Supplemental Material

AGReMA statement checklist for the mediation analysis.

Section/Topic	Item Number	Item Description	Reported on page No
Introduction			
Objectives	1	State the objectives of the study specific to the mechanisms of interest. The objectives should specify whether the study aims to test or estimate the mechanistic effects	Page 6, line 10-12
Methods			
Effects of interest	2	Specify the effects of interest	Page 11 line 11-16
Causal assumptions	3	Specify assumptions about the causal model	Figure 2A
Measurement	4	Clearly describe the interventions or exposures, mediators, outcomes, confounders, and moderators that were used in the analyses. Specify how and when they were measured, the measurement properties, and whether blinded assessment was used	Page 8, line 2-9; page 9, line 13-page 10 line 8
Statistical methods	5	Describe the statistical methods used to estimate the causal relationships of interest. This description should specify analytical strategies used to reduce confounding, model building procedures, justification for the inclusion or exclusion of possible interaction terms, modelling assumptions, and methods used to handle missing data. Provide a reference to the statistical software and package used	Page 11, line 11-16 Figure 2A
Results			
Participants	6	Describe baseline characteristics of participants included in mediation analyses. Report the total sample size and number of participants lost during follow-up or with missing data	Table 1; Page 12 line 7- page 13 line 6
Outcomes and estimates	7	Report point estimates and uncertainty estimates for the exposure-mediator and mediator-outcome relationships. If inference concerning the causal relationship of interest is considered feasible given the causal assumptions, report the point estimate and uncertainty estimate	Figure 2B. 2C
Discussion			
Limitations	8	Discuss the limitations of the study including potential sources of bias	Page 22, line 10-13
Interpretation	9	Interpret the estimated effects considering the study's magnitude and uncertainty, plausibility of the causal assumptions, limitations, generalizability of the findings, and results from relevant studies	Page 22, line 13-16

From: Lee H, Cashin AG, Lamb SE, Hopewell S, Vansteelandt S, VanderWeele TJ, et al. A Guideline for Reporting Mediation Analyses of Randomized Trials and Observational Studies. The AGReMA Statement. JAMA. 2021;326(11):1045–1056. doi:10.1001/jama.2021.14075

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Table S1, Pathogenic *NOTCH3* variants of the enrolled individuals.

Exon	EGFR	Amino acid change	Coding sequence change	Case number (%)
2	1	p.S60C	c.179c>G	1 (0.4)
3	2	p.R90C	c.268C>T	1 (0.4)
3	2	p.R110C	c.328C>T	4 (1.6)
4	2	p.S118C	c.353C>G	4 (1.6)
4	3	p.R133C	c 397C>T	3 (1.2)
4	3	p.R141C	c.421C>T	2 (0.8)
4	3	p.R153C	c.457C>T	1 (0.4)
4	3	p.C155Y	c.464G>A	2 (0.8)
6	8	p.R332C	c.994C>T	3 (1.2)
8	10	p.G420C	c.1258G>T	1 (0.4)
8	10	p.R427C	c.1279C>T	1 (0.4)
11	13/14	p.R544C	c.1630C>T	210 (85.4)
11	14	p.R558C	c.1672C>T	3 (1.2)
11	15	p.R587C	c.1759C>T	2 (0.8)
18	25	p.C977S	c.2929T>A	4 (1.6)
22	31	p.R1231C	c.3691C>T	1 (0.4)
23	32	p.C1250R	c.3748A>G	2 (0.8)

EGFR = epidermal-growth-factor-like repeats.

Table S2. Univariate analysis of clinical variables associated with global cognitive performance, measured by MMSE, in patients harboring cysteine-altering *NOTCH3* variants.

	Univariate analysis		
	B	B 95% CI	P value
Age, per 10 years	-2.535	-3.314, -1.755	<0.0001
Sex	0.637	-1.150, 2.425	0.483
Hospital	-1.697	-3.512, 0.117	0.067
Education			
7-12 years vs. < 6 years	4.174	2.065, 6.283	0.0001
>=13 years vs. < 6 years	6.835	4.692, 8.978	<0.0001
NOTCH3 variant position			
EGFR 1-6 vs. EGFR 7-34	0.440	-2.994, 3.874	0.801
p.R544C vs. other variants	0.875	-1.624, 3.375	0.491
APOE genotype			
ε2 carrier (ε2ε3) vs. ε3ε3	-4.517	-7.620, -1.413	0.0045
ε4 carrier (ε3ε4 or ε4ε4) vs. ε3ε3	-1.091	-3.444, 1.263	0.362
Stroke	-3.664	-5.439, -1.890	<0.0001
Ischemic stroke	-3.570	-5.317, -1.824	<0.0001
Hemorrhagic stroke	-0.402	-2.785, 1.980	0.740
Diabetes mellitus	-2.030	-4.277, 0.216	0.076
Hypertension	-1.453	-3.247, 0.342	0.112
Hyperlipidemia	1.077	-0.792, 2.947	0.257
Smoking	0.865	-1.248, 2.979	0.421
Alcohol	1.394	-1.099, 3.868	0.273
DWM hyperintensity score	-4.655	-5.903, -3.407	<0.0001
PVWM hyperintensity score	-4.515	-6.213, -2.817	<0.0001
MTA score	-3.919	-4.805, -3.032	<0.0001

DWM = deep white matter; EGFR = epidermal-growth-factor-like repeats; MTA = mesial temporal atrophy; PVWM = periventricular white matter.

Table S3. Association between MMSE and *NOTCH3* variant position.

NOTCH3 variant position	Univariate analysis			Model adjusted for age		
	B	(95% CI)	p-value	Adjusted B	(95% CI)	p-value
EGFR 1-6 vs. 7-34	0.440	(-2.994, 3.874)	0.801	-2.982	(-6.304, 0.340)	0.078
EGFR 1-8 vs. 9-34	0.862	(-2.344, 4.067)	0.597	-2.888	(-6.043, 0.268)	0.073
EGFR 1-10 vs. 11-34	0.246	(-2.833, 3.324)	0.875	-3.271	(-6.276, -0.265)	0.033
p.R544C vs. Other variants	0.875	(-1.624, 3.375)	0.491	2.979	(0.610, 5.347)	0.014

EGFR = epidermal-growth-factor-like repeats.

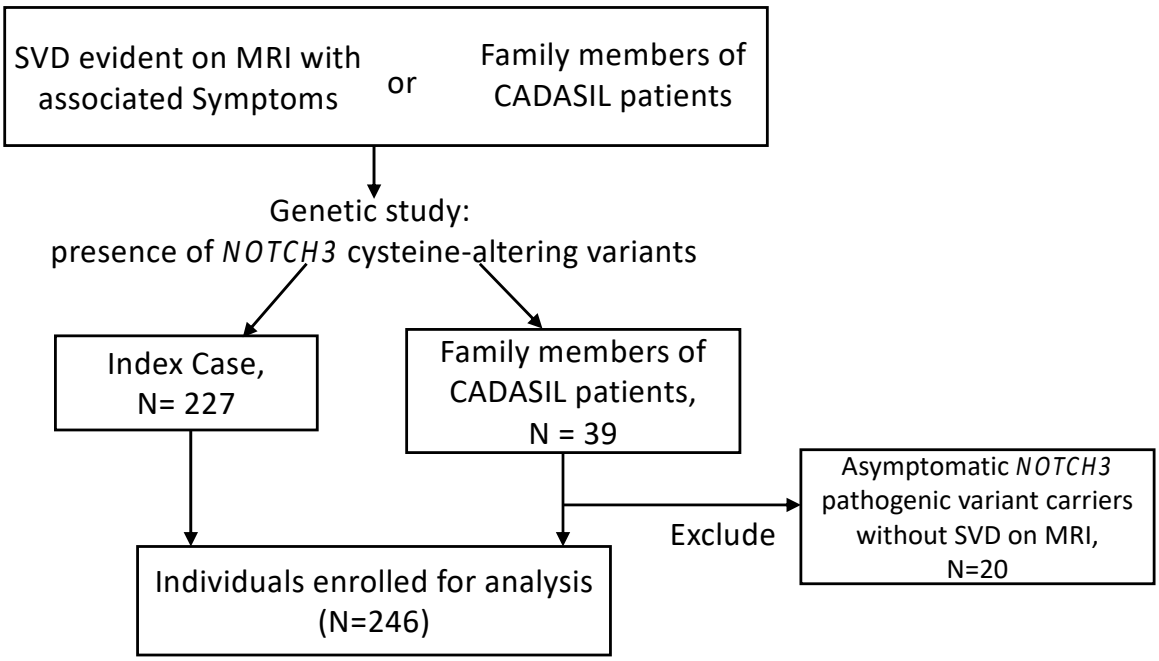
Table S4. Comparison of imaging characteristics between *NOTCH3* variant and *APOE* genetic groups.

	Mesial temporal atrophy		**DWM hyperintensity**	
	B estimate (95% CI) *	P-value	B estimate (95% CI) *	P-value
***APOE* genotype**		0.008		0.216
ε2 carrier (ε2ε3)	0.401 (0.052, 0.749)	0.025	0.046 (-0.226, 0.319)	
ε4 carrier (ε3ε4 or ε4ε4)	0.320 (0.066, 0.574)	0.014	-0.173 (-0.377, 0.032)	
ε3ε3 (Reference)				
***NOTCH3* variant position**				
EGFR 1-6	0.158 (-0.242, -0.558)	0.437	0.540 (0.242, 0.838)	0.0004
EGFR 7-34 (Reference)				
***NOTCH3* p.R544C**	-0.108 (-0.395, 0.108)	0.460	-0.376 (-0.162, -0.590)	0.001

* Models were adjusted for age and sex

DWM = deep white matter; EGFR = epidermal-growth-factor-like repeats.

Figure S1. Flowchart of subject enrollment.



SVD = small vessel disease.