identified by whole exome sequencing (WES). We determined their associations with both prevalent and incident stroke and dementia. We also compared clinical characteristics and longitudinal changes in cognitive function between participants with and without rare *NOTCH3* variants. This study adds to our understanding of the impact of rare *NOTCH3* variants.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Participants

All participants were from the Shunyi Study, an ongoing prospective community-based cohort study designed to investigate the risk factors and consequences of brain alterations in older people in China. The detailed study protocol has been previously published.[8] Inclusion criteria for participants were permanent local residents ≥35 years old, living independently, and volunteering to participate. From June 2013 to September 2014, a total of 1586 participants were enrolled in the Shunyi Study. Complete standard baseline assessments, including structured questionnaires, physical examination, and laboratory tests, were collected as previously described. All participants were invited to undergo baseline brain magnetic resonance imaging (MRI) and carotid ultrasound (1147 completed both assessments). The cohort was followed up annually between 2017 and 2020, with cognitive assessments every 3 years.

The study flowchart is shown in the Figure. Of the 1586 participants, 579 were excluded due to refusal of MRI, poor-quality MRI images, and incomplete baseline data, leaving 1007 participants for baseline analysis. Baseline demographic characteristics of included (n=1007) and excluded (n=579) individuals are compared in Table S1. Participants were divided into 3 groups according to rare *NOTCH3* variants, the group without rare *NOTCH3* variants, the group with epidermal growth factor-like repeats (EGFr)-involving rare *NOTCH3* variants, and the group with EGFr-sparing rare *NOTCH3* variants. All subjects at baseline completed a stroke follow-up assessment, and 1007 subjects were included in the analysis of incident stroke. For the analysis of incident dementia, we excluded 11 subjects with a baseline diagnosis of dementia and 126 subjects who did not complete the cognitive follow-up assessment, leaving 870 subjects for the longitudinal analysis of dementia. The average follow-up time for stroke and dementia was 4.3 (3.8–4.7) and 4.0 (3.8–4.9) years, respectively.

This study was approved by the ethics committee of Peking Union Medical College Hospital. Written informed consent was obtained from all participants.

Genetic Analysis

WES analysis of the *NOTCH3* gene was performed according to the previously described protocol.[7] We extracted all the sites of the *NOTCH3* gene based on the WES data, and 258 rare *NOTCH3* variants were

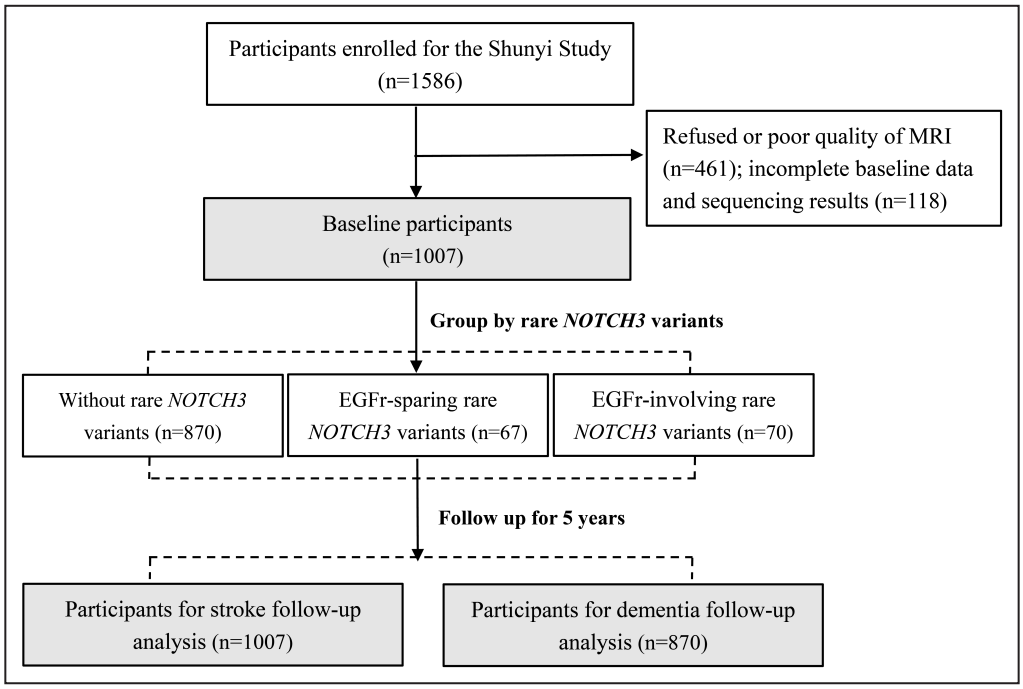

Figure. Flowchart of study population recruitment.
EGFr indicates epidermal growth factor-like repeats; and MRI, magnetic resonance imaging.

detected. Rare *NOTCH3* variants were defined as variants with minor allele frequency <1% in all 4 public population databases. Of these, 193 rare *NOTCH3* variants were further excluded, including 158 variants located in the intronic region and 35 synonymous single-nucleotide polymorphisms. Finally, 65 rare *NOTCH3* variants that could lead to functional changes by changing amino acids (n=60) or affecting splicing (n=5) were included in our analysis (Figure S1). The functionality of the detected rare *NOTCH3* single-nucleotide polymorphisms was assessed using Sorting Intolerant From Tolerant, Polyphen2, Mutation Taster, and Combined Annotation Dependent Depletion in silico. The selection of rare *NOTCH3* was performed blinded to clinical data.

Evaluation of Intracranial and Carotid Arteries

Intracranial atherosclerotic stenosis (ICAS) was assessed by time-of-flight magnetic resonance angiography at the site of the most severe stenosis according to established criteria.[9] The definition of ICAS was any degree of stenosis in at least 1 of the following arteries: internal carotid artery, middle cerebral artery, anterior cerebral artery, intracranial segment of the vertebral artery, basilar artery, posterior cerebral artery. The presence of carotid plaque was determined in bilateral common, internal, and bifurcation sites of the carotid arteries in a supine position with a color Doppler ultrasound diagnostic system (Esaote, Firenze, Italy) using a 5- to 13-MHz vascular probe LA523 according to a standardized scanning protocol. Plaques were identified as focal structures encroaching at least 1.5 mm into the arterial lumen.[10]

Cognitive Function Assessments

Cognitive function assessments were conducted twice, with a baseline assessment from 2013 to 2014 and a follow-up assessment from 2017 to 2020. At both time points, participants underwent a comprehensive neuropsychological assessment to measure overall cognition and to examine the cognitive domains of memory, language, attention, visuospatial perception, and executive function. Cognitive testing included the Mini-Mental State Examination, the Montreal Cognitive Assessment, the Fuld Object Memory Test, the Rapid Verbal Retrieval Test, the Digit Span Test, the Wechsler Intelligence Scale for Children: Block Design subtest, and the Clock Draw Test.

Diagnosis of Stroke and Dementia

To diagnose previous strokes, a structured questionnaire was used to ask each participant about their history of stroke and associated clinical symptoms, and a physical examination was performed. For participants who reported a stroke or suspected stroke, further information was obtained from their medical records. We obtained and recorded detailed information, including signs and symptoms, date of onset, duration, discharge letters, and brain MRI or computed

tomography scans from the hospital where they were treated. The diagnosis of stroke was confirmed by an experienced vascular neurologist. Participants were followed up annually for new-onset stroke through on-site interviews. In addition, we continuously monitored the medical records of all participants at the community health centers. Stroke was defined as a syndrome of rapidly evolving clinical signs of focal or global deficits in cerebral function documented by neurological examination and lasting >24 hours. New-onset stroke was defined as a stroke event occurring during the follow-up period, without excluding individuals with baseline stroke. Follow-up began on the date participants underwent brain MRI. Participants were followed until the date of stroke occurrence, date of death, or date of last contact.

Participants' cognitive status was determined by a review panel consisting of 2 neurologists specializing in cognitive disorders and cerebrovascular disease. All available source documents in each study cycle, including medical history, neurological examination, neuropsychological testing, and stroke review results, were used to determine cognitive status and, in the case of dementia, to assign a possible date of onset. The diagnosis of dementia needs to satisfy the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*) criteria for dementia (or the 2011 National Institute on Aging and the Alzheimer's Association criteria for all-cause dementia: core clinical criteria[11]). The start time is the date of the first cognitive assessment, and the end time is the date of dementia diagnosis. For disease-free subjects, time was censored at the date of last contact or date of death.

Assessment of Other Baseline Characteristics

Demographic and clinical information, including age, sex, apolipoprotein E (*APOE*) ε4 allele carrier status, smoking status, alcohol intake, history of hypertension, diabetes, hyperlipidemia, and current medication, was collected using a structured questionnaire and physical examination. Definitions of these baseline characteristics are described in Data S1.

Statistical Analysis

Continuous variables are expressed as mean (SD) or median (interquartile range), and categorical variables are expressed as frequencies (percentages). We used ANOVA, Kruskal-Wallis test, Student *t* test, or Mann-Whitney *U* test for continuous variables and the χ^2 test or Fisher exact test for categorical variables to analyze differences in baseline characteristics. The associations between baseline prevalent stroke, dementia, ICAS, and carotid plaques (dependent variables) and rare *NOTCH3* variants (independent variables) were investigated using binary logistic regression. Three models were used to adjust for confounders in a stepwise manner. Model 1 was univariate. Model 2 was adjusted for age and sex. Model 3, based on Model 2, was additionally adjusted for hypertension and diabetes for stroke, ICAS, and carotid plaques. We corrected for multiple testing in the 3 models using the Benjamini-Hochberg false discovery rate correction.

The scores of each cognitive test were normalized to *Z* scores [(individual test score–mean score)/SD]. We assessed longitudinal changes in cognitive function by differences in the subtraction *Z* scores of these cognitive scales at baseline and follow-up. Cox regression models were used to compare the risk of incident stroke and dementia between groups, and the hazard ratio and 95% CI were estimated. All analyses were conducted using SPSS (version 26.0) and R (version 4.0.3). Statistical significance was set at $P<0.05$ by false discovery rate correction.

RESULTS

Demographic Characteristics of the Study Population

The baseline characteristics of the study population are shown in Table 1. Of the 1007 participants, the mean age was 55.1 years (SD, 8.6), and 35.7% were men. At baseline, there were 61 participants (6.06%) with stroke and 11 participants (1.09%) with dementia in the total population. The carrier rate of the *APOE* ε4 allele was 15.5%. A total of 137 rare *NOTCH3* carriers were enrolled in the baseline study. Of the 137 individuals with rare variants, most were cysteine sparing. In this population, we found only 2 individuals carrying cysteine-altering variants of *NOTCH3* (p.R587C and p.C738Y). These 2 carriers of rare cysteine-altering variants did not develop stroke or dementia and performed normally on cognitive tests.

In the follow-up analysis of incident dementia, participants who were lost to follow-up were significantly older, less educated, and had a higher prevalence of cardiovascular risk factors than those who were followed up (Table S2).

Association of Rare *NOTCH3* Variants With Stroke

The association between rare *NOTCH3* variants and prevalent stroke is shown in Table 2. At baseline, stroke prevalence was not significantly higher in rare *NOTCH3* variant carriers (12/137, 8.8% versus 49/870, 5.6%, $P=0.539$). By further stratified analysis, we found that EGFr-involving *NOTCH3* variant carriers were associated with a higher risk of stroke, and the difference remained after correction for associated vascular risk

Table 1. Baseline Characteristics of the Study Population

Characteristic	Without rare *NOTCH3* variant	With rare *NOTCH3* variant		*P* value*	*P* value†	*P* value‡	*P* value§
		EGFr-sparing	EGFr-involving				
	N=870	N=67	N=70				
Age, y	55 (49–63)	56 (49–62)	58 (48–65)	0.728	0.896	0.929	0.544
Men	311 (35.7%)	25 (37.3%)	29 (41.4%)	0.947	0.972	0.797	0.597
Education, y	8 (5–9)	8 (4–9)	8 (5–9)	0.789	0.965	0.838	0.851
BMI	26.3 (23.8–29.0)	26.2 (24.1–29.4)	26.9 (23.9–29.8)	0.767	0.948	0.791	0.592
Hypertension	461 (53.0%)	35 (52.2%)	40 (57.1%)	0.819	0.849	0.906	0.542
Diabetes	142 (16.3%)	11 (16.4%)	15 (21.4%)	0.884	0.956	0.987	0.635
Hyperlipidemia	430 (49.5%)	34 (50.7%)	30 (42.9%)	0.766	1.000	0.842	0.572
Cardiovascular disease	132 (15.2%)	13 (19.4%)	13 (18.6%)	0.714	1.000	0.356	0.629
Current smoker	181 (20.9%)	19 (28.4%)	19 (27.1%)	0.327	0.665	0.149	0.605
APOE ε4 carriers	133 (16.2%)	8 (12.1%)	15 (22.1%)	0.844	0.806	0.381	0.749
ICAS	126 (14.5%)	10 (14.9%)	13 (18.6%)	0.852	0.918	0.927	0.557
Carotid plaques	381 (50.2%)	35 (61.4%)	42 (65.6%)	0.084	0.147	0.103	0.126
Stroke	49 (5.6%)	2 (2.99%)	10 (14.3%)	0.539	0.112	0.574	0.126
Dementia	7 (0.8%)	1 (1.5%)	3 (4.3%)	0.350	0.117	0.449	0.154

Data represent median (interquartile range) or frequency (percentage). Multiple corrections for *P* values were made using the false discovery rate method. BMI indicates body mass index; EGFr, epidermal growth factor-like repeats; and ICAS, intracranial atherosclerotic stenosis.

*Significance test between the group without rare *NOTCH3* variant and group with rare *NOTCH3* variant, using the *t* test, χ^2 test, or Mann-Whitney *U* test.

†Significance test among 3 groups, using the ANOVA, χ2 test or Kruskal-Wallis test.

‡Significance test between the EGFr-sparing group and group without rare *NOTCH3* variant, using the *t* test, χ^2 test, or Mann-Whitney *U* test.

§Significance test between the EGFr-involving group and group without rare *NOTCH3* variant, using the *t* test, χ^2 test, or Mann-Whitney *U* test.

factors (odds ratio [OR], 2.697 [95% CI, 1.266–5.745]; *P*=0.040). In addition, we found that the prevalence of carotid plaque was higher in carriers of rare *NOTCH3* variants compared with noncarriers, and the difference remained after correction for associated vascular risk factors (OR, 1.885 [95% CI, 1.188–2.990]; *P*=0.028). There was no difference in the prevalence of ICAS between the 2 groups.

After 5 years of follow-up, 3 (3/137, 2.2%) of the rare *NOTCH3* variant carriers and 30 (30/870, 3.4%) of the noncarriers had a new stroke. Rare *NOTCH3* variants were not associated with the risk of incident stroke (hazard ratio [HR], 0.590 [95% CI, 0.180–1.937]; *P*=0.384), as shown in Table 3. The Kaplan-Meier survival curve of stroke is shown in Figure S2.

Association of Rare *NOTCH3* Variants With Dementia

At baseline, 4 of the rare *NOTCH3* variant carriers (4/137, 2.9%) had dementia, and 7 of the noncarriers (7/870, 0.8%) had dementia. The association between rare *NOTCH3* variants and prevalent dementia is shown in Table 2. The prevalence of dementia was significantly higher in carriers of rare EGFr-involving *NOTCH3* variants than in those without rare *NOTCH3* variants, and this difference persisted after adjusting for age and sex (OR, 8.498 [95% CI, 1.727–41.812]; *P*=0.032). There was no difference in the prevalence of dementia between EGFr-sparing rare *NOTCH3* variant carriers and noncarriers. When comparing the cognitive scores, there was no difference among the 3 groups, as shown in Table S3. The median Mini-Mental State Examination score was 27 for both rare *NOTCH3* carriers and noncarriers, and the median Montreal Cognitive Assessment score was 19 for both carriers and noncarriers.

In the dementia-free population, after 5 years of follow-up, 3 of the rare *NOTCH3* variant carriers (3/114, 2.6%) had incident dementia and 19 of the noncarriers (19/756, 2.5%) had incident dementia. Rare *NOTCH3* variants were not associated with the risk of incident dementia (HR, 0.992 [95% CI, 0.283–3.470]; *P*=0.989), as shown in Table 3. The Kaplan-Meier survival curve of dementia is shown in Figure S3. There were no between-group differences in longitudinal changes in cognitive scores, as shown in Table S4.

DISCUSSION

In a sample of Chinese community-dwelling rural residents, we found that EGFr-involving rare *NOTCH3* variants were associated with prevalent stroke and dementia. In addition, after correcting for associated vascular risk factors, the rate of carotid plaques was higher in rare *NOTCH3* variant carriers. This suggests that rare *NOTCH3* variant carriers not only have more

Table 2. Association Between Rare *NOTCH3* Variants and Prevalent ICAS, Carotid Plaque, Stroke, and Dementia

Variable	Model 1		Model 2		Model 3	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
With vs without rare *NOTCH3* variants (n=137 and n=870)						
ICAS	1.188 (0.731–1.932)	0.487	1.136 (0.683–1.888)	0.623	1.104 (0.655–1.861)	0.947
Carotid plaque	1.736 (1.168–2.582)	0.024*	1.903 (1.203–3.011)	0.024*	1.885 (1.188–2.990)	0.028*
Stroke	1.608 (0.832–3.108)	0.209	1.563 (0.800–3.051)	0.255	1.550 (0.788–3.048)	0.408
Dementia	3.704 (1.070–12.823)	0.078	4.511 (1.176–17.297)	0.056	…	…
EGFr+ vs without rare *NOTCH3* variants (n=70 and n=870)						
ICAS	1.343 (0.714–2.525)	0.360	1.265 (0.654–2.447)	0.485	1.211 (0.617–2.378)	0.769
Carotid plaque	1.894 (1.109–3.234)	0.025*	1.959 (1.063–3.611)	0.041*	1.893 (1.022–3.504)	0.084
Stroke	2.793 (1.347–5.788)	0.024*	2.712 (1.287–5.715)	0.018*	2.697 (1.266–5.745)	0.040*
Dementia	5.514 (1.394–21.811)	0.030*	8.498 (1.727–41.812)	0.032*	…	…
EGFr– vs without rare *NOTCH3* variants (n=67 and n=870)						
ICAS	1.033 (0.514–2.076)	0.927	1.005 (0.484–2.083)	0.990	0.991 (0.467–2.104)	0.982
Carotid plaque	1.578 (0.909–2.741)	0.105	1.842 (0.968–3.503)	0.063	1.8623 (0.976–3.555)	0.059
Stroke	0.516 (0.123–2.168)	0.366	0.496 (0.117–2.108)	0.342	0.492 (0.115–2.104)	0.339
Dementia	1.866 (0.226–15.392)	0.562	2.160 (0.235–19.833)	0.496	…	…

Model 1 was unadjusted. Model 2 was adjusted for age and sex. Model 3 was based on Model 2 and was additionally adjusted for hypertension and diabetes for stroke, ICAS, and carotid plaques. Multiple corrections for *P* values were made using the false discovery rate method. EGFr indicates epidermal growth factor-like repeats; ICAS, intracranial atherosclerotic stenosis; and OR, odds ratio.

*Surviving a false discovery rate-corrected threshold of *P*=0.05.

severe imaging features of cerebral small vessel disease but also present with clinical symptoms.

NOTCH3 variants are associated with stroke in patients with CADASIL.[12] Cho et al found that the presence of cysteine-altering *NOTCH3* variants was associated with at least a 2-fold increase in the odds of stroke in the general UK population using WES.[13,14] Consistent with previous studies, our results showed that carriers of the rare *NOTCH3* variants had a 2.7-fold risk of prevalent stroke in the general Chinese population. We found that this association is primarily driven by variants located in the EGFr domain, which is known to be the major modifier in CADASIL.[15,16] The *NOTCH3* gene, located on chromosome 19p13,[17] encodes a single-pass transmembrane receptor expressed primarily in smooth muscle cells and pericytes.[18] EGFr is a major component of the extracellular fragment of NOTCH3 protein and is associated with essential physiological processes such as ligand binding and glycosylation.[19,20] Therefore, we suggest that carriers of EGFr-involving

Table 3. Cox Regression Analyses of Rare *NOTCH3* Variants for the Risk of Incident Stroke and Dementia

Variable	Model 1		Model 2		Model 3	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
With vs without rare *NOTCH3* variants						
Stroke	0.636 (0.194–2.083)	0.454	0.573 (0.175–1.880)	0.359	0.590 (0.180–1.937)	0.384
Dementia	1.133 (0.335–3.833)	0.840	0.992 (0.283–3.470)	0.989	…	…
EGFr+ vs without rare *NOTCH3* variants						
Stroke	0.407 (0.056–2.985)	0.377	0.344 (0.047–2.523)	0.294	0.333 (0.045–2.446)	0.280
Dementia	1.462 (0.340–6.283)	0.610	1.740 (0.397–7.627)	0.462	…	…
EGFr– vs without rare *NOTCH3* variants						
Stroke	0.884 (0.211–3.698)	0.866	0.864 (0.206–3.621)	0.842	0.955 (0.227–4.022)	0.950
Dementia	0.789 (0.106–5.899)	0.817	0.511 (0.064–4.082)	0.527	…	…

Model 1 was unadjusted. Model 2 was adjusted for age and sex. Model 3 was based on Model 2 and was additionally adjusted for hypertension and diabetes for stroke. The total number of subjects for incident stroke analysis was 1007, including EGFr-involving rare *NOTCH3* variant carriers (n=70), EGFr-sparing rare *NOTCH3* variant carriers (n=67), and noncarriers (n=870). The total number of subjects for incident dementia analysis was 870, including EGFr-involving rare *NOTCH3* variant carriers (n=58), EGFr-sparing rare *NOTCH3* variant carriers (n=56), and noncarriers (n=756). EGFr indicates epidermal growth factor-like repeats; and HR, hazard ratio.

rare *NOTCH3* variants should be considered a high-risk group for cerebral small vessel disease.

The association of rare *NOTCH3* variants with small-vessel intracranial lesions has received attention in the past,[4,7] but how they relate to large-vessel disease has not been investigated. In this study, we found that carotid plaque rates were higher in rare *NOTCH3* variant carriers, suggesting that rare variants are also associated with large vessel lesions. Intracranial and extracranial vascular stenosis has been reported in patients with CADASIL.[21–24] Although neglected, infarction associated with large intracranial artery disease may be one of the clinical manifestations of CADASIL, at least in East Asia.[22] One autopsy study[25] reported disruption of vascular smooth muscle cells and the presence of granular osmiophilic material in the aorta, carotid, and renal arteries. It can be speculated that granular osmiophilic material deposition in large vessels may accelerate atherosclerosis, although direct evidence is lacking.

In addition to the association with stroke, we demonstrated a strong association between rare *NOTCH3* variants and prevalent dementia. Previously, Cho et al[13,14] reported that cysteine-altering *NOTCH3* variants increased the risk of vascular dementia by ≈5-fold. They found that all cases of dementia in cysteine-altering *NOTCH3* carriers were of vascular origin, whereas all-cause dementia was not significantly associated with the presence of *NOTCH3* variants. In a case–control study, Guo et al[26] found that pathogenic mutations in *NOTCH3* were enriched in subcortical vascular dementia patients by targeted capture sequencing, but neither common variants nor rare missense variants in *NOTCH3* were associated with AD in the Chinese population. In contrast, Sassi et al[5] identified that rare *NOTCH3* coding variants were associated with AD in the UK and North American populations. Another study[6] reported that the missense mutation in *NOTCH3* (rs149307620) was enriched in patients with AD compared with controls in individuals of European ancestry. The inconsistent results of these studies may be due to differences in sequencing methods, sample size, statistical methods, and ethnicity. In our study, due to the small number of dementia cases, there was insufficient statistical power to distinguish whether the association between rare *NOTCH3* variants and dementia was primarily vascular dementia or AD, so further investigation is needed in the future.

Importantly, rare *NOTCH3* variants affecting cysteine residues, a hallmark of CADASIL,[2] were not found in the Shunyi Study cohort with stroke and dementia. Among the rare *NOTCH3* variant carriers, patients who develop stroke and dementia are cysteine-sparing variant carriers. Therefore, we suggest that genetic research on *NOTCH3* should focus not only on EGFr cysteine-altering *NOTCH3* mutations but also on the possible pathogenic mechanism of rare *NOTCH3* cysteine-sparing variants, especially those involving EGFr. Although most pathogenic *NOTCH3* mutations are currently thought to cause CADASIL by affecting the number of cysteines in the EGFr structural domain, some cysteine-sparing mutations in the EGFr structural domain have been identified in patients with suspected CADASIL.[27–29] Muino et al[30] showed that some cysteine-sparing mutations may be potentially pathogenic, and that patients carrying these mutations have typical clinical CADASIL syndrome and diffuse white matter hyperintensities. Previous studies have shown that amino acids other than cysteine may play a role in the secondary structure of EGFr,[31,32] and some cysteine-sparing missense variants in EGFr can lead to abnormal regulation of NOTCH3 O-glycosylation,[4] thereby affecting its function. In addition, certain cysteine-sparing mutations can lead to aggregation of the NOTCH3 protein,[33] resulting in a corresponding clinical phenotype, similar to the mechanisms observed for typical cysteine mutations.

In the longitudinal follow-up analysis, we found no association between rare *NOTCH3* variants and incident stroke and dementia. There was extensive phenotypic variation among participants carrying rare *NOTCH3* mutations,[7] and significant differences in disease severity and age of onset even among individuals carrying the same *NOTCH3* mutation.[34,35] Due to the short follow-up period and the relatively young study population, the cumulative incidence of stroke and dementia caused by rare *NOTCH3* variants may not be sufficient to reach statistical significance. To the best of our knowledge, this is the first study on the association of rare *NOTCH3* variants with prevalent and incident stroke and dementia, and further validation in other populations is needed in the future. We did not find any longitudinal cognitive changes in the rare *NOTCH3* variant carriers. Several previous studies have compared cognitive function in patients with and without *NOTCH3* variants in the general population,[13] patients with subcortical vascular mild cognitive impairment,[36] patients with subcortical vascular dementia,[26] and patients with Parkinson disease.[37,38] These studies also did not observe cognitive changes in participants with the *NOTCH3* variants.

The main strength of our study is that it is a community-based longitudinal study cohort with a comprehensive, standardized protocol and high-quality clinical, MRI, and WES data. However, the study has several limitations. First, 45.1% (716/1586) of the original study sample was excluded from the final analysis due to incomplete clinical information, MRI data, or follow-up cognitive scores. These individuals were generally older and in poorer health than those included in the study, which may lead to selection bias. Second, this was a single-center study with a small sample size of rare *NOTCH3* variant carriers due to the low frequency of rare *NOTCH3* variants (minor allele frequency <1%). The

participants in our study were relatively young, with a mean follow-up of 5 years, so further validation of the role of rare *NOTCH3* variants is needed in multicenter studies with larger sample sizes, longer follow-up, and different races. Third, the study population was divided into groups based on whether they carried rare *NOTCH3* variants to explore the association between the variants and dementia and stroke. The results obtained by this statistical method represent the cumulative effect of all rare variants in the *NOTCH3* gene. However, the direction and magnitude of the effects of individual variants on stroke and dementia may vary, leading to reduced statistical power. Fourth, we selected several vascular risk factors for adjustment in the logistic models for stroke and dementia, but other unmeasured relevant risk variables may also cause confounding.

CONCLUSIONS

Carriers of rare *NOTCH3* variants involving the EGFr domain have a higher prevalence of stroke and dementia compared with the general Chinese population. They should be regarded as predisposed for stroke and dementia and deserve further investigation in other populations.

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Disclosures

None.

Supplemental Material

Data S1
Tables S1–S4
Figures S1–S3

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