

# Clinical features and spectrum of *NOTCH3* variants in Finnish patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)

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**Objectives:** Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a cerebral small vessel disease caused by pathogenic variants in the *NOTCH3* gene. In Finland, the majority of CADASIL patients carry the pathogenic founder variant c.397C>T, (p.Arg133Cys), but the spectrum of other *NOTCH3* variants has not been investigated previously. The aim of the study was to investigate the spectrum and prevalence of *NOTCH3* variants in Finnish CADASIL patients and to examine the clinical features associated with them.

**Materials and Methods:** The spectrum of *NOTCH3* variants and the clinical features associated with them were retrospectively examined in 294 Finnish CADASIL patients tested during January 1996 to October 2021 in the Medical Genetics laboratory of Department of Genomics of Turku University Hospital, where practically all samples of patients with suspected CADASIL in Finland are investigated.

**Results:** The most common *NOTCH3* variants in the study cohort were c.397C>T, (p.Arg133Cys) (68%) and c.3206A>G p.(Tyr1069Cys) (18%), but other less common *NOTCH3* variants were detected in as many as 14% of the patients. Eight of the detected *NOTCH3* variants were novel: c.520T>A,p.(Cys174Ser), c.836A>G,p.(Gln279Arg), c.1369T>G,p.(Cys457Gly), c.1338C>G,p.(Cys446Trp), c.1564T>G,p.(Cys522Gly), c.2848T>G,p.(Cys950Gly), c.6102dup,p.(Gly2035Argfs\*60), and c.2410+6C>G. Other *NOTCH3* variants than p.Arg133Cys and p.Tyr1069Cys were more often associated with more severe clinical features.

**Conclusion:** This study revealed the genetic and clinical spectrum of CADASIL in the Finnish population. Sequencing of the whole *NOTCH3* gene performing a gene-panel or exome sequencing is recommended when suspecting CADASIL.

## KEYWORDS

CADASIL, Finnish, genotype, mutation, *NOTCH3*, phenotype

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## 1 | INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited cerebral small vessel disease caused by pathogenic variants in the *NOTCH3* gene located in the chromosome region 19p13.<sup>1,2</sup> CADASIL typically presents in young or mid-adulthood and the characteristic clinical features include migraine with aura, recurrent cerebral ischemic events, cognitive impairment and dementia, and mood disturbances.<sup>3</sup> Clinical presentation of CADASIL is highly variable even between patients with the same pathogenic variant and within families.<sup>4–6</sup>

The *NOTCH3* gene consists of 33 exons and encodes a transmembrane receptor Notch3. The Notch3 protein is comprised of an extracellular domain with 34 epidermal growth factor repeats (EGFR), three Notch/Lin12 repeats (LNR), a transmembrane domain and an intracellular domain.<sup>2,7</sup> The majority of disease-causing variants in *NOTCH3* occur in exons 2–24. Typically, the disease-causing variants are missense variants causing an uneven number of cysteine residues within one of the 34 EGFRs of the Notch3 extracellular domain.<sup>1</sup> However, other types of variants, such as deletions, duplications, and splice-site variants, including cysteine-sparing variants have also been described in CADASIL families.<sup>8,9</sup> In Finland, most of the CADASIL patients carry the variant c.397C>T,(p.Arg133Cys), due to the founder effect.<sup>10</sup> Until now, the spectrum and prevalence of other *NOTCH3* variants in Finnish CADASIL patients has been unknown.

The aim of this study was to study the spectrum of disease-causing *NOTCH3* variants in the Finnish population and to assess genotype–phenotype correlations of the variants.

## 2 | MATERIALS AND METHODS

The Ethical Committee of the Hospital District of Southwest Finland and Helsinki University Hospital Ethics Committee of Medicine approved the study. Approvals for the access to medical records were obtained from the Hospital District of Southwest Finland and Helsinki University Hospital.

### 2.1 | Patients

This retrospective study included all genetically confirmed Finnish CADASIL patients examined in the Medical Genetics laboratory of Department of Genomics of Turku University Hospital from January 1996 to October 2021 ( $n = 294$ ). In all, 2324 patient samples were sent for *NOTCH3* gene testing to the Medical Genetics laboratory of Department of Genomics of Turku University Hospital from January 1996 to October 2021. Of these, 1861 samples were studied at least for the Finnish founder variant c.397C>T,(p.Arg133Cys), and in part of the cases also for variants c.622C>T,(p.Arg182Cys) and c.3206A>G,p.(Tyr1069Cys). Of the 2324 samples, 463 samples were studied for other *NOTCH3* variants using Sanger sequencing

of specific *NOTCH3* exons or sequencing the whole *NOTCH3* gene using next-generation sequencing (NGS). All patients with reported *NOTCH3* variants were included in the study, including predictive cases. In Finland, practically all samples of patients with suspected CADASIL are sent for gene testing to the Medical Genetics laboratory of Department of Genomics of Turku University Hospital.

### 2.2 | *NOTCH3* gene testing

In Finland, genetic testing for *NOTCH3* began in 1996 by testing for the Finnish founder mutation c.397C>T,(p.Arg133Cys) and the c.622C>T,(p.Arg182Cys) variant, and since 2008 also for the c.3206A>G,p.(Tyr1069Cys) variant, using the restriction fragment length polymorphism (RFLP) technique. Sanger sequencing of *NOTCH3* exons was gradually introduced and since 2019, next-generation sequencing (NGS) has been used for screening all *NOTCH3* exons in the Laboratory of Medical Genetics of Turku University Hospital.

Hence, the patients in this study were studied for at least the variant c.397C>T,(p.Arg133Cys) and in most cases also for variant c.622C>T,(p.Arg182Cys) ( $n = 131$ ), using RFLP or Sanger sequencing. Some of the patients were also studied for the variant c.3206A>G p.(Tyr1069Cys), using RFLP or Sanger sequencing ( $n = 116$ ). Some of the samples were Sanger sequenced for variants in exon 4 ( $n = 2$ ), exons 3 and 4 ( $n = 1$ ), exon 10 ( $n = 2$ ), exon 19 ( $n = 1$ ), or for variants in exons 3–8, 11, and 18–20 ( $n = 24$ ), or for variants in exons 3–8, 11–12, and 18–20 ( $n = 8$ ). A minority ( $n = 7$ ) of the samples were studied across all *NOTCH3* exons using NGS. DNA samples of two patients with variant c.1660G>T,p.(Gly528Cys) were first analyzed in the Laboratory of Medical Genetics of Turku University Hospital, but the variant was detected in an additional analysis in France. Some uncommon variants were detected in Sanger sequencing of variants c.397C>T,(p.Arg133Cys) and c.622C>T,(p.Arg182Cys) (exon 4), or c.3206A>G,p.(Tyr1069Cys) (exon 20). In predictive cases, only the variant in the family was analyzed. Clinical laboratory geneticists analyzed and interpreted the gene test results, in some cases together with a clinical geneticist. In this study, variants were re-interpreted based on the American College of Medical Genetics and Genomics (ACMG) criteria.<sup>11</sup> *NOTCH3* variants were considered pathogenic when they resulted in cysteine-alteration in an EGFR and they were previously reported in a CADASIL patient.

### 2.3 | Collection of clinical data

Clinical and demographic data were collected retrospectively from medical records or genetic testing referrals. Age of onset for migraines and age of onset for ischemic events were recorded separately, as migraine usually occurs earlier than other symptoms. Risk factors included hypertension, smoking, diabetes, and hyperlipidemia. Family history was considered positive if a patient had family members with CADASIL, migraine, stroke, or dementia.

TABLE 1 NOTCH3 variants detected in CADASIL patients in Finland. Reference sequence: NM\_000435.2

Number of patients (total n = 294)	Variant	Variant type	Note	dbSNP	Reference (PMID)	ClinVar/LOVD	Exon	EGFR	ACMG
1	c.200G>C,p.(Cys67Ser)	Missense			19174371 (different nucleotide position)	Not reported	3	2	5
1	c.259T>C,p.(Cys87Arg)	Missense		rs1568362232	15364702	VCV000585601.1	3	2	5
1	c.323G>A,p.(Cys108Tyr)	Missense		rs1555729584	15364702 33268848	VCV000872948.2	3	2	5
2	c.328C>T,p.(Arg110Cys)	Missense		rs775836288	9388399	VCV000447831.25	3	2	5
1	c.341-2A>G	Splice site	Causes in-frame deletion including cysteine residue	rs2046935672	10802807 25982499	VCV000995318.1	intr 3	2	5
1	c.421C>T,p.(Arg141Cys)	Missense		rs1174625611	9388399	VCV000447846.13	4	3	5
1	c.391G>T,p.(Gly131Cys)	Missense			19006080	#0000081682	4	3	5
200	c.397C>T,p.(Arg133Cys)	Missense		rs137852642	9388399 15378071	VCV000009225.18	4	3	5
1	c.464G>A,p.(Cys155Tyr)	Missense			22664156	#0000081573	4	3	5
1 <sup>a</sup>	c.520T>A,p.(Cys174Ser)	Missense		rs1599394806	Novel	VCV000872534.9	4	4	4
1	c.622C>T,p.(Arg182Cys)	Missense		rs28933697	8878478	VCV000009220.22	4	4	5
5	c.580T>C,p.(Cys194Arg)	Missense		rs1568361818	12146805	VCV000585612.3	4	4	5
1	c.752G>A,p.(Cys251Tyr)	Missense		rs1555729405	19174371 19372454	VCV000447872.1	5	6	5
1	c.836A>G,p.(Gln279Arg)	Missense	Cysteine-sparing		Novel	Not reported	6	7	3
1	c.931T>G,p.(Cys311Gly)	Missense		rs781158121	27844030	#0000832811	6	7	4
1	c.1012T>A,p.(Cys338Ser)	Missense			29188607 (different nucleotide position)	Not reported	6	8	5
1	c.1261C>T,p.(Arg421Cys)	Missense		rs1555729068	15364702 16009764	VCV000447779.4	8	10	5
1	c.1300_1308dupGAGTGTCTG,p.(Glu434_Leu436dup)	In-frame insertion	Causes insertion of three additional amino acids, including cysteine		19174371	Not reported	8	11	5
1	c.1369T>G,p.(Cys457Gly)	Missense			Novel	Not reported	8	11	4
1	c.1338C>G,p.(Cys446Trp)	Missense			Novel	Not reported	8	11	4
2	c.1564T>G,p.(Cys522Gly)	Missense			Novel	#0000832896	10	13	4
3	c.1660G>T,p.(Gly528Cys)	Missense			19174371	Not reported	10	13	5

(Continues)

TABLE 1 (Continued)

Number of patients (total n = 294)	Variant	Variant type	Note	dbSNP	Reference (PMID)	ClinVar/LOVD	Exon	EGFR	ACMG
1	c.2410+6C>G	Splice site	Effect unknown	rs1479891295	Novel	Not reported	intr 15	n/a	3
1	c.2848T>G.p.(Cys950Gly)	Missense		rs1378535955	Novel	Not reported	18	24	4
2	c.3043T>C.p.(Cys1015Arg)	Missense		rs1599382214	10371548	VCV000803539.6	19	26	5
52	c.3206A>G p.(Tyr1069Cys)	Missense		19174371		VCV001510760.1	20	27	5
6	c.3226C>T.p.(Arg1076Cys)	Missense		rs1438626607	11571335 12861102	VCV000447830.4	20	27	5
1	c.3298C>T.p.(Arg1100Cys)	Missense		34222332		Not reported	20	28	5
1	c.5510G>A.p.(Arg1837His)	Missense	Cysteine-sparing	rs138265894	32573853	Not reported	30	intracellular domain	3
1	c.6102dup.p.(Gly2035Argfs*60)	Frameshift	Causes premature stop codon	rs771517374	Novel	#0000660245	33	intracellular domain	3

Abbreviations: EGFR, epidermal growth factor repeat; LOVD, Leiden Open Variation Database; n/a, information not available.

Note: ACMG variant classification (5 = pathogenic, 4 = likely pathogenic, 3 = variant of unknown significance, 2 = likely benign, and 1 = benign).

<sup>a</sup>African ethnicity.

## 2.4 | Statistical analyses

Statistical analyses were conducted using SPSS version 27.0 (SPSS Inc.). Age at onset and clinical features among patients carrying the variants p.Arg133Cys, p.Tyr1069Cys, and other variants were compared using Fisher's exact test for categorical variables and unpaired t-test for continuous variables. Values of  $p < .05$  were considered statistically significant.

## 3 | RESULTS

### 3.1 | Variant spectrum in Finnish CADASIL patients

This study identified 30 different *NOTCH3* variants in a cohort of 294 Finnish patients with suspected CADASIL, including 24 missense variants, two splice-site variants, one frameshift variant, and one in-frame insertion (Table 1). The most common pathogenic *NOTCH3* variants in the study cohort were c.397C>T,p.(Arg133Cys) ( $n = 200$ , 68%) in exon 4 and c.3206A>G,p.(Tyr1069Cys) ( $n = 52$ , 18%) in exon 20. Another 28 variants were identified in 42 patients (14%) and were classified either pathogenic, likely pathogenic or as of unknown significance, using the ACMG criteria. Of these, eight were novel variants that have not been reported earlier in the literature, to the best of our knowledge.

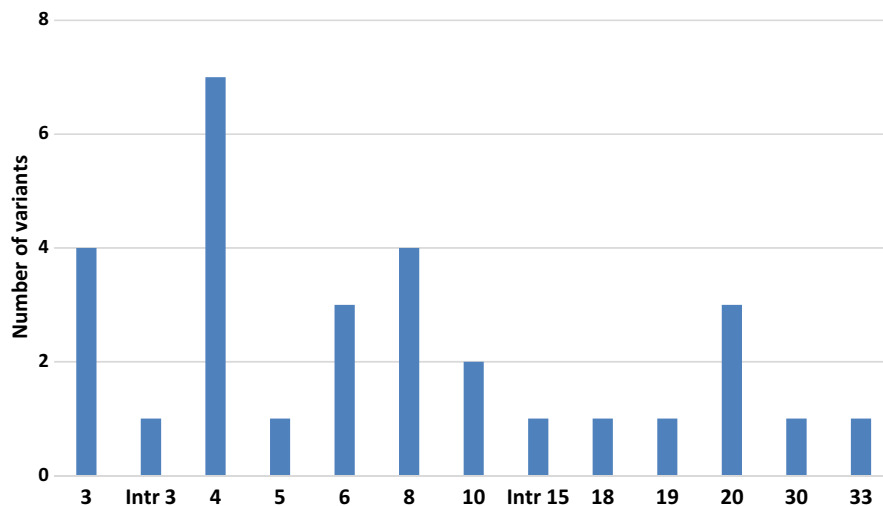
The majority of all variants were found in exon 4 (6/29, 21%), followed by exon 3 (4/29, 14%) and 8 (4/29, 14%) (Figure 1). Variants c.5510G>A,p.(Arg1837His) and c.6102dup,p.(Gly2035Argfs\*60) affected the intracellular part of Notch3, while the remaining variants affected the extracellular EGFR region. Of the 28 variants affecting the extracellular domain with EGFRs, 26 were cysteine-altering variants. Atypical variants affecting the extracellular EGFR region included a cysteine-sparing variant c.836A>G,p.(Gln279Arg) in exon 6, and a splice-site variant c.2410+6C>G whose effect on gene transcription and translation is unknown.

### 3.2 | Clinical features of the CADASIL patients

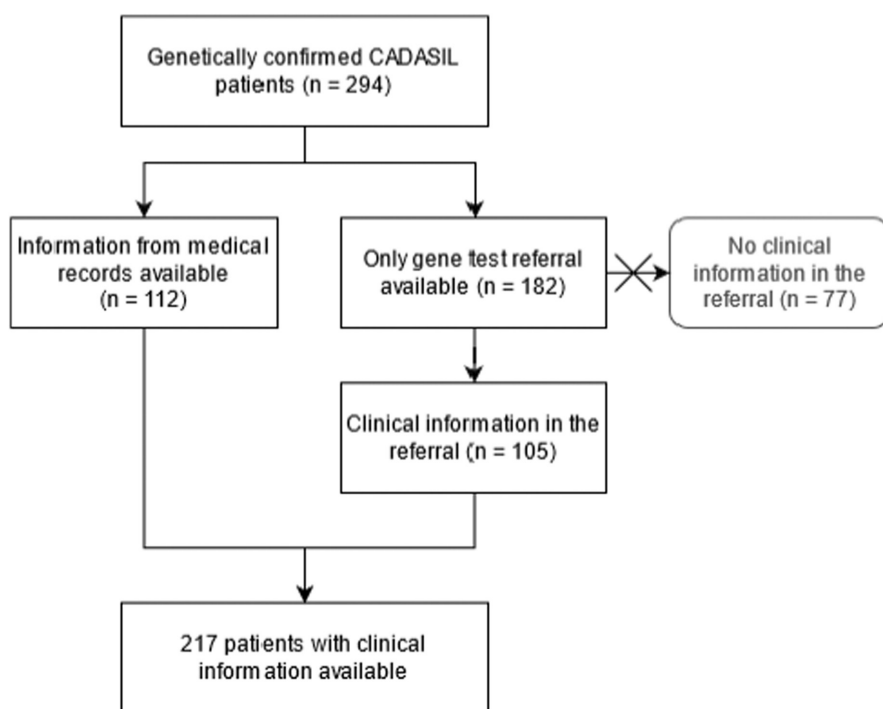
Clinical data from medical records were available for 38% (112/294) of the patients (Figure 2). For the remaining 62% (182/294) of patients, only information from the genetic testing referral was available. In 77 cases of the 182 with only genetic testing referral available, 26 cases were predictive gene tests and in 51 cases there was no clinical information in the referral. Hence, the study cohort for analyzing clinical characteristics included 217 patients (Figure 2).

Of the patients, 53% were women (155/294). Mean age at the time of gene testing was 50 (SD  $\pm$  13.7) years old, ranging from 17 to 87. The age at onset information was available in 103 cases: mean age at onset was 47 (SD  $\pm$  11.5) years old, ranging from 17 to 77. Family history was reported positive in the referral or medical records in 59% of patients (173/294). A skin biopsy result was available for 19 patients (19/294, 6%), and GOM was detected in all the cases.

**FIGURE 1** Exonic distribution of *NOTCH3* variants identified in the Finnish CADASIL cohort. Total number of different variants was 30.



**FIGURE 2** Schematic representation describing the study cohort and available clinical data



All patients were Finnish, but the patient with variant c.520T>A,p.(Cys174Ser) had African ethnicity. The demographic and clinical features of the patients with variants p.Arg133Cys, p.Tyr1069Cys, and other *NOTCH3* variants are summarized in Table 2. Clinical features of the 42 patients with less common *NOTCH3* variants in Finland are presented in more detail in Table S1. Of these 42 patients, eight had a skin biopsy and/or autopsy report available (Table 3).

## 4 | DISCUSSION

The spectrum of *NOTCH3* variants in CADASIL patients and clinical features associated with them have been studied only in a few studies focusing mainly on Asian<sup>5,12–14</sup> and some South-European<sup>4,15,16</sup> populations. The aim of this study was to retrospectively investigate

the prevalence of different *NOTCH3* variants in Finnish CADASIL patients and examine whether there are phenotypic differences between carriers of different variants. In Finland, practically all samples of patients with suspected CADASIL are sent for gene testing to the Medical Genetics laboratory of Department of Genomics of Turku University Hospital, and therefore, this study material therefore represents the great majority of Finnish CADASIL patients. The Finnish population is genetically homogeneous due to isolation and population bottlenecks. Thus, in Finland, until the implementation of more developed genetic testing methods, patients with clinically suspicious CADASIL were often screened only for the most common *NOTCH3* variants (p.Arg133Cys and p.Tyr1069Cys) or variants in specific exons encoding EGFRs and sequencing of the whole *NOTCH3* gene has become routine only in recent years. The estimated prevalence of CADASIL in Finland is 4/100000,<sup>17</sup> which is similar to

TABLE 2 Comparison of clinical features between Finnish CADASIL patients carrying the *NOTCH3* variants p.Arg133Cys and p.Tyr1069Cys and other *NOTCH3* variants

	p.Arg133Cys (n = 200)	p.Tyr1069Cys (n = 52)	Other (n = 42)	p-value (p.Arg133Cys vs. other)	p-value (p.Tyr1069Cys vs. other)
Sex (F/M)	104/96	28/24	23/19		
Family history	118 (59%)	29 (56%)	26 (62%)		
Predictive cases	16 (8%)	7 (13%)	3 (7%)		
Age at the time of predictive testing, mean ± SD	33 ± 10.8	43 ± 10.8	41 ± 18.0		
Clinical features					
Clinical information available	142 (71%)	35 (67%)	40 (95%)		
Age at onset, mean ± SD	46 ± 12.5 (n = 68)	53 ± 5.7 (n = 8)	49 ± 9.2 (n = 27)	.208	.235
Risk factors <sup>a</sup>	32/142 (23%)	6/35 (17%)	13/40 (33%)	.216	.184
Migraine/headache	48/142 (34%)	8/35 (23%)	21/40 (52%)	<b>.042</b>	<b>.010</b>
Ischemic stroke/TIA	59/142 (42%)	8/35 (23%)	23/40 (58%)	.105	<b>.004</b>
ICH	1/142 (1%)	2/35 (6%)	5/40 (13%)	<b>.002</b>	.438
Epilepsy	6/142 (4%)	1/35 (3%)	5/40 (13%)	.066	.206
Psychiatric symptom	15/142 (11%)	3/35 (9%)	7/40 (18%)	.272	.321
Cognitive impairment	44/142 (31%)	11/35 (31%)	15/40 (38%)	.449	.633
GOM detected in skin biopsy <sup>b</sup>	11/142 (8%)	1/35 (3%)	7/40 (18%)	.078	.061

Abbreviations: F, female; GOM, granular osmiophilic material; ICH, intracerebral hemorrhage; M, male; SD, standard deviation; TIA, transient ischemic attack.

<sup>a</sup>Risk factors include hypertension, smoking, diabetes, and hyperlipidemia.

<sup>b</sup>GOM was detected in all patients with skin biopsy result available.

other populations.<sup>4,18–20</sup> In the present study, 28 *NOTCH3* variants other than the most common *NOTCH3* variants, p.Arg133Cys and p.Tyr1069Cys, were identified in as much as 14% of patients, despite the fact that only the minority of the patients were studied for the whole *NOTCH3* gene. These results indicate that CADASIL may be somewhat underdiagnosed in Finland, as less common *NOTCH3* variants may have been missed in constricted DNA analyses.<sup>21–23</sup>

Overall, this study identified 30 different *NOTCH3* variants in Finnish CADASIL patients. All detected variants were distributed throughout the 33 exons of the gene, including two intronic variants between exons. Of the variants, 26 were typical disease-causing variants resulting in an uneven number of cysteine residues within one of the EGFRs. This study detected four atypical *NOTCH3* variants: a cysteine-sparing variant c.836A>G,p.(Gln279Arg) in exon 6, a cysteine-sparing variant c.5510G>A,p.(Arg1837His) in exon 30 affecting the intracellular part of the protein, a frame-shift variant c.6102dup,p.(Gly2035Argfs\*60) causing premature stop codon in exon 33, and a splice-site variant c.2410+6C>G with an unknown effect in intron 15. The clinical significance of atypical *NOTCH3* variants is uncertain.<sup>24</sup> Unfortunately, we had very limited clinical information for patients with cysteine-sparing missense variants; therefore, comparing them with patients carrying cysteine-altering variants was not possible. *NOTCH3* variants causing premature stop codons have been reported only in a few patients with suspected CADASIL and interpreted controversially.<sup>25–27</sup> Furthermore, truncating variants located in the last exon of *NOTCH3*, as in our case, have been linked to lateral meningocele

syndrome, which is a rare neurological disorder affecting connective tissues, distinct from CADASIL.<sup>28</sup> Interestingly, the skin biopsy of the patient with c.6102dup,p.(Gly2035Argfs\*60) in this study showed GOM deposits typical for CADASIL supporting the pathogenic role of the variant, unlike other cases with truncating *NOTCH3* variants (nonsense variant in exon 3 and intragenic deletion of exons 3–16) reported in the literature that did not show GOM.<sup>25,26</sup> Furthermore, segregation analysis revealed that the patient's unaffected mother and sibling did not carry the variant c.6102dup,p.(Gly2035Argfs\*60).

There were no remarkable differences in the clinical manifestations between patients with different rare *NOTCH3* variants, between patients with variants in specific exons or between patients with variants in EGFR domains 1–6 versus patients with variants in EGFR domains 7–34. However, some observations could be made from the available clinical data. Age at onset of patients with a variant affecting EGFR 1–6 was 46 ± 11.4, which was somewhat lower than patients with an EGFR 7–34 variant, whose age at onset was 50 ± 7.8. This supports the study of Rutten et al.,<sup>23</sup> which suggested that EGFR 1–6 pathogenic variants were associated with an earlier age at onset of stroke than variants affecting EGFRs 7–34. Some of the detected variants were identified in more than one patient, which revealed a few possible genotype-phenotype relationships. Four out of five patients (80%) with the variant c.580T>C,p.(Cys194Arg) suffered from migraine or headache. Both patients with the variant c.1660G>T,p.(Gly528Cys) had severe psychiatric symptoms including depression or anxiety and

TABLE 3 Clinical features of the patients with rare NOTCH3 variants and deposition of GOM in skin biopsy, and/or neuropathological autopsy data available

Patient	c.341-2A>G C6	c.391G>T,p. (Gly131Cys) C7	c.1012T>A,p. (Cys338Ser) C19	c.1300_1308dupGAGTGTCTG,p. (Glu434_Leu436dup) C21	c.1660G>T,p. (Gly528Cys) C28	c.1660G>T,p. (Gly528Cys) C29	c.3226C>T,p. (Arg1076Cys) C38	c.6102dup,p. (Gly2035Argfs*60) C42
Sex	F	F	M	M	F	F	F	F
Age at onset	52	35	40	49	59	50?	45	52
Age at death	62	44			68	87		
Source of clinical information	Medical records	Referral	Medical records	Medical records	Medical records	Medical records	Referral and skin biopsy report	Medical records
WM changes in MRI	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Migraine/headache	Yes	n/a	Yes	No	n/a	Yes	n/a	No
Ischemic stroke/TIA	Yes	n/a	No	Yes	n/a	n/a	Yes	n/a
ICH	No	n/a	No	No	Yes	No	n/a	No
Epilepsy	No	n/a	Yes	No	No	Yes	n/a	No
Psychiatric symptom	No	n/a	n/a	n/a	Yes	Yes	n/a	Yes
Cognitive impairment	Yes	n/a	No	No	Yes	Yes	n/a	Yes
Hypertension	Yes	n/a	Yes	Yes	n/a	n/a	No	n/a
Hyperlipidemia	Yes	n/a	No	Yes	n/a	n/a	No	n/a
Smoking	No	n/a	No	Yes	n/a	n/a	No	n/a
Diabetes	No	n/a	No	No	n/a	n/a	No	n/a
Family history	Yes	n/a	n/a	Yes	Yes	n/a	n/a	Yes
Skin biopsy	GOM+	GOM+	Not performed	GOM+	GOM+	GOM+	GOM+	GOM+
Neuropathology		Lacunar infarcts, WM changes, thickened arterioles; vascular changes consistent with CADASIL. Purkinje cell loss in cerebellum, gliotic foci in hippocampus	Lacunar infarcts, WM changes; most prominent lesions in basal ganglia. Thickened vessels, especially arterioles, consistent with CADASIL. Findings milder than usually found in CADASIL patients		Lacunar infarcts, WM changes, multiple infarcts in basal ganglia, infarcts in nucleus caudatus and putamen. Thickened vessels, especially arterioles, consistent with CADASIL. Ischemic lesion in cerebellum, AD changes in temporal lobe, $\beta$ -amyloid positivity, Braak II.			

Abbreviations: AD, Alzheimer's disease; F, female; GOM, granular osmiophilic material; ICH, intracerebral hemorrhage; M, male; n/a, information not available or not applicable; TIA, transient ischemic attack; WM, white matter.

changes in behavior, and one also suffered from hallucinations and paranoia. In addition, both patients with c.1660G>T,p.(Gly528Cys) developed dementia. Of the five patients carrying the variant c.3226C>T,p.(Arg1076Cys) with clinical information available, all had ischemic strokes or TIAs, two patients also had ICH, suggesting that cerebrovascular events are common in CADASIL patients with the variant c.3226C>T,p.(Arg1076Cys).

The frequency of ischemic strokes and/or TIA was higher in the patients carrying the rare *NOTCH3* variants (58%) than in patients with p.Arg133Cys (42%,  $p = .105$ ) or p.Tyr1069Cys (23%  $p = .004$ ), though the difference was statistically significant only when comparing to the patients carrying p.Tyr1069Cys. Intracerebral hemorrhage (ICH) was distinguished from microbleeds and was reported in 13% of the patients with rare variants, whereas in the patients carrying p.Arg133Cys and p.Tyr1069Cys the frequencies were 1% ( $p = .002$ ) and 6%, respectively. Most ICH cases had cysteine-altering missense variants, and the ICHs were located in capsula interna and thalamus ( $n = 1$ ), in thalamus ( $n = 1$ ), in nucleus caudatus ( $n = 1$ ), and in basal ganglia ( $n = 1$ ). Furthermore, recurrent ICH was detected in a patient with the cysteine-sparing variant c.836A>G,p.(Gln279Arg) according to the gene test referral, but detailed information was unfortunately not available. Taken together, the results indicate that ICH is an important manifestation of CADASIL. Similarly, epilepsy was also reported more frequently in the patients carrying rare variants than in patients carrying p.Arg133Cys or p.Tyr1069Cys, although the differences were not statistically significant. All patients with epilepsy had cysteine-altering missense variants, indicating that seizures might be more common in patients with typical *NOTCH3* variants. Furthermore, migraine or headache were also reported more frequently in patients with rare variants (50%) compared to patient groups with p.Arg133Cys (34%,  $p = .042$ ) or p.Tyr1069Cys (23%,  $p = .01$ ). Psychiatric manifestations were also more common in patients with rare variants (18%) than in patients carrying p.Arg133Cys (11%) or p.Tyr1069Cys (9%), although the differences were not statistically significant.

Because we were unable to acquire clinical information from all 294 patients in the study cohort, the analysis of clinical features was performed on only 217 patients. In half of cases, the clinical information was available only from the gene test referral. Thus, due to the small sample size with limited statistical power, results of phenotype comparison between variant groups should be interpreted with caution. Although the amount of patient clinical information for the study cohort was limited, the results of the study suggest that the less common *NOTCH3* variants are more often associated with more severe clinical features than the variants p.Arg133Cys and p.Tyr1069Cys. However, percentages of cognitive impairment did not differ between variant groups. This may be due to the limited clinical information available for the study. Cognitive decline may be present at the later stages of disease and therefore may not have been mentioned in a gene test referral or in medical records if only some patient notes were available.

In conclusion, this is the first study revealing the *NOTCH3* variant spectrum among CADASIL patients in Finland. Although the majority of Finnish CADASIL patients carry the founder mutation

p.Arg133Cys, less common variants were detected in a significant portion (14%) of patients. Sequencing of the whole *NOTCH3* gene, or performing a gene panel or exome sequencing is recommended when suspecting CADASIL. More patients should be identified, and functional studies are needed to clarify the role of the novel *NOTCH3* variants.

#### AUTHOR CONTRIBUTIONS

SM involved in design of the study, data collection, interpretation of the data, and preparation of the first draft of the study, and revised the manuscript. LK involved in design of the study and interpretation of the data, and drafted and revised the manuscript. JS involved in data collection, and drafted and revised the manuscript. LM involved in design, supervision and funding of the study and interpretation of the data, and drafted and revised the manuscript. MP involved in design and supervision of the study, data collection and interpretation of the data, and drafted and revised the manuscript.

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#### CONFLICT OF INTEREST

None of the authors has any conflicts of interest to disclose.

#### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ane.13703>.

#### DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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