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ORIGINAL RESEARCH

Contribution of the *APOE* Genotype to Cognitive Impairment in Individuals With *NOTCH3* Cysteine-Altering Variants

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BACKGROUND: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most prevalent monogenic cerebral small-vessel disease. Phenotype variability in CADASIL suggests the possible role of genetic modifiers. We aimed to investigate the contributions of the *APOE* genotype and Neurogenic locus notch homolog protein 3 (*NOTCH3*) variant position to cognitive impairment associated with CADASIL.

METHODS AND RESULTS: Patients with the cysteine-altering *NOTCH3* variant were enrolled in a cross-sectional study, including the Mini-Mental State Examination (MMSE), brain magnetic resonance imaging, and *APOE* genotyping. Cognitive impairment was defined as an MMSE score <24. The associations between the MMSE score and genetic factors were assessed using linear regression models. Bayesian adjustment for confounding was used to identify clinical confounders. A total of 246 individuals were enrolled, among whom 210 (85%) harbored the p.R544C variant, 96 (39%) had cognitive impairment, and 150 (61%) had a history of stroke. The *APOE* ε2 allele was associated with a lower MMSE score (adjusted *B*, −4.090 [95% CI, −6.708 to −1.473]; *P*=0.023), whereas the *NOTCH3* p.R544C variant was associated with a higher MMSE score (adjusted *B*, 2.854 [95% CI, 0.603–5.105]; *P*=0.0132) after adjustment for age, education, and history of ischemic stroke. Mediation analysis suggests that the associations between the *APOE* ε2 allele and MMSE score and between the *NOTCH3* p.R544C variant and MMSE score are mediated by mesial temporal atrophy and white matter hyperintensity, respectively.

CONCLUSIONS: *APOE* genotype may modify cognitive impairment in CADASIL, whereby individuals carrying the *APOE* ε2 allele may present a more severe cognitive impairment.

Key Words: *APOE* ■ CADASIL ■ cerebral small-vessel disease ■ *NOTCH3* ■ vascular cognitive impairment

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most prevalent monogenic cerebral small-vessel disease (SVD). It is caused by pathogenic variants in the Neurogenic locus notch homolog protein 3 (*NOTCH3*) gene mapped to the short arm of chromosome 19 (19p13.2-p13.1) (Online Mendelian Inheritance in Man number 600276). Pathogenic *NOTCH3* variants are almost always stereotyped missense variants leading to loss or gain of a cysteine residue within 1 of the epidermal growth factor–like repeats (EGFRs) in the extracellular domains of the NOTCH3 protein.[1] The clinical manifestations of CADASIL, including migraine, ischemic and hemorrhagic stroke, cognitive impairment, gait disturbance, and psychiatric disorders, partially overlap with sporadic cerebral SVD but often have a younger age of onset and tend to be more multifaceted. Although

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CLINICAL PERSPECTIVE

What Is New?

- In this cross-sectional analysis of patients with the cysteine-altering *NOTCH3* variant, the study provides evidence that the *APOE* ε2 genotype may contribute to a more severe cognitive impairment associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL); this association may be mediated by mesial temporal atrophy.

What Are the Clinical Implications?

- *APOE* genotype may modify the cognitive manifestation among patients with CADASIL.
- The mechanism underlying this clinical association needs to be explored in further studies and may shed light on future drug development.

Nonstandard Abbreviations and Acronyms

BAC	Bayesian adjustment for confounding
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
DWM	deep white matter
EGFR	epidermal growth factor–like repeat
MTA	mesial temporal atrophy
SVD	small-vessel disease
WMH	white matter hyperintensity

CADASIL is a monogenic disease, substantial phenotypic heterogeneity exists on the age of symptom onset, predominant clinical presentation, and imaging severity among patients with CADASIL. The phenotypic heterogeneity is present even among individuals sharing the same pathogenic variant and within families.[2,3] Previous studies suggested that both *NOTCH3* pathogenic variant positions[4,5] and concurrent vascular risk factors[6–8] may contribute to the risk of stroke and disability among patients with CADASIL. Conversely, factors that influence the clinical manifestations other than cerebrovascular events remain largely unknown. Some studies suggest that hypertension and smoking are associated with dementia among patients with CADASIL.[6,9]

Cognitive impairment is 1 of the core manifestations and the major cause of disability among patients with CADASIL. It has been presumed that cognitive impairment in CADASIL is a consequence of cerebral vasculopathy. However, cerebral SVD burden, measured by structural brain magnetic resonance imaging (MRI), only has a weak correlation with cognitive performance in patients with CADASIL.[10] Furthermore, cerebral cortical atrophy was associated with cognitive impairment in patients with CADASIL independent of their cerebral SVD burden.[11,12] These observations suggest that pathomechanisms in addition to vasculopathy may also contribute to neurodegeneration in CADASIL. Investigation of the genetic and environmental factors that contribute to the variable severity of cognitive dysfunction among patients with CADASIL may reveal the mechanism underlying neurodegeneration in CADASIL. Polymorphism in the apolipoprotein E (*APOE*) gene is the most important genetic risk factor associated with sporadic Alzheimer disease, with the presence of 1 ε4 allele associated with 3.7 times increased risk and 1 ε2 allele associated with 40% reduced risk of developing Alzheimer disease, compared with the most common ε3ε3 genotype.[13] In addition to its association with Alzheimer pathology, more and more evidence suggests that *APOE* may play a role in cerebrovascular function and blood-brain barrier integrity.[14,15] Meta-analysis showed a robust, dose-dependent association between *APOE* ε4 allele and risk of cerebral amyloid angiopathy,[16] 1 of the common pathologic features of age-related cerebral small-vessel disease. Besides, both of the *APOE* ε4 and ε2 alleles are associated with increased risk of intracerebral hemorrhage.[17] Whether the polymorphism of *APOE* contributes to the clinical heterogeneity of cognitive impairment related to CADASIL remained controversial.[2,18]

In the present study, we aimed to explore (1) the contributions of the *APOE* genotype and *NOTCH3* pathogenic variant position to the severity of cognitive impairment among patients harboring cysteine-altering *NOTCH3* variants; and (2) the possible mediation effect of imaging characteristics on the relationship between genetic factors and cognitive impairment.

METHODS

Anonymized data sets analyzed during the current study are available from the corresponding author on reasonable request.

Participants and Cognitive Assessment

The study participants include genetically confirmed patients harboring cysteine-altering *NOTCH3* variants enrolled from 2 medical centers in Taiwan, the Taipei Veterans General Hospital and the National Taiwan University Hospital, between April 2015 and April 2021. The criteria for *NOTCH3* genetic analysis included the following: (1) SVD evident on brain MRI and having at least 1 of the associated clinical manifestations (ie,

cerebrovascular event, cognitive impairment, psychotic symptoms, gait disturbance, and migraine with aura); or (2) the presence of a family history of cysteine-altering *NOTCH3* variants. SVD evident on brain MRI was defined as a moderate-to-severe white matter hyperintensity (WMH) (Fazekas score 2 or 3 on the deep white matter [DWM] regions),[24] or at least mild WMH (Fazekas score ≥1 on the DWM regions) for subjects aged <50 years. Individuals who were asymptomatic (ie, received genetic study because of family history alone) and not having evident SVD on brain MRI were excluded from this study (Figure S1). Enrolled subjects received a standardized questionnaire to document their demographic data, the presence of specific symptoms related to CADASIL, concurrent vascular risk factors, family history for at least 3 generations, and current medication list. In addition, a fasting blood sample was collected for total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose, and hemoglobin A1c. Stroke was defined on the basis of the subject's reported history and verified by his/her medical record when available. Only symptomatic stroke presenting as acute-onset focal neurologic deficit with symptoms lasting for ≥24 hours was included. The types of strokes (ie, ischemic stroke and hemorrhagic stroke) were documented and verified by their medical record when available.

The cognitive performance of the enrolled subjects was assessed by the Taiwanese version of the Mini-Mental State Examination (MMSE) score.[19,20] Cognitive impairment was defined as an MMSE score <24 for patients harboring cysteine-altering *NOTCH3* variants. The educational level of participants was stratified into 3 groups, <6 years, 6 to 12 years, and >12 years. Less than 6 years of education indicates that the individual did not complete elementary school education. A total of 6 to 12 years of education indicates that the individual had completed the elementary, junior high, or senior high school education. More than 12 years of education indicates that the individual has received college or university education. The educational policy in Taiwan started to cover 6 years of mandatary education since 1947. According to the publicly available statistical data from the Taiwan Ministry of the Interior, updated in 2022, the proportions of individuals aged 50 to 64 years who received <6 years, 6 to 12 years, and >12 years of education were 0.2%, 64%, and 35%, respectively. The proportions of individuals aged ≥65 years who received <6 years, 6 to 12 years, and >12 years of education were 5%, 77%, and 18%, respectively.[21]

This study was approved by the local ethics committees of the participating hospitals (National Taiwan University Hospital: No. 201807044RIND; Taipei Veterans General Hospital: No. 2015-04-005A). All investigations were conducted according to the principles expressed in the Declaration of Helsinki. All of the participants or their proxies provided written informed consent before enrollment.

Genetic Analysis

Genomic DNA was extracted from peripheral blood samples. Genetic analysis of *NOTCH3* was performed using Sanger sequencing. *NOTCH3* exon 11 was analyzed first because the p.R544C variant in exon 11 is the most common pathogenic variant in Taiwanese patients with CADASIL.[22] Then, for patients without pathogenic variants in exon 11, *NOTCH3* exons 2 to 10 and 12 to 24 were also investigated. Only patients with cysteine-altering pathogenic *NOTCH3* variants were included in this study. Genotyping for *APOE* was performed by Sanger sequencing to determine the ε2, ε3, and ε4 variants.

Brain MRI Acquisition and Visual Rating for Imaging Characteristics

Brain MRI was performed on a 1.5- or 3.0-T scanner. The scanning protocols included axial T2-weighted fluid-attenuated inversion recovery and coronal T1-weighted imaging or 3-dimensional T1-weighted imaging. For each brain MRI, the severity of mesial temporal atrophy (MTA) and WMH was rated semiquantitatively. MTA was rated using the visual scoring system proposed by Scheltens et al.[23] Each side of the medial temporal lobe and hippocampus was scored from 0 to 4 on the coronal views of T1-weighted imaging. A higher MTA score indicated more severe medial temporal lobe atrophy. The extent of WMH was rated using the visual scoring system developed by Fazekas et al.[24] The severity of WMH was rated from 0 to 3 on the T2-weighted fluid-attenuated inversion recovery images for periventricular white matter and DWM regions, separately. A higher WMH score indicated a more extensive white matter lesion. The MTA and WMH scores were rated for each hemisphere separately, and the average score from both hemispheres was calculated for each participant.

The above-mentioned visual rating for MTA and WMH was performed by an experienced neurologist (Y.-W.C.). To evaluate the interrater reliability of the visual rating, a subset of 30 MRI scans from this cohort was independently reviewed by another experienced neurologist (C.-H.C.). The interrater agreements were calculated using the quadratic weighted κ values. The weighted κ values were 0.90 (95% CI, 0.82–0.98) for MTA, 0.88 (95% CI, 0.74–0.99) for DWM hyperintensity, and 0.80 (95% CI, 0.66–0.94) for periventricular white matter hyperintensity, indicating strong interrater agreements for the above ratings.

Statistical Analysis

We used independent *t* tests for continuous variables and χ^2 tests or Fisher exact tests for categorical variables to compare the between-group differences in demographic variables. First, we tested the effects of genetic factors and clinical variables on the MMSE score using univariate linear regression analysis. Bayesian adjustment for confounding (BAC)[25,26] was used to identify clinical confounders before running the multiple regression models. The 10 clinical confounders that were used for BAC included age, sex, educational level, history of ischemic stroke, history of hemorrhagic stroke, hypertension, diabetes, hyperlipidemia, and enrollment hospital. The algorithm was implemented in the bac function in the R package bacr.[26] The number of Markov chain Monte Carlo iterations was set to 100 000, with a burn-in of 100 000 and a thinning parameter of 1000. Confounders selected by the BAC method were used as covariates in the multiple regression model to estimate the adjusted effects of genetic factors on the MMSE score. To compare the imaging characteristics between different *APOE* genotypes or *NOTCH3* variants at different positions, ANCOVA was performed using a univariate general linear model with adjustment for age and sex. To explore the possible mediation effect of imaging characteristics on the relationship between genetic factors and cognitive impairment, we performed mediation analysis with linear regression models and included age, sex, and educational level as covariates, following the A Guideline for Reporting Mediation Analyses statement[27] (see Supplemental Material). The PROCESS macro, version 3.5, for SPSS was used to perform the mediation analysis.[28] The significance of the mediation effect was tested by calculating the 95% CIs using nonparametric bootstrapping of 10 000 resamples. P <0.05 was considered statistically significant. Data were analyzed using SPSS, version 23.0 (IBM Corp, Armonk, NY).

RESULTS

Demographics and Clinical Characteristics

A total of 246 patients harboring cysteine-altering *NOTCH3* variants were enrolled, among whom 150 patients had normal cognitive performance and 96 had cognitive impairment. The demographic data of the study participants are shown in Table 1. Patients who had cognitive impairment were older (67.4±9.8 versus 59.7±10.1 years; P<0.0001), had a lower educational level (9.4±4.8 versus 12.2±3.7 years; P<0.0001), and more frequently had a history of ischemic stroke (65% versus 44%; P=0.001) or concurrent gait disturbance (52% versus 26%; P<0.0001). Migraine was less frequent in patients who had cognitive impairment than in those with normal cognitive function (1% versus 10%; P=0.015). The frequencies of *APOE* ε3ε3, ε2 carriers (ε2ε3), and ε4 carriers (including ε3ε4 and ε4ε4) were 66%, 13%, and 22%, respectively, in patients with cognitive impairment, and 80%, 6%, and 14%, respectively, in patients with normal cognitive performance. One patient in the normal cognition group harbored the *APOE* ε2ε4 genotype and was excluded from further analysis considering the possible differential effect of the ε2 and ε4 alleles on cognitive and vascular outcomes. The distributions of the *APOE* genotypes differed significantly between patients with and without cognitive impairment (P=0.029). For imaging characteristics, patients with cognitive impairment had more advanced WMH in both the DWM and periventricular white matter and more advanced MTA than patients without cognitive impairment. The frequency of anterior temporal WMH involvement did not differ between patients with and without cognitive impairment (47% versus 40%; P=0.341).

The demographics among patients with *APOE* ε3ε3, ε2 carriers, and ε4 carriers are shown in Table 2. The distributions of age, sex, and educational level did not differ among the 3 groups. Clinical presentations and concurrent vascular risk factor profile did not differ among the 3 groups, except for an increased frequency of hemorrhagic stroke observed in *APOE* ε3ε3 carriers (19%, 5%, and 8% for ε3ε3, ε2 carriers, and ε4 carriers, respectively; P=0.049). The mean MMSE score was lower in ε2 carriers compared with the other 2 groups (23.9±6.4, 18.7±10.4, and 22.4±7.3 for ε3ε3, ε2 carriers, and ε4 carriers, respectively; P=0.005). For imaging characteristics, the severity of WMH did not differ among the 3 *APOE* groups. *APOE* ε2 and ε4 carriers had a more advanced MTA than patients carrying the *APOE* ε3ε3 genotype (MTA score, 1.19±0.90, 1.58±0.71, and 1.51±0.82 for ε3ε3, ε2 carriers, and ε4 carriers, respectively; P=0.037).

The pathogenic *NOTCH3* variants of the enrolled subjects are shown in Table S1. Most (228/246 [93%]) of the enrolled subjects harbored pathogenic variants located in EGFR 7 to 34, and 210 (85%) of the enrolled subjects harbored the p.R544C variant. Patients carrying pathogenic variants located in EGFR 7 to 34 were older than those carrying pathogenic variants in EGFR 1 to 6 (63.6±10.3 versus 51.2±8.0 years; P<0.0001). Therefore, age was considered as a possible confounding factor when inferring the relationships between the *NOTCH3* variant position and cognitive performance in the following analysis.

The frequencies of patients having cognitive impairment in different age strata and across different *APOE* and *NOTCH3* genotypes are shown in Figure 1.

APOE ε2 Allele and the *NOTCH3* p.R544C Variant Are Associated With Cognitive Performance in Patients Harboring Cysteine-Altering *NOTCH3* Variants

In the univariate analysis, an older age, *APOE* ε2 allele carrier status, and a history of ischemic stroke

Table 1. Demographics of Enrolled Patients Harboring Cysteine-Altering *NOTCH3* Variants

Variable	Total patients (N=246)	Patients without cognitive impairment (N=150)*	Patients with cognitive impairment (N=96)*	*P* value
Age, y	62.7 (10.6)	59.7 (10.1)	67.4 (9.8)	<0.0001
Male sex, n (%)	132 (54)	85 (49)	47 (51)	0.561
Education, y	11.2 (4.3)	12.2 (3.7)	9.4 (4.8)	<0.0001
NOTCH3 variant position, n (%)				0.990
EGFR 1–6	18 (7)	11 (7)	7 (7)	
EGFR 7–34	228 (93)	139 (93)	89 (93)	
NOTCH3 p.R544C, n (%)	209 (85)	130 (87)	79 (82)	0.349
APOE genotype, n (%)				0.029†
ε3ε3	183 (74)	120 (80)	63 (66)	
ε2ε3	21 (9)	9 (6)	12 (13)	
ε3ε4	40 (16)	19 (13)	21 (22)	
ε4ε4	1 (0.4)	1 (1)	0 (0)	
ε2ε4	1 (0.4)	1 (1)	0 (0)	
MMSE score	23.2 (7.1)	28.0 (1.8)	15.7 (5.5)	<0.0001
Clinical presentations, n (%)				
Stroke	150 (61)	81 (54)	69 (72)	0.005
Stroke type				
Ischemic stroke	123 (52)	64 (44)	59 (65)	0.001
Hemorrhagic stroke	38 (16)	21 (14)	17 (19)	0.368
Gait disturbance	89 (36)	39 (26)	50 (52)	<0.0001
Psychiatric symptoms	49 (20)	25 (17)	24 (25)	0.110
Migraine	13 (6)	12 (10)	1 (1)	0.015
Medical history, n (%)				
Hypertension	132 (54)	78 (53)	54 (56)	0.587
Diabetes	48 (20)	25 (17)	23 (24)	0.175
Dyslipidemia	87 (35)	58 (39)	29 (31)	0.169
Smoking	56 (24)	37 (26)	19 (21)	0.415
Alcohol	37 (16)	27 (19)	10 (11)	0.127
Laboratory data				
Total cholesterol, mg/dL	176.5 (38.9)	179.2 (37.4)	171.7 (41.1)	0.209
LDL cholesterol, mg/dL	103.8 (34.5)	104.9 (33.8)	102.0 (35.9)	0.579
HDL cholesterol, mg/dL	47.9 (12.3)	49.1 (11.5)	46.4 (13.2)	0.303
Triglyceride, mg/dL	121.1 (69.3)	120.9 (74.6)	121.4 (60.8)	0.962
Fasting plasma glucose, mg/dL	106.6 (30.4)	107.6 (34.6)	105.1 (22.7)	0.605
HbA1c, %	5.89 (0.90)	5.88 (0.91)	5.90 (0.88)	0.889
Imaging characteristics‡				
DWM hyperintensity score				<0.0001
1–1.5	21 (9)	21 (14)	0 (0)	
2–2.5	82 (33)	64 (43)	18 (19)	
3	143 (58)	65 (43)	78 (81)	
PVWM hyperintensity score				<0.0001
1–1.5	7 (3)	7 (5)	0 (0)	
2–2.5	50 (20)	44 (29)	6 (6)	
3	189 (77)	99 (66)	90 (94)	
MTA score				<0.0001
0–0.5	55 (22)	52 (37)	3 (4)	

(*Continued*)

Table 1. Continued

Variable	Total patients (N=246)	Patients without cognitive impairment (N=150)*	Patients with cognitive impairment (N=96)*	*P* value
1–1.5	87 (35)	59 (42)	28 (33)	
2–2.5	66 (27)	26 (19)	40 (48)	
3–4	15 (6)	2 (1)	13 (16)	
Anterior temporal WMH, n (%)	95 (43)	52 (40)	43 (47)	0.341

Data are shown as mean (SD) unless otherwise indicated. DWM indicates deep white matter; EGFR, epidermal growth factor–like repeat; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MMSE, Mini-Mental State Examination; MTA, mesial temporal atrophy; PVWM, periventricular white matter; and WMH, white matter hyperintensity.

*Cognitive impairment was defined by an MMSE score <24.

†Comparison among *APOE* subgroups of the following: ε3ε3, ε2 carrier (ε2ε3), and ε4 carrier (ε3ε4 and ε4ε4).

‡DWM and PVWM hyperintensities were rated by the score of Fazekas et al,[24] and MTA was rated by the score of Scheltens et al.[23] Each semiquantitative score was presented as the average score from both hemispheres.

were significantly associated with a lower MMSE score (Table S2). A higher educational level was associated with a higher MMSE score. After adjusting for age, the *NOTCH3* p.R544C variant was associated with a higher MMSE score than the other pathogenic variants (adjusted *B*, 2.979 [95% CI, 0.610–5.347]; *P*=0.014; Table S3).

To estimate the effect of the *APOE* ε2 allele carrier status on the MMSE score, clinical confounders selected by the BAC method were adjusted in the multiple regression model. The result of the multiple regression model was shown in Table 3. The association between the *APOE* ε2 allele and a lower MMSE

Table 2. Comparison of Clinical Information in Each *APOE* Genotype

Variable	ε3ε3 (N=183)	ε2 Carrier (N=21)	ε4 Carrier (N=41)	*P* value
Age y	62.7 (10.8)	62.5 (11.7)	62.3 (9.5)	0.977
Male sex, n (%)	91 (50)	11 (52)	20 (49)	0.964
Education, y	11.2 (4.2)	9.7 (5.2)	11.7 (4.4)	0.235
NOTCH3 variant position, n (%)				0.731
EGFR 1–6	12 (7)	2 (10)	4 (10)	
EGFR 7–34	171 (93)	19 (90)	37 (90)	
MMSE score	23.9 (6.4)	18.7 (10.4)	22.4 (7.3)	0.005
MMSE-recall score	2.0 (1.1)	1.6 (1.4)	1.6 (1.3)	0.198
Clinical presentations, n (%)				
Ischemic stroke	88 (49)	13 (62)	22 (58)	0.402
Hemorrhagic stroke	34 (19)	1 (5)	3 (8)	0.049
Gait disturbance	66 (36)	10 (48)	13 (32)	0.463
Psychiatric symptoms	41 (22)	5 (24)	3 (7)	0.083
Migraine	9 (6)	0 (0)	3 (8)	0.599
Medical history, n (%)				
Hypertension	103 (57)	11 (55)	17 (42)	0.213
Diabetes	37 (20)	3 (15)	8 (20)	0.850
Dyslipidemia	67 (37)	4 (20)	16 (40)	0.279
Smoking	43 (24)	5 (25)	8 (21)	0.906
Alcohol	26 (15)	4 (20)	7 (18)	0.760
Imaging characteristics*				
DWM hyperintensity score	2.52 (0.63)	2.57 (0.60)	2.34 (0.76)	0.230
PVWM hyperintensity score	2.75 (0.48)	2.76 (0.44)	2.66 (0.62)	0.533
MTA score	1.19 (0.90)	1.58 (0.71)	1.51 (0.82)	0.037
Anterior temporal involvement, n (%)	68 (41)	9 (45)	18 (50)	0.616

Data are shown as mean (SD) unless otherwise indicated. DWM indicates deep white matter; EGFR, epidermal growth factor–like repeat; MMSE, Mini-Mental State Examination; MTA, mesial temporal atrophy; and PVWM, periventricular white matter.

*DWM and PVWM hyperintensities were rated by the score of Fazekas et al,[24] and MTA was rated by the score of Scheltens et al.[23] Each semiquantitative score was presented as the average score from both hemispheres.

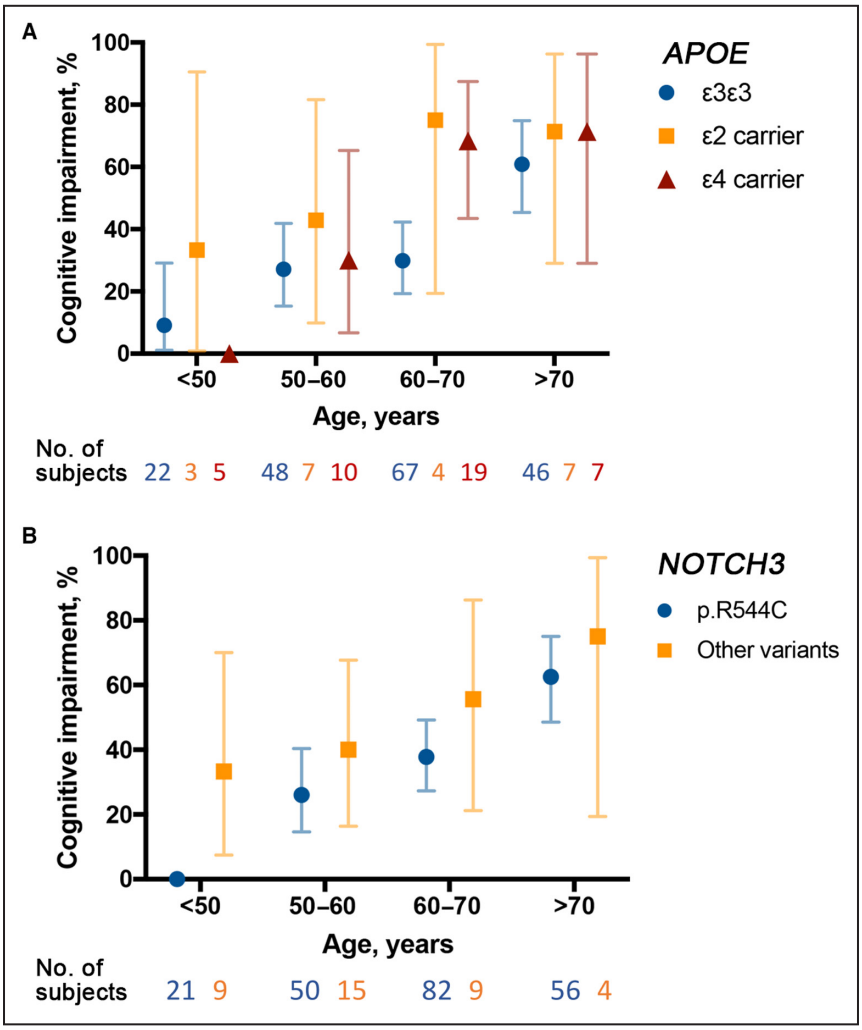


Figure 1. The frequency of cognitive impairment among patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), stratified by age.
A, Patients were stratified according to their *APOE* genotype and age group. **B**, Patients were stratified according to their pathogenic *NOTCH3* variant and age group. The 95% CI for the proportion of cognitive impairment in each subgroup was calculated using the binomial distribution method. Cognitive impairment was defined as a Mini-Mental State Examination score <24 for patients with CADASIL.

score remained statistically significant (adjusted *B*, 4.090 [95% CI, −6.708 to −1.473]; *P*=0.023) after controlling for age, educational level, and history of ischemic stroke. When assessing the effect of the *NOTCH3* p.R544C variant on the MMSE score, the BAC model selected the same set of clinical confounders (namely, age, educational level, and history of ischemic stroke). The result of the multiple regression model that estimates the effect of *NOTCH3* p.R544C variant on the MMSE score was shown in Table 4. The *NOTCH3* p.R544C variant was significantly associated with a higher MMSE score (adjusted *B*, 2.854 [95% CI, 0.603–5.105]; *P*=0.0132) compared with other cysteine-altering *NOTCH3* variants, after controlling for age, educational level, and history of ischemic stroke.

Effects of the *APOE* ε2 Allele and the *NOTCH3* p.R544C Variant on Cognitive Performance Were Mediated by MTA and WMH

The imaging characteristics were compared among *APOE* ε2 carriers, ε4 carriers, and ε3ε3 carriers using ANCOVA, between patients with *NOTCH3* variants residing in EGFR 1 to 6 and those with variants residing in EGFR 7 to 34, and between the *NOTCH3* p.R544C variant and other pathogenic variants (Table S4). After adjusting for age and sex, patients harboring either the ε2 or ε4 allele had a more advanced MTA than *APOE* ε3ε3 carriers (*B*, 0.401 [95% CI, 0.052–0.749]; *P*=0.025 for *APOE* ε2 carriers, and *B*, 0.320 [95% CI,

Table 3. Effect Estimates of the APOE *ε2* Allele and Confounding Factors on Global Cognitive Performance Measured by MMSE Score, in Patients Harboring Cysteine-Altering *NOTCH3* Variants

Clinical variables	*B**	(95% CI)	*P* value
APOE ε2 carrier vs ε3ε3	−4.090	(−6.708 to −1.473)	0.0023
Age, every 10 y	−2.006	(−2.746 to −1.265)	<0.0001
Education >12 vs ≤12 y	2.481	(0.812 to 4.151)	0.0038
Ischemic stroke	−2.617	(−4.193 to −1.042)	0.0012

MMSE indicates Mini-Mental State Examination.
*The *B* estimates were derived from the multiple linear regression model with confounders selected using the Bayesian adjustment for confounding method. A negative *B* estimate for a clinical variable indicates that the variate is associated with a lower MMSE score (ie, a poorer global cognitive performance).

Table 4. Effect Estimates of the *NOTCH3* p.R544C Variant and Confounding Factors on Global Cognitive Performance Measured by MMSE Score, in Patients Harboring Cysteine-Altering *NOTCH3* Variants

Clinical variables	*B**	(95% CI)	*P* value
NOTCH3 p.R544C variant	2.854	(0.603 to 5.105)	0.0132
Age, every 10 y	−2.246	(−3.011 to −1.480)	<0.0001
Education >12 vs ≤12 y	2.822	(1.122 to 4.522)	0.0012
Ischemic stroke	−2.381	(−3.985 to −0.777)	0.0038

MMSE indicates Mini-Mental State Examination.
*The *B* estimates were derived from the multiple linear regression model with confounders selected using the Bayesian adjustment for confounding method. A negative *B* estimate for a clinical variable indicates that the variate is associated with a lower MMSE score (ie, a poorer global cognitive performance).

0.066–0.574]; *P*=0.014 for *APOE* ε4 carriers). There was no statistically significant difference in the MTA score neither between *NOTCH3* pathogenic variant located in EGFR 1 to 6 and variants located in EGFR 7–34 (*P*=0.437), nor between the *NOTCH3* p.R544C variant and other pathogenic variants (*P*=0.460). Conversely, *NOTCH3* pathogenic variants located in EGFR 1 to 6 were associated with a more advanced DWM hyperintensity than pathogenic variants located in EGFR 7 to 22 (*B*, 0.540 [95% CI, 0.242–0.838]; *P*=0.0004). The *NOTCH3* p.R544C variant was associated with a less advanced DWM score (*B*, −0.376 [95% CI, −0.162 to −0.590]; *P*=0.001). The DWM hyperintensity score did not differ among patients harboring different *APOE* genotypes (*P*=0.216).

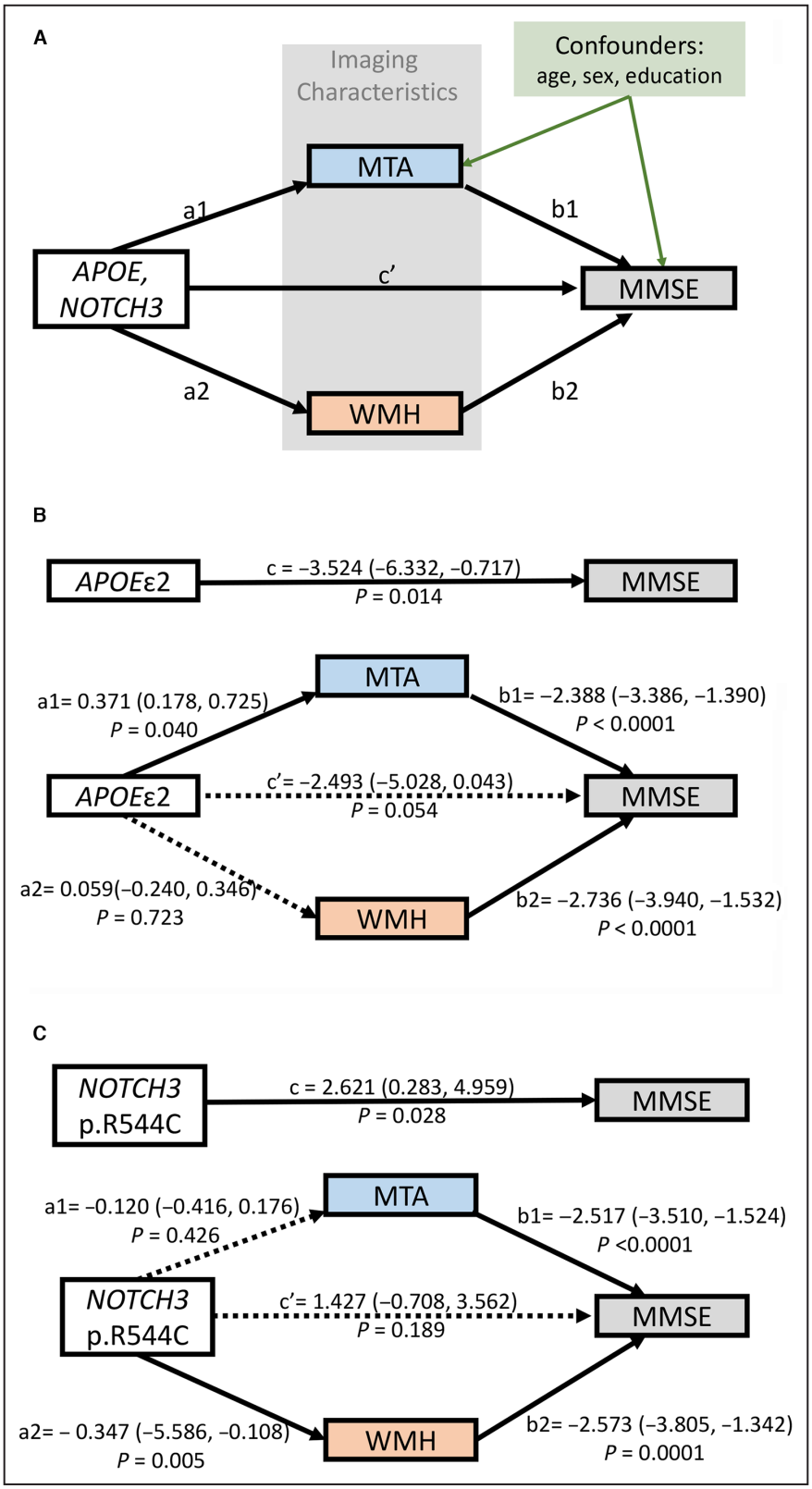
To explore how imaging characteristics influenced the relationship between genetic factors and cognitive performance, mediation analysis was performed, and the model of the hypothetical causal pathway was shown in Figure 2A. The mediation analysis revealed a significant indirect effect of MTA on the association between the *APOE* ε2 allele and the MMSE score (linear regression *B* estimate of indirect effect, −1.0314 [bootstrap 95% CI for the indirect effect, −2.4213 to −0.1199]). The indirect effect of WMH on the association between the *APOE* ε2 allele and the MMSE score was not statistically significant (*B* estimate of indirect effect, −0.1146 [bootstrap 95% CI for the indirect effect, −0.8600 to 0.6528]; Figure 2B). In contrast, there was a significant indirect effect of WMH on the association between the *NOTCH3* p.R544C variant and a milder cognitive impairment (*B* estimate of indirect effect, 0.8928 [bootstrap 95% CI for the indirect effect, 0.2377–1.6772]), whereas the indirect effect of MTA on the association between the *NOTCH3* p.R544C variant and MMSE score was not statistically significant (*B* estimate of indirect effect, 0.3014 [bootstrap 95% CI for the indirect effect, −0.4620 to 1.1257]; Figure 2C).

DISCUSSION

In the present study, we demonstrated 3 major findings among patients harboring cysteine-altering *NOTCH3* variants. First, the *APOE* ε2 allele was associated with worse cognitive performance, and the association was mediated by MTA but was independent of the stroke history and WMH burden. Second, patients harboring the *NOTCH3* p.R544C showed a milder cognitive impairment, and this relationship was mediated by the severity of WMH. Third, MTA and WMH were independently associated with cognitive impairment in patients harboring cysteine-altering *NOTCH3* variants.

The role of *APOE* genotypes in the disease severity of CADASIL has been explored in a few studies with

Figure 2. Relationship between genetic factors, imaging markers, and cognitive performance, revealed by mediation analysis.
A, Model of the hypothetical causal pathway in cognitive impairment of patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. The total effect of genetic factors on the Mini-Mental State Examination (MMSE) score was designated as c. The direct effects of genetic factors on imaging markers were designated as a1 (for mesial temporal atrophy [MTA]) and a2 (for white matter hyperintensity [WMH]), and the direct effects of these imaging markers on the MMSE score were designated as b1 (for MTA) and b2 (for WMH). The remaining effect of genetic factors on the MMSE score after adjusting for imaging markers was designated c′. Total effect (c)=direct effect (c′)+indirect effects (a1b1+a2b2). The effect estimates of the model were derived from the *B* estimate of linear regression analysis. Age, sex, and educational level were adjusted for all of the linear regression models. **B**, *APOE ε2* carrier status was associated with a lower MMSE score than the other *APOE* genotypes. The relationship between the *APOE ε2* allele and a lower MMSE score was mediated by MTA but not WMH. **C**, The *NOTCH3* p.R544C variant was associated with a higher MMSE score than the other pathogenic variants. The relationship between the p.R544C variant and a higher MMSE score was mediated by WMH but not MTA.



inconsistent results. A CADASIL cohort from the United Kingdom including 123 patients found no significant association between *APOE* genotypes and dementia risks.[2] However, in a Korean cohort that enrolled 87 patients with CADASIL mostly harboring the *NOTCH3* p.R544C variant, the *APOE* ε4 allele was associated with an increased risk of incident dementia during an average follow-up of 67 months.[18] In the present study,

we enrolled 246 patients harboring cysteine-altering *NOTCH3* variants and found a significant association between the *APOE* ε2 allele and a lower MMSE sore. Although the *APOE* ε4 allele was not a significant contributor to cognitive impairment in our cohort, it was associated with more advanced MTA. Whether the *APOE* ε4 allele is associated with accelerated conversion to dementia cannot be assessed in this cross-sectional study and should be investigated in additional longitudinal follow-up studies.

Interestingly, the relationship between the *APOE* ε2 allele and cognitive impairment was mediated by MTA rather than WMH. In a few studies investigating subjects with sporadic SVD[29] and Alzheimer disease,[30] the *APOE* ε2 allele was associated with a more advanced white matter disease burden. In a large European CADASIL cohort of 488 patients with a median age of ≈50 years, Gesierich et al showed that the *APOE* ε2 allele was associated with an increased WMH volume compared with the ε3ε3 genotype.[31] Compared with the European cohort, participants in the present study were older (mean age, 63 years) and predominantly harbored the p.R544C variant on EGFR13/14. In addition, the severity of WMH was measured by the semiquantitative score of Fazekas et al in the present study, whereas WMH volume was quantitatively measured by Gesierich et al. We cannot exclude the possibility that the insignificant association between the *APOE* ε2 allele and WMH severity was related to the ceiling effect of the score of Fazekas et al used in the present study.[32] Alternatively, we found that the association between the *APOE* ε2 allele and cognitive impairment was mediated by MTA in the present study, suggesting the possibility of other neurodegenerative processes in addition to arteriopathy. The *APOE* ε4 allele is known to be associated with an increased risk of developing Alzheimer disease, whereas the ε2 allele is protective against developing Alzheimer disease.[18] Alternatively, a few studies have suggested a role of the *APOE* ε2 allele in primary τ pathology. The *APOE* ε2 allele was associated with increased τ pathology in progressive supranuclear gaze palsy, a neurodegenerative disease caused by primary tauopathy.[33] The *APOE* ε2 allele was more frequent in primary age-related tauopathy[34] and in mild cognitive impairment related to suspected non–Alzheimer disease pathophysiology.[35] Whether τ pathology plays a role in the neurodegeneration of CADASIL is worth exploring in future studies. The contribution of other neurodegenerative processes in addition to NOTCH3-related arteriopathy may be more influential for aged patients with CADASIL than for younger subjects.

The influence of the *NOTCH3* variant position on disease severity has been reported in several recent studies. Patients harboring *NOTCH3* variants located in EGFR 1 to 6 had a higher vascular NOTCH3 protein aggregation load than those harboring *NOTCH3* variants in EGFR 7 to 34.[36] Clinically, *NOTCH3* variants located in EGFR 1 to 6 are associated with an earlier age of stroke onset,[4] a higher white matter disease and lacune burden,[4,5] and worse survival.[4] Consistent with previous studies, we found a greater WMH burden for patients harboring *NOTCH3* variants located in EGFR 1 to 6. Meanwhile, the severity of cognitive impairment was comparable between patients with *NOTCH3* variants located in EGFR 7 to 34 and those with variants in EGFR 1 to 6. Consistent with our findings, in a recent investigation that enrolled 176 patients with CADASIL, among whom 73% harbored *NOTCH3* variants located in EGFR 1 to 6, the association between *NOTCH3* variant position (located in EGFR 1–6 versus EGFR 7–34) and vascular cognitive impairment was nonsignificant.[37] Interestingly, we found a better cognitive performance in patients harboring the *NOTCH3* p.R544C variant than other pathogenic variants in the present study. Previous epidemiologic studies showed that the *NOTCH3* p.R544C variant was associated with a later age of onset and less frequent anterior temporal pole involvement.[22,38,39] Cognitive impairment was reported to be more frequent in patients harboring the p.R544C variant, although patients harboring the p.R544C variant were older than those with other *NOTCH3* pathogenic variants in these patient cohorts.[22,38] In the present study, the p.R544C variant was associated with milder cognitive impairment after adjusting for age. Unlike other cysteine-altering pathogenic variants that located within the 34 EGFR domains, the p.R544C variant is located at the boundary of EGFR 13 and EGFR 14 of the NOTCH3 protein (https://www.uniprot.org/uniprotkb/Q9UM47). The unique position of the p.R544C variant may contribute to specific conformational changes in the mutant protein, therefore causing specific clinical features associated with the p.R544C variant.

There are some limitations of the present study. First of all, the association between genetic factors and cognitive performance was assessed cross-sectionally in the present study. Further longitudinal follow-up studies are crucial to investigate the role of *APOE* and *NOTCH3* genotypes on cognitive dysfunction among patients with CADASIL. Second, most of the enrolled subjects harbored pathogenic variants located in EGFR 7 to 34. Therefore, this study may be underpowered to investigate the role of *NOTCH3* variant position on cognitive performance.

CONCLUSIONS

The *APOE* ε2 allele was associated with worse cognitive function and more advanced MTA, whereas the *NOTCH3* p.R544C variant is associated with a milder cognitive impairment and less severe WMH. The *APOE*

genotype and *NOTCH3* pathogenic variant position may contribute to different aspects of neurodegeneration in CADASIL.

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Disclosures

None.

Supplemental Material

Table S1–S4
Figure S1

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