Original Article

Comparative phenotypic spectrum of the *NOTCH3* p.R75P variant and cysteine-altering mutations: A systematic review and meta-analysis

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Abstract

The Notch receptor 3 gene (*NOTCH3*) p.R75P variant, a cysteine-sparing missense substitution, has been frequently reported in East Asian patients with cerebral small vessel disease resembling cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). While classical *NOTCH3* mutations involve cysteine alterations, emerging evidence suggests phenotypic overlap between p.R75P and cysteine-altering variants. The aim of this review is to compare the clinical and imaging features of p.R75P with those of cysteine-altering *NOTCH3* mutations. Following PRISMA 2020 guidelines, a comprehensive search was conducted up to September 2025 and in August 2026. Meta-analyses were performed using random-effects models to calculate odds ratios (ORs) with 95% confidence intervals (CIs). In addition, genetic and pathological evidence was described narratively. Nineteen studies involving 150 p.R75P carriers were included. Clinical manifestations, including stroke, symptomatic intracerebral hemorrhage (ICH), cognitive impairment, psychiatric disorders, and migraine, were comparable between *NOTCH3* p.R75P and cysteine-altering variants. Neuroradiological features were also largely similar, including white matter hyperintensities (WMH) involving the anterior temporal lobe (ATL) and external capsule (EC), as well as cerebral microbleeds (CMBs). In subgroup analyses, p.R75P was associated with a significantly higher prevalence of migraine compared with the R544C variant (OR: 2.98; 95%CI: 1.12–7.94; I^2=0.0%) and substantially lower ATL involvement reported than cysteine-altering mutations (OR: 0.01; 95%CI: 0.00–0.05; I^2=0.0%). However, these subgroup findings were based on limited and selectively reported datasets and should therefore be interpreted cautiously. The *NOTCH3* p.R75P variant demonstrates substantial clinical and imaging overlap with cysteine-altering mutations, supporting its potential pathogenic relevance in CADASIL-type arteriopathy, particularly in East Asian populations. Further multicenter, genotype-confirmed, and mechanistic studies are warranted to validate these findings, clarify underlying mechanisms, and inform diagnostic and counseling strategies across diverse populations.

Keywords: *NOTCH3*, p.R75P, cysteine-sparing variant, cerebral small vessel disease, East Asia

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a monogenic cerebral small-vessel disease attributed to pathogenic variants of the

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Notch receptor 3 gene (*NOTCH3*). Clinically, it is characterized by recurrent subcortical ischemic events, migraines (frequently accompanied by aura), mood disturbances, and progressive cognitive impairment, ultimately leading to vascular dementia [1-3]. Pathologically, CADASIL is marked by the accumulation of the *NOTCH3* extracellular domain (N3ECD) within small arterial walls and granular osmiophilic material (GOM). Neuroimaging typically reveals confluent white-matter hyperintensities (WMH), particularly in the external capsule (EC) and anterior temporal lobe (ATL), as well as lacunar infarcts and cerebral microbleeds (CMBs) [1,4-6]. These clinical, pathological, and imaging features have historically defined diagnostic criteria associated with the "cysteine rule" of *NOTCH3* mutation pathogenicity.

The "cysteine rule" suggests that pathogenic *NOTCH3* missense substitutions modify the number of cysteine residues within one of the 34 epidermal growth factor-like repeats (EGFr) in the NOTCH3 extracellular domain. This alteration leads to abnormal disulfide bonding, protein misfolding, and N3ECD aggregation, ultimately resulting in arteriopathy [1-3]. This hypothesis is corroborated by the clustering of classical CADASIL mutations within EGFr domains and by genotype-phenotype correlations that associate mutation position and cysteine alteration with disease severity and imaging findings [4,6]. Consequently, frameworks for variant interpretation and clinical testing have traditionally prioritized cysteine-altering mutations as likely pathogenic.

Contrary to the prevailing paradigm, a growing body of literature has identified cysteine-sparing *NOTCH3* missense variants in patients exhibiting CADASIL-like syndromes, prompting discussions regarding their biological and clinical significance [7-9]. Systematic evaluations of cysteine-sparing mutations have demonstrated variability: some studies report atypical imaging features, such as reduced involvement of the ATL, while others present clinical and radiological findings consistent with classic CADASIL [6,9]. To address this variability, researchers have proposed stringent criteria for determining the pathogenicity of cysteine-sparing mutations. These criteria include comprehensive exon screening to exclude canonical mutations, evidence of familial co-segregation, low population allele frequency, and confirmatory pathology, such as GOM or vascular N3ECD deposits [6,9].

In this context, the *NOTCH3* p.R75P variant is a cysteine-sparing missense substitution in which arginine is replaced by proline at codon 75 without altering the number of cysteine residues within the EGFr domain [10]. It has emerged as a recurrent, region-specific variant that challenges the cysteine rule. Initially identified in Japanese and Korean families, p.R75P carriers exhibit vascular N3ECD deposits and, in some instances, GOM on skin biopsies, thereby providing initial pathological evidence of CADASIL-type arteriopathy despite the mutation's cysteine-sparing nature [1,4,11,12]. More recent, extensive analyses of East Asian cohorts revealed that p.R75P carriers present a broad CADASIL clinical-radiological spectrum, commonly presenting with migraines and cognitive impairment, along with a higher frequency of hemorrhagic events, such as symptomatic intracerebral hemorrhage (ICH) and multiple CMBs, and less prominent temporal pole lesions [13]. The accumulated pathological, co-segregation, and cohort data suggest that p.R75P exhibits a phenotypic profile similar to that of canonical cysteine-altering *NOTCH3* mutations. However, despite growing evidence, no systematic review has yet synthesized the phenotype of p.R75P compared with cysteine-altering *NOTCH3* mutations.

Given the prevalence of the *NOTCH3* p.R75P mutation in East Asian populations and the challenges in classifying this cysteine-sparing variant, a direct comparison with cysteine-altering mutations is essential to clarify its pathogenic relevance and inform diagnostic and counseling strategies. Therefore, the aim of this meta-analysis is to compare the clinical and imaging characteristics of p.R75P and cysteine-altering variants. This synthesis directly engages with emerging research on cysteine-sparing mutations and supports findings that the p.R75P mutation may present the classical CADASIL phenotype within East Asian populations.

Methods

Protocol registration

This meta-analysis was performed in accordance with the PRISMA 2020 guideline [14]. The review protocol was registered in Open Science Framework: 10.17605/OSF.IO/S6V7Q. Reference management and full-text screening were carried out using the Rayyan platform [15].

Search strategy

A comprehensive literature search was conducted in PubMed, ScienceDirect, and Google Scholar from database inception to September 2025. An updated search was subsequently conducted as of August 12, 2026, across the three mentioned databases, with the addition of the Cochrane Library and Scopus. The search strategy employed a combination of Medical Subject Headings (MeSH) and free-text keywords, utilizing the Boolean operators AND and OR. The complete search strategies are presented in **Table 1**. No language restrictions were applied during the initial database search; however, only articles published in English or Japanese were considered during the screening process. The reference lists of included studies were also manually screened to identify additional eligible publications.

Table 1. Search strategies for each database

Databases	Database-specific search strategy	Date of final search	Records retrieved
PubMed	("notch3 protein human"[Supplementary Concept] OR "notch3 protein human"[All Fields] OR "notch3"[All Fields]) AND ("cadasil"[MeSH Terms] OR "cadasil"[All Fields]) AND ("R75P"[All Fields] OR "Arg75Pro"[All Fields] OR "224G>C" [All Fields] OR "c.224G>C"[All Fields])	12 August 2026	24
ScienceDirect	"NOTCH3" AND "CADASIL" AND ("R75P" OR "Arg75Pro" OR "224G>C" OR "p.Arg75Pro" OR "c.224G>C")	12 August 2026	18
Google Scholar	NOTCH3 AND CADASIL AND (R75P OR Arg75Pro OR 224G>C OR p.Arg75Pro OR c.224G>C)	12 August 2026	245
Cochrane Library	#1 MeSH descriptor: [NOTCH3] explode all trees	12 August 2026	24
Scopus	TITLE-ABS-KEY(NOTCH3 AND CADASIL AND (R75P OR Arg75Pro OR "224G>C" OR "p.Arg75Pro" OR "c.224G>C"))	12 August 2026	22

Eligibility criteria and study selection

The study selection followed a Population, Intervention, Comparison, Outcomes, and Study design (PICOS) framework tailored to the protocol's single-variant focus on *NOTCH3* c.224G>C (p.Arg75Pro; p.R75P). Studies of patients with a CADASIL or CADASIL-like presentation in whom the p.R75P variant was identified by genetic testing (targeted panels, exome sequencing, or full 33-exon sequencing) were included. Comparator groups comprised patients with cysteine-altering *NOTCH3* variants. Studies without comparator groups were also included for proportion analyses.

Eligible outcomes encompassed clinical features, including stroke/transient ischemic attack (TIA), symptomatic ICH, migraine, psychiatric symptoms, and cognitive impairment or dementia defined clinically or using screening instruments such as the Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA); neuroimaging features classified according to the Standards for Reporting Vascular Changes on Neuroimaging (STRIVE), including WMHs, with particular emphasis on ATL and EC involvement, CMBs, and other abnormalities; and genetic or pathological evidence, including familial co-segregation, GOM identified by electron microscopy, and sequencing completeness. Case reports, case series, and observational reports that allowed extraction of genotype–phenotype linkage for p.R75P were considered eligible.

Articles with suspected overlapping cohorts were assessed by cross-referencing the institution, recruitment period, and number of p.R75P carriers. Reports originating from the same institution with overlapping recruitment periods and potentially overlapping patient populations were excluded, with preference given to the most comprehensive dataset. Publications from the same institution were retained when they reported distinct outcomes that were not available in the other publication, while avoiding duplicate inclusion of the same participants within individual pooled analyses. The screening process was independently conducted by three investigators (YAF, NPL, AIA) utilizing Rayyan for blinded evaluation of titles, abstracts, and full texts. Discrepancies were resolved through consensus with a senior investigator (GVS).

Data extraction and variables

The data were extracted by YAF, AKB, and NPL using a standardized data extraction form. Data from each study were extracted by one investigator and independently verified by the senior investigator (GVS). Extracted data included study identifiers (first author, year, and country), institution, number of p.R75P carriers, sex distribution (male/female), age (mean or median, when reported), family history, and vascular risk factors (smoking, hypertension, diabetes, dyslipidemia, and prior stroke). Data on clinical domains and magnetic resonance imaging (MRI) characteristics were also collected. Where available, genetic/pathology evidence (full 33-exon sequencing, GOM, co-segregation) from texts, tables, figures, and supplementary files was recorded. All variables mirrored the headers and coding used in the consolidated inclusion table (Y/N/NA or "+ / – / NA"), and missing items were entered as NR/NA without imputation. In multi-paper cohorts, the most complete/non-overlapping dataset was prioritized, and discrepancies were reconciled by re-reading full texts and supplements.

Risk of bias assessment and variant adjudication

A qualitative risk-of-bias assessment was conducted using Joanna Briggs Institute (JBI) Critical appraisal tools, tailored to the respective study designs (case report, case series, cross-sectional, and cohort) [16-19]. Risk-of-bias assessments were used to evaluate the methodological limitations of included studies and were not used as a proxy for study eligibility. Depending on the study design, the JBI tools assessed domains including participant selection, validity and reliability of measurements, identification and management of confounding, outcome assessment, adequacy and completeness of follow-up, and appropriateness of statistical analysis.

Statistical analysis

For comparative analyses, pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were estimated using random-effects models with the Mantel–Haenszel method. Between-study variance (τ^2) was estimated using the restricted maximum likelihood (REML) method, and statistical heterogeneity was quantified using the I^2 statistic. Heterogeneity was interpreted as low (<25%), moderate (25–49%), or substantial (≥50%).

Two prespecified comparisons were conducted for each eligible outcome: p.R75P versus p.R544C and p.R75P versus other cysteine-altering *NOTCH3* variants. A continuity correction of 0.5 was applied to studies containing zero cell frequencies. To assess the robustness of the findings to the choice of continuity correction, additional sensitivity analyses were performed using a correction of 0.25 for sparse and zero-event data. Leave-one-out sensitivity analyses were also conducted to evaluate the influence of individual studies on the pooled estimates.

For outcomes reported as frequencies among p.R75P carriers, pooled proportions were estimated using the Freeman–Tukey double-arcsine transformation. Given the limited number of available studies, case reports and case series were included in the primary proportion analyses to maximize data availability. Additional sensitivity analyses were performed by restricting the dataset to observational studies and, separately, to cohort studies to evaluate the robustness of the pooled frequencies to study design and case-based ascertainment.

Publication bias was assessed visually using funnel plots when a sufficient number of studies contributed to an outcome. Because relatively few studies were available for most outcomes, funnel plot assessments were considered exploratory. Statistical significance was set at $p<0.05$. All analyses were performed using R software version 4.3.2 (Foundation for Statistical Computing, Vienna, Austria) with the meta and metafor packages [20,21].

Results

Study selection and characteristics

The literature search identified 333 records, of which 24 records were derived from PubMed, 18 from ScienceDirect, 245 from Google Scholar, 24 from Cochrane, and 22 from Scopus using analogous search terms. After removing 89 duplicate entries, 244 unique records were subjected to title and abstract screening. Of these, 192 records were excluded based on predefined criteria, including background articles (n=2), wrong population (n=84), inappropriate study design (n=72), wrong outcome (n=10), and foreign language (n=24). The remaining 52 reports were

assessed for full-text eligibility, of which 7 could not be retrieved because the full texts were unavailable. Thus, 45 studies underwent full-text eligibility assessment. A total of 17 studies were excluded because variant-specific data were not reported separately, while 11 studies were excluded because of potential overlap in study populations, particularly among reports from the same institutions with overlapping recruitment periods. Consequently, 17 studies met the eligibility criteria through database searching. Backward and forward citation searching identified 22 additional records, of which 20 were excluded, and two met the eligibility criteria, resulting in a total of 19 included studies encompassing 150 carriers of the *NOTCH3* p.R75P variant [3,4,6,13,22-36]. The detailed study selection process is presented in **Figure 1**.

Following assessment of potential cohort overlap, no reports with suspected overlapping populations were simultaneously included in the same pooled analysis. Of the 19 included studies, 14 included cysteine-altering *NOTCH3* variants as comparator groups, whereas five had no comparator group. The detailed characteristics of the included studies are presented in **Table 2**. The studies were conducted between 2006 and 2025, including the earliest case reports from Korea [31]. A notable increase in publications was observed after 2020, coinciding with the broader adoption of next-generation sequencing and the increasing recognition of cysteine-sparing *NOTCH3* variants as clinically significant. The majority of studies originated from Japan (n=9) and South Korea (n=8), with China contributing a smaller number of series (n=2). All included studies originated from different institutions, with the exception of two publications from Asan Medical Center [6,22]. Both were retained because they reported distinct outcomes, while the more complete dataset was used when the same outcome was reported. In terms of study design, three were cohort studies, nine were cross-sectional studies, five were case reports, and two were case series (**Table 2**).

Across studies, the weighted mean age of individuals carrying the p.R75P variant at the time of evaluation was approximately 57.9 years, with ages ranging from the early 50s to the mid-70s. In contrast, individuals with cysteine-altering variants had a slightly lower mean age of 53.8 years, ranging from the late 40s to the mid-60s.

Family history and vascular risk factors were variably documented across the included studies. Family history was reported in 18 of the 19 included studies. A positive family history was reported in 11 studies, whereas seven studies reported no family history, and one study did not provide information on family history. Vascular risk factors, including smoking, hypertension, diabetes, and dyslipidemia, were variably reported across the included studies, with limited information available from several case reports and small case series. Among studies reporting these characteristics, the corresponding frequencies of smoking, hypertension, diabetes, and dyslipidemia were approximately 24%, 33%, 10%, and 38% among p.R75P carriers and 24%, 36%, 11%, and 37% among comparator participants, respectively. Detailed characteristics of the included studies are provided in **Table 2** and **Table 3**.

Clinical manifestations

The clinical manifestations associated with the *NOTCH3* p.R75P variant were compared with those of cysteine-altering variants known to cause CADASIL to evaluate its potential pathogenic relevance, focusing on five key domains: stroke, symptomatic ICH, cognitive impairment, psychiatric symptoms, and migraine. No statistically significant differences were observed between the p.R75P variant and cysteine-altering variants across clinical outcomes (all $p>0.05$), indicating broadly comparable clinical profiles. The odds of stroke were similar (OR: 1.19; 95%CI: 0.58–2.44; $I^2=39.6\%$), as were those for cognitive impairment (OR: 0.78; 95%CI: 0.29–2.08; $I^2=54.1\%$), symptomatic ICH (OR: 2.94; 95%CI: 0.47–18.52; $I^2=45.2\%$), psychiatric symptoms (OR: 1.34; 95%CI: 0.61–2.92; $I^2=0.0\%$), and migraine (OR: 1.58; 95%CI: 0.79–3.15; $I^2=5.6\%$). Substantial heterogeneity was observed for cognitive impairment, likely reflecting differences in definitions and assessment methods across studies (**Figure 2**).

Given that p.R544C has been proposed to exhibit milder genotype–phenotype characteristics [2,37], this variant was explored separately from other cysteine-altering variants through subgroup analyses. Subgroup analyses revealed notable findings.

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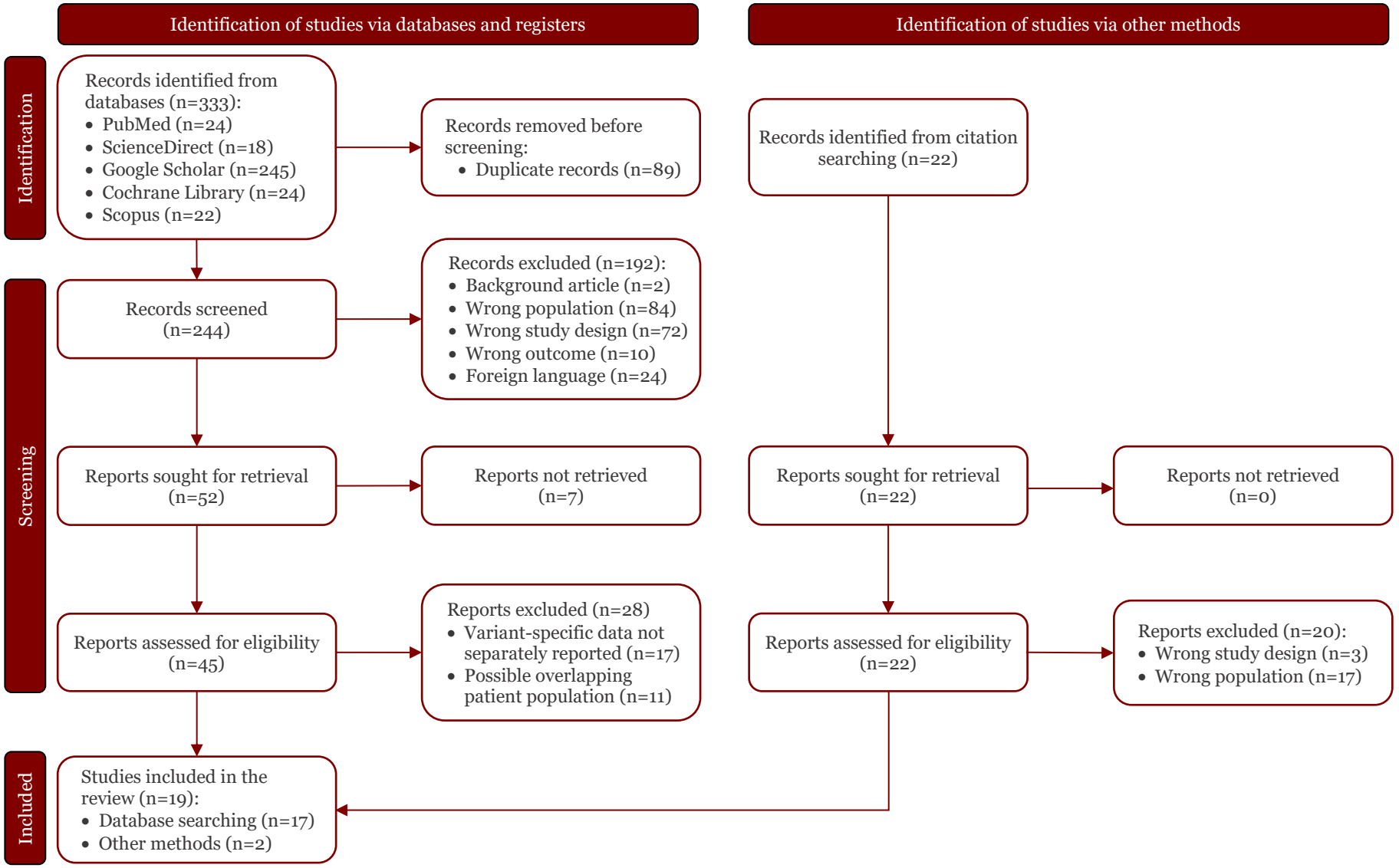


Figure 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2020 flow chart of the study selection.

Table 2. Characteristics of the included studies and p.R75P carriers

Author (year)	Study design (period)	Country	Carriers		Mean age (year)		Family history		Smoking		Hypertension		Diabetes		Dyslipidemia	
			R75P	C	R75P	C	R75P	C	R75P	C	R75P	C	R75P	C	R75P	C
Ishiyama *et al.* (2024) [13]	Cohort (Oct 2015–Oct 2022)	Japan	15	48	55*	54*	Y	Y	1/15	9/48	11/15	15/48	1/15	7/48	12/15	33/48
Park *et al.* (2025) [22]	Cohort (Jan 2002–Dec 2022)	South Korea	25	23	58.6	59.1	Y	Y	9/25	6/23	4/25	7/23	2/25	4/23	9/25	8/23
Takei *et al.* (2023) [23]	Cross-sectional (2006–2021)	Japan	24	8	61	54.5	Y	Y	-	-	9/23	1/8	3/23	1/8	9/23	1/8
Mukai *et al.* (2020) [24]	Cohort (Mar 2019)	Japan	14	32	58.0	52.19	Y	Y	4/14	16/32	-	-	0/14	1/32	6/14	9/32
Ueda *et al.* (2015) [4]	Cross-sectional (Apr 1997–Mar 2014)	Japan	7	44	57.4	49.1	N	N	-	-	-	-	-	-	-	-
Kim *et al.* (2014) [26]	Cross-sectional (Jan 2003–Dec 2011)	South Korea	6	7	55.7	57.1	Y	Y	-	-	2/6	4/7	2/6	1/7	1/6	2/7
Kim *et al.* (2012) [25]	Case report (NR)	South Korea	3	-	56	-	Y	-	-	-	-	-	-	-	-	-
Hu *et al.* (2021) [3]	Cross-sectional (2014–2020)	China	2	14	71	-	Y	-	-	-	-	-	-	-	-	-
Min *et al.* (2021) [27]	Cross-sectional (Apr 2010–Apr 2020)	South Korea	22	29	51.5	55.1	Y	Y	3/22	2/29	5/22	7/29	4/22	4/29	2/22	4/29
Kim *et al.* (2020) [6]	Cross-sectional (Sep 1999–Sep 2017)	South Korea	18	12	58.5	63.6	-	-	5/18	-	5/18	-	2/18	-	-	-
Yamashiro *et al.* (2025) [35]	Case report (NR)	Japan	1	-	53	-	N	-	1/1	-	0/1	-	0/1	-	0/1	-
Kim *et al.* (2019) [36]	Cross-sectional (2005–2015)	South Korea	5	25	52.6	48.2	N	Y	-	-	2/5	10/25	0/5	1/25	3/5	8/25
Matsushima *et al.* (2017) [28]	Cross-sectional (2015–2016)	Japan	1	7	78	56.6	Y	Y	-	-	0/1	2/7	0/1	1/7	0/1	3/7
Shimizu *et al.* (2014) [29]	Case report (NR)	Japan	1	-	60	-	Y	-	0/1	-	1/1	-	0/1	-	1/1	-
Liu *et al.* (2015) [30]	Cross-sectional (Mar 2003–May 2014)	China	1	1	NA	-	N	-	-	-	-	-	-	-	-	-
Choi *et al.* (2006) [31]	Case series (Jan 2000–Dec 2005)	South Korea	2	15	67	57.7	N	N	0/2	2/15	2/2	11/15	0/2	2/15	0/2	1/15
Kunii *et al.* (2018) [32]	Case series (NR)	Japan	1	1	75	59	N	Y	-	-	-	-	-	-	-	-
Jung *et al.* (2022) [33]	Case report (NR)	South Korea	1	-	61	-	Y	-	-	-	-	-	-	-	-	-
Tachiyama *et al.* (2018) [34]	Case report (NR)	Japan	1	-	55	-	N	-	-	-	0/1	-	0/1	-	1/1	-

C: comparator group; N: no; NR: not reported; R75P: p.R75P group; Y: yes; -: not reported
* Present in median

Table 3. Clinical and neuroimaging characteristics of p.R75P carriers in the included studies

Author (year)	MAO	MAA	Stroke	sICH	Cognitive impairment	Psychiatric symptoms	Migraine	Bulbar symptoms	ATL	External capsule	Lacunar infarction	CMB
Ishiyama *et al.* (2024) [13]	N/A	N/A	8/15	5/15	5/15	1/15	7/15	-	1/15	15/15	15/15	15/15
Park *et al.* (2025) [22]	-	58.6	17/25	-	-	-	-	-	-	16/25	22/25	16/25
Takei *et al.* (2023) [23]	55.1	61.0	19/24	5/24	9/24	2/24	6/24	9/23	4/24	22/24	22/24	24/24
Mukai *et al.* (2020) [24]	53.6	58.0	6/14	-	7/14	4/14	6/14	5/14	2/14	11/14	11/14	2/14
Ueda *et al.* (2015) [4]	53.6	57.4	5/7	-	4/7	1/7	3/7	-	2/7	6/7	-	-
Kim *et al.* (2014) [26]	-	55.7	4/6	-	4/6	0/6	2/6	-	2/6	5/6	-	-
Kim *et al.* (2012) [25]	-	56.0	1/2	-	1/2	-	-	-	-	-	-	-
Hu *et al.* (2021) [3]	63.0	71.0	4/6	-	4/6	0/6	2/6	-	2/4	4/4	-	4/4
Min *et al.* (2021) [27]	47.8	51.5	14/22	1/22	6/22	0/22	12/22	-	12/20	17/20	17/20	-
Kim *et al.* (2020) [6]	-	58.5	11/18	-	6/18	6/18	7/18	-	5/18	9/18	-	11/13
Yamashiro *et al.* (2025) [35]	43.0	53.0	0/1	-	1/1	-	-	1/1	-	1/1	1/1	1/1
Kim *et al.* (2019) [36]	-	52.6	4/5	-	-	-	0/5	-	-	1/5	2/5	-
Matsushima *et al.* (2017) [28]	76.0	78.0	0/1	-	1/1	1/1	0/1	0/1	-	0/1	1/1	1/1
Shimizu *et al.* (2014) [29]	54.0	60.0	1/1	-	1/1	1/1	0/1	1/1	-	0/1	1/1	1/1
Liu *et al.* (2015) [30]	-	-	1/1	-	0/1	0/1	0/1	-	1/1	1/1	-	-
Choi *et al.* (2006) [31]	65.0	67.0	2/2	0/2	2/2	0/2	0/2	0/2	-	-	-	-
Kunii *et al.* (2018) [32]	62.0	75.0	-	-	-	-	-	-	0/1	0/1	0/1	1/1
Jung *et al.* (2022) [33]	-	61.0	1/1	-	1/1	0/1	1/1	-	1/1	0/1	-	-
Tachiyama *et al.* (2018) [34]	-	55.0	1/1	-	1/1	1/1	-	-	-	0/1	1/1	1/1

ATL: anterior temporal lobe; CMB: cerebral microbleeds; MAA: mean age at assessment; MAO: mean age of onset; sICH: symptomatic intracerebral hemorrhage; WMH: white-matter hyperintensities; -: not reported

Migraine was significantly more frequent in individuals with p.R75P compared with those carrying the p.R544C variant (OR: 2.98; 95%CI: 1.12–7.94; I^2=0.0%). ICH was also significantly more frequent among individuals with p.R75P than among those carrying typical cysteine-altering variants; however, the wide confidence interval indicates substantial uncertainty around the magnitude of this association (OR: 12.71; 95%CI: 2.09–77.28). No heterogeneity was observed in this outcome (I^2=0.0%) (**Figure 2**).

Neuroimaging findings

The MRI characteristics of the *NOTCH3* p.R75P variant were compared with those of cysteine-altering variants, which are well established to cause CADASIL. Across most studies, MRI findings were described according to STRIVE criteria, with particular emphasis on the ATL, EC, lacunes, and CMBs.

No statistically significant differences were identified between the p.R75P variant and cysteine-altering variants across neuroimaging outcomes (all p>0.05), suggesting largely comparable imaging patterns. The odds of WMH in the ATL tended to be lower in p.R75P, although this did not reach statistical significance (OR: 0.13; 95%CI: 0.01–1.18; p=0.07; I^2=87.0%), with substantial heterogeneity observed. Likewise, no significant differences were found for WMH in the EC (OR: 1.74; 95%CI: 0.83–3.63; p=0.14; I^2=0.0%) or for CMBs (OR: 2.12; 95%CI: 0.76–5.91; p=0.15; I^2=0.0%).

Further insights were obtained from subgroup analyses. The frequency of WMH in the ATL was similar between individuals with p.R75P and those with the p.R544C variant (OR: 1.21; 95%CI: 0.22–6.82; I^2=71.7%), but was significantly lower when compared with other cysteine-altering variants (OR: 0.01; 95%CI: 0.00–0.05; I^2=0.0%) (**Figure 3**).

A summary of the meta-analysis comparing the p.R75P variant with cysteine-altering variants across clinical and radiological findings, together with the additional sensitivity analyses using a 0.25 continuity correction, is provided in **Table 4** and **Table 5**. The sensitivity analysis across outcomes are presented in **Figure 4**. The exploratory assessment of publication bias is presented in **Figure 5**. Given the small number of studies contributing to several outcomes, these funnel plots were interpreted as exploratory.

Pooled frequencies of clinical and neuroimaging features

To complement these comparative findings, pooled proportion analyses were performed to characterize the reported clinical and neuroimaging profile of the *NOTCH3* p.R75P variant among published, clinically ascertained cases. Among the reported p.R75P cases, stroke was the most frequently reported clinical manifestation (69.2%), followed by cognitive impairment (43.7%), migraine (34.2%), bulbar symptoms (33.6%), and psychiatric symptoms (10.2%). WMH in the ATL was reported in 25.6% of cases, whereas WMH in the EC was more frequently reported (82.2%). Lacunes were reported in all participants contributing data to the corresponding analysis, and CMBs were reported in 93.2% of cases (**Table 6**).

Sensitivity analyses restricted to observational studies yielded broadly similar pooled frequencies for most clinical and neuroimaging features. When further restricted to cohort studies, pooled frequencies were generally lower for several outcomes, particularly stroke and CMBs, although these estimates were based on a limited number of studies.

These pooled estimates represent reported frequencies among published, clinically ascertained p.R75P cases rather than population-level prevalence or penetrance. Leave-one-out sensitivity analyses of the pooled proportion estimates are presented in **Figure 6**.

Genetic and pathological evidence

Genetic confirmation was consistently available across all included studies. While earlier investigations utilized exon-specific sequencing, the majority of recent studies employed comprehensive 33-exon *NOTCH3* sequencing or targeted exome panels, confirming the p.R75P variant in isolation. This variant was either absent from large population databases or present at an extremely low frequency.

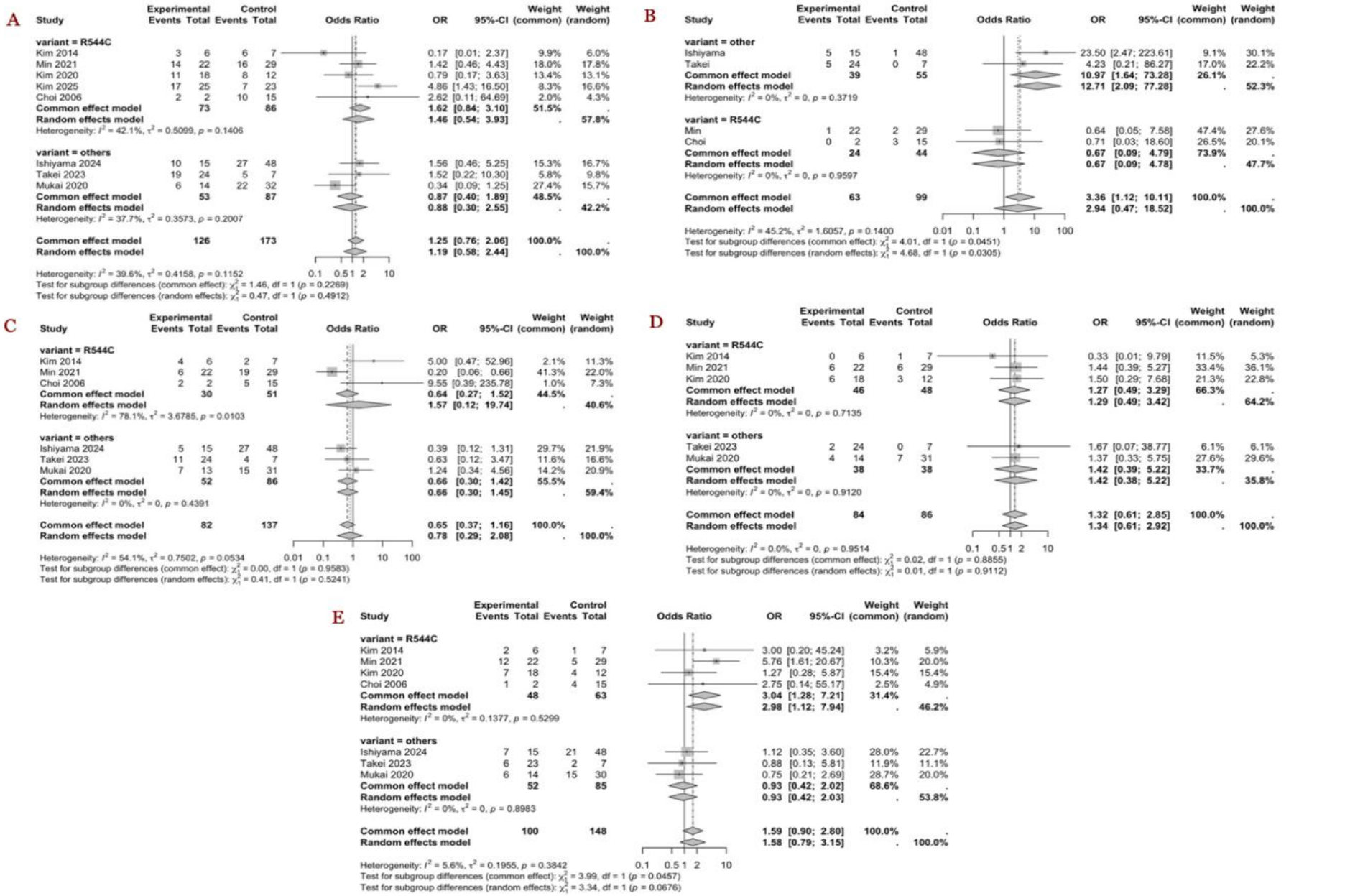


Figure 2. Meta-analysis comparing p.R75P and cysteine-altering variants in clinical manifestation: (A) stroke, (B) symptomatic intracerebral hemorrhage, (C) cognitive impairment, (D) psychiatric symptoms, and (E) migraine.

Table 4. Meta-analysis and subgroup analysis comparing p.R75P and cysteine-altering variants

Outcomes	All studies				Subgroup analysis			
					R544C		Typical cysteine-altering variants	
	Studies	OR (95%CI)	I^2	*p*-value	OR (95%CI)	I^2	OR (95%CI)	I^2
Stroke	8	1.19 (0.58–2.44)	39.6	0.63	1.46 (0.54–3.93)	42.1	0.88 (0.30–2.55)	37.7
Symptomatic ICH	4	2.94 (0.47–18.52)	45.2	0.14	0.67 (0.09–4.78)	0	12.71 (2.09–77.28)*	0.0
Cognitive impairment	6	0.78 (0.29–2.08)	54.1	0.62	1.57 (0.12–19.74)	78.1	0.66 (0.30–1.45)	0.0
Psychiatric symptoms	5	1.34 (0.61–2.92)	0.0	0.47	1.29 (0.49–3.42)	0.0	1.42 (0.38–5.22)	0.0
Migraine	7	1.58 (0.79–3.15)	5.6	0.11	2.98 (1.12–7.94)*	0.0	0.93 (0.42–2.03)	0.0
WMH in ATL	6	0.13 (0.01–1.18)	87.0	0.07	1.21 (0.22–6.82)	71.7	0.01 (0.00–0.05)*	0.0
WMH in EC	6	1.74 (0.83–3.63)	0.0	0.14	2.17 (0.90–5.22)	0.0	1.02 (0.26–3.95)	0.0
Cerebral microbleeds	3	2.12 (0.76–5.91)	0.0	0.15	2.14 (0.71–6.49)	0.0	2.00 (0.13–29.81)	NA

ATL: anterior temporal lobe; CI: confidence interval; EC: external capsule; ICH: intracerebral hemorrhage; NA: not applicable; OR: odds ratio; WMH: white matter hyperintensities

Table 5. Sensitivity analysis comparing p.R75P and cysteine-altering variants using a continuity correction of 0.25

Outcomes	Primary (0.5)	Sensitivity (0.25)
Symptomatic ICH	All studies: 2.94 (0.47–18.52, I^2=45.2)	All studies: 3.17 (0.38–26.50, I^2=46.7)
	R544C: 0.67 (0.09–4.78, I^2=0)	R544C: 0.58 (0.07–4.92, I^2=0)
	Typical cysteine-altering: 12.71 (2.09–77.28, I^2=0)*	Typical cysteine-altering: 18.26 (2.53–131.54, I^2=0)*
Psychiatric symptoms	All: 1.34 (0.61–2.92, I^2=0.0)	All: 1.38 (0.62–3.07, I^2=0.0)
	R544C: 1.29 (0.49–3.42, I^2=0)	R544C: 1.32 (0.49–3.56, I^2=0)
	Typical cysteine-altering: 1.42 (0.38–5.22, I^2=0)	Typical cysteine-altering: 1.48 (0.38–5.76, I^2=0)

ICH: intracerebral hemorrhage
* Statistically significant at $p<0.05$

Co-segregation of the mutation with the clinical phenotype was documented in multiple families [1,4,13,27], providing supportive familial evidence for pathogenicity. In instances where biopsies were conducted, GOM deposits were observed in vascular smooth-muscle cells, representing a characteristic histopathological feature of CADASIL.

Collectively, the reported clinical and radiological features, together with genetic, familial, and histopathological findings, provide convergent evidence supporting the pathogenicity of p.R75P and warrant formal reassessment of its current classification as a variant of uncertain significance (VUS) in East Asian populations.

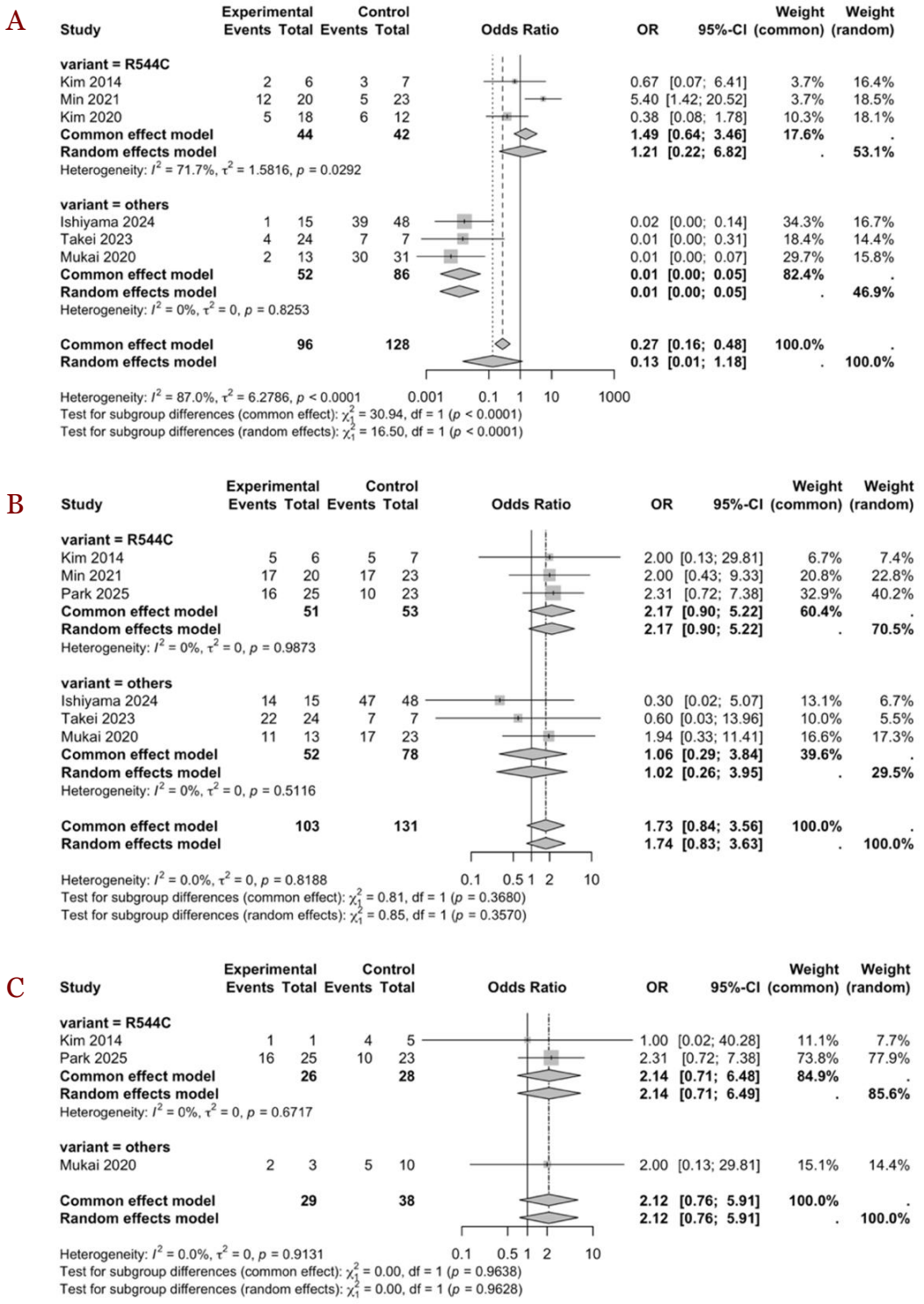
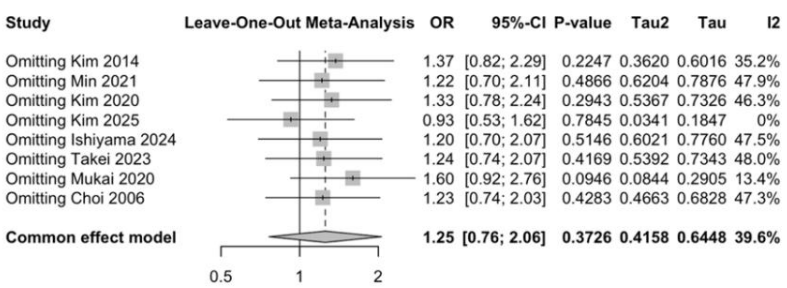


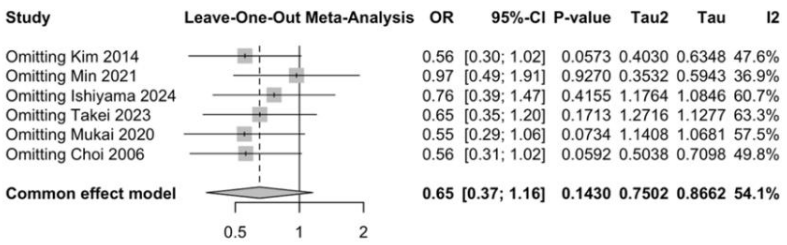
Figure 3. Meta-analysis comparing p.R75P and cysteine-altering variants across neuroimaging findings: (A) white matter hyperintensities in the anterior temporal lobe, (B) white matter hyperintensities in the external capsule, and (C) cerebral microbleeds.

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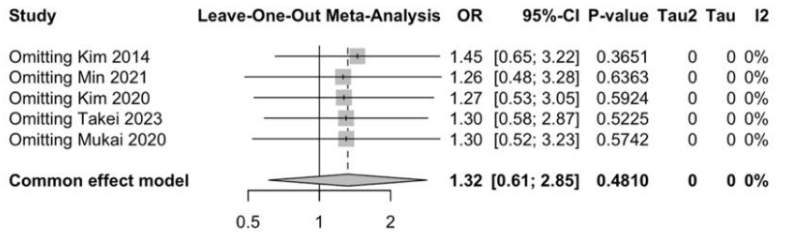
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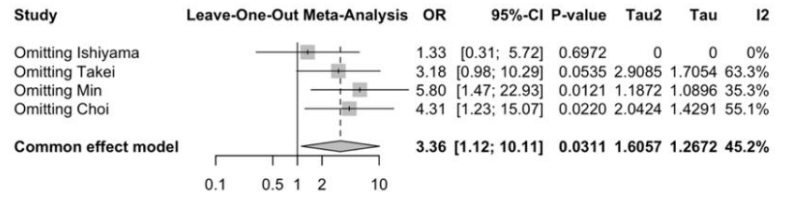
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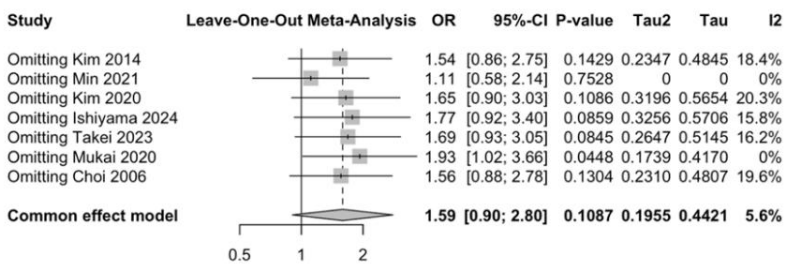
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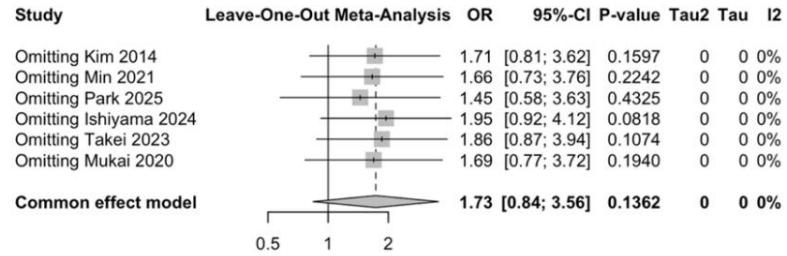
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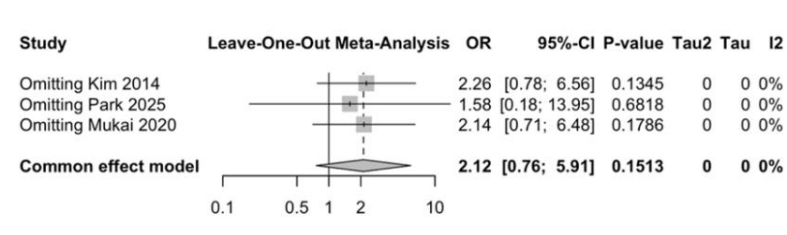
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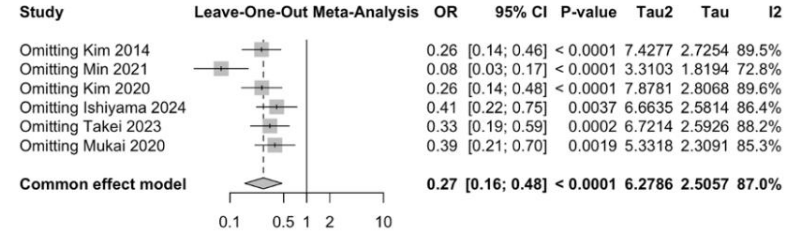


Figure 4. Sensitivity analysis: (A) stroke, (B) cognitive impairment, (C) psychiatric symptoms, (D) symptomatic intracerebral hemorrhage, (E) migraine, (F) white matter hyperintensities in the external capsule, (G) cerebral microbleeds, and (H) white matter hyperintensities in the anterior temporal lobe.

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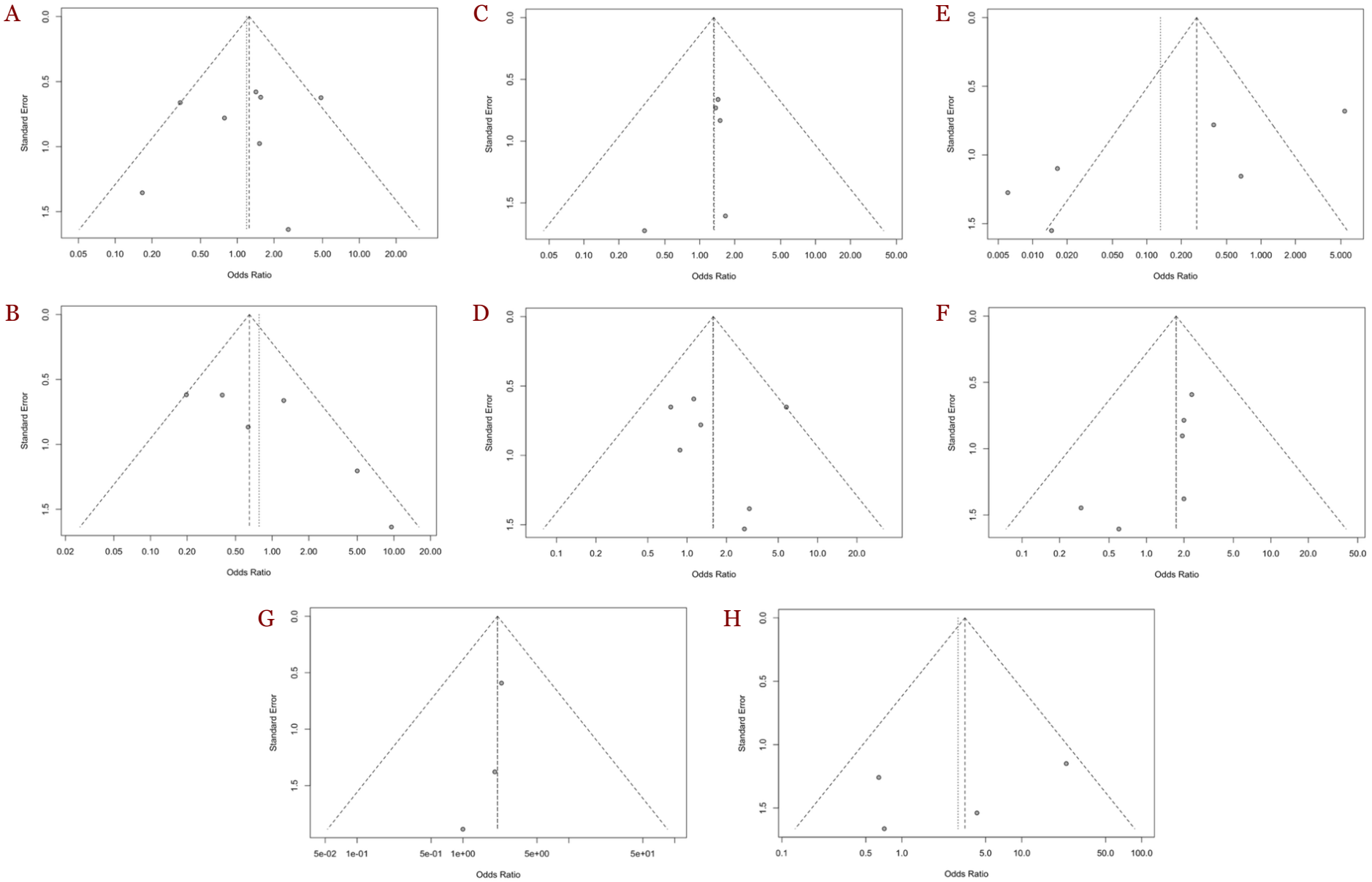


Figure 5. Funnel plot: (A) stroke, (B) cognitive impairment, (C) psychiatric symptoms, (D) migraine, (E) white matter hyperintensities in the anterior temporal lobe, (F) white matter hyperintensities in the external capsule, (G) cerebral microbleeds, and (H) symptomatic intracerebral hemorrhage.

Table 6. Pooled proportions of clinical and neuroimaging features among reported p.R75P cases

Outcomes	All eligible studies				Observational studies				Cohort studies			
	Studies	Events/ total	Pooled proportion (95%CI)	I^2	Studies	Events/ total	Pooled proportion (95%CI)	I^2	Studies	Events/ total	Pooled proportion (95%CI)	I^2
Stroke	17	95/147	69.23 (58.67–79.13)	0.0	11	89/139	66.44 (56.64–75.73)	0.0	3	31/54	57.06 (41.44–72.06)	14.7
Cognitive impairment	16	53/122	43.73 (30.76–57.01)	10.8	10	46/114	38.45 (27.78–49.57)	0.0	2	12/29	41.26 (23.40–60.24)	0.0
Psychiatric disorder	14	23/119	10.24 (1.70–22.54)	36.1	10	21/114	11.44 (2.89–22.94)	35.0	2	5/29	16.14 (0.67–41.73)	54.9
Migraine	14	47/123	34.19 (22.62–46.49)	11.9	11	45/119	34.57 (23.54–46.25)	3.1	2	13/29	44.83 (26.57–63.75)	0.0
Bulbar symptoms	6	16/42	33.59 (14.35–55.07)	0.0	3	14/38	33.45 (15.17–53.81)	0.0	1	5/14	NR	NR
WMH ATL	11	32/111	25.57 (10.32–43.46)	52.1	9	31/109	26.41 (12.04–43.09)	56.7	2	3/29	10.08 (0.88–24.87)	0.0
WMH EC	16	107/140	82.22 (63.69–96.20)	58.3	11	106/135	85.91 (71.17–96.82)	59.2	3	42/54	84.27 (54.88–99.98)	81.2
Lacunes	6	41/44	100.00 (93.53–100.00)	0.0	3	38/41	100.00 (86.36–100.00)	18.5	2	37/40	95.30 (77.91–100.00)	58.0
Cerebral microbleeds	11	77/100	93.22 (65.89–100.00)	80.8	7	73/96	86.90 (54.37–100.00)	88.5	3	33/54	64.98 (9.90–100.00)	93.7

ATL: anterior temporal lobe; CI: confidence interval; EC: external capsule; NR: not reported; WMH: white-matter hyperintensities

Pooled estimates and I^2 were not calculated when fewer than two studies contributed data. Events/total represents the number of participants with the reported feature divided by the total number of participants contributing data for that outcome across the included studies; Pooled proportions were estimated using random-effects meta-analysis and may therefore differ from the crude proportion calculated from aggregated event counts.

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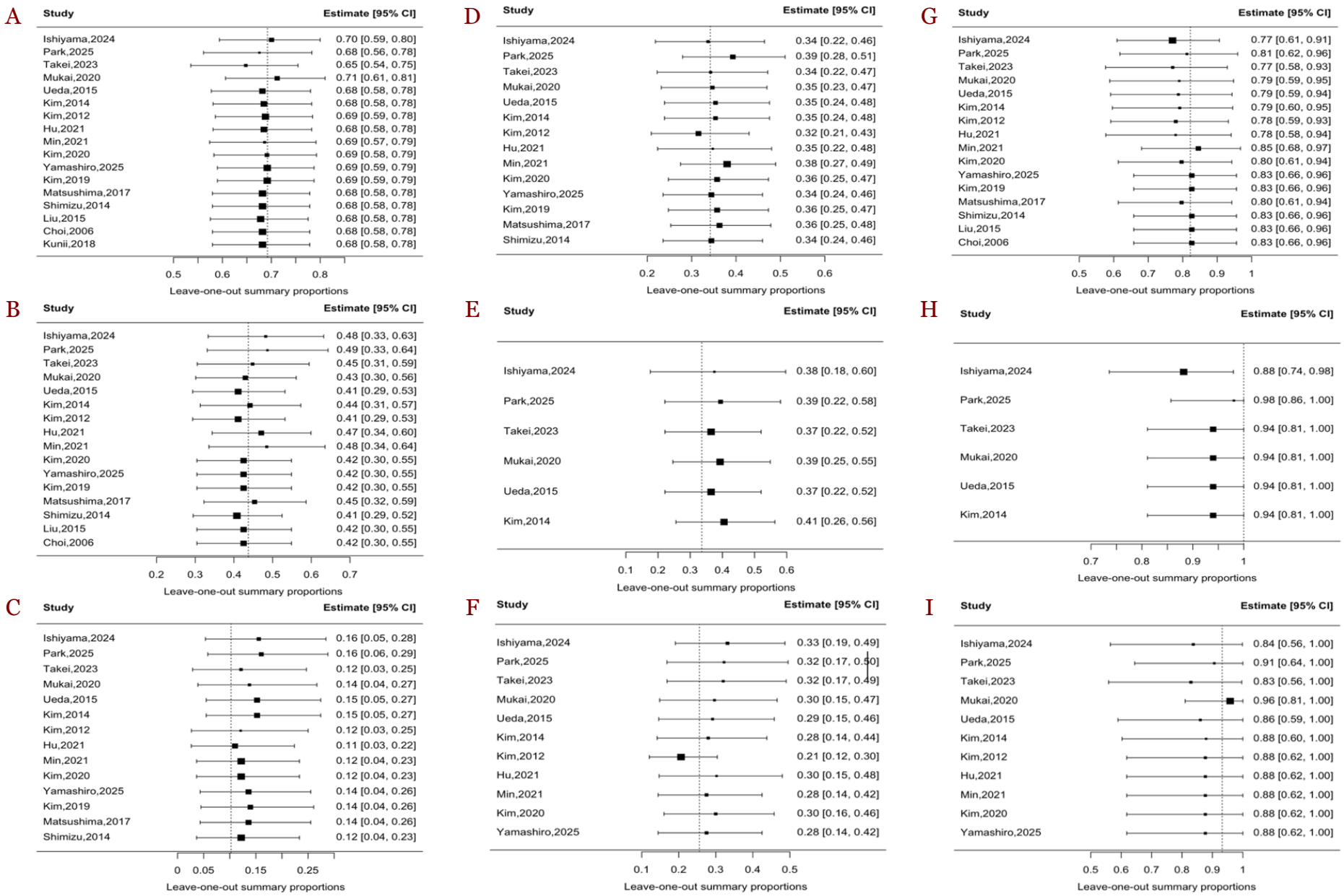


Figure 6. Leave-one-out sensitivity analysis of meta-proportion: (A) stroke, (B) cognitive impairment, (C) psychiatric symptoms, (D) migraine, (E) bulbar symptoms, (F) white matter hyperintensities in the anterior temporal lobe, (G) white matter hyperintensities in the external capsule, (H) lacunes, and (I) cerebral microbleeds.

Risk of bias assessment and variant adjudication

The methodological quality of the included studies was generally acceptable across study designs (**Table 7**). Case reports and case series fulfilled most of the JBI appraisal criteria, with minor concerns related to incomplete patient history descriptions. Cross-sectional studies exhibited several methodological limitations, primarily due to the lack of clearly reported strategies to address confounding factors. Cohort studies generally met key methodological criteria, although some concerns remained regarding confounding and exposure assessment. Follow-up-related domains were frequently not applicable depending on the study contexts.

Table 7. Risk of bias assessment using the Joanna Briggs Institute critical appraisal checklists

Author, year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
Hee-Jin Kim *et al.* (2012) [25]	Y	N	Y	Y	N/A	N/A	N/A	Y	–	–	–
Yamashiro *et al.* (2025) [35]	Y	Y	Y	Y	Y	Y	N	Y	–	–	–
Shimizu *et al.* (2014) [29]	Y	Y	Y	Y	Y	Y	Y	Y	–	–	–
Jung *et al.* (2022) [33]	Y	N	Y	Y	N/A	N/A	N/A	Y	–	–	–
Tachiyama *et al.* (2018) [34]	Y	Y	Y	Y	Y	Y	U	Y	–	–	–
Kunii *et al.* (2018) [32]	Y	Y	Y	N	Y	Y	Y	N/A	Y	N/A	–
Choi *et al.* (2006) [31]	U	Y	Y	Y	Y	Y	Y	N/A	Y	N/A	–
Takei *et al.* (2023) [23]	Y	Y	Y	Y	Y	N	Y	Y	–	–	–
Ueda *et al.* (2015) [4]	Y	Y	Y	Y	Y	N	Y	Y	–	–	–
Kim *et al.* (2014) [26]	N	Y	Y	Y	Y	N	Y	Y	–	–	–
Hu *et al.* (2021) [3]	Y	Y	Y	Y	Y	N	Y	Y	–	–	–
Min *et al.* (2021) [27]	Y	Y	Y	Y	Y	Y	Y	Y	–	–	–
Liu *et al.* (2015) [30]	Y	Y	Y	Y	N	N	Y	U	–	–	–
Kim *et al.* (2020) [6]	Y	Y	Y	Y	Y	Y	Y	Y	–	–	–
Kim *et al.* (2019) [36]	Y	Y	Y	Y	Y	N	Y	Y	–	–	–
Matsushima *et al.* (2017) [28]	Y	Y	Y	Y	Y	N	Y	Y	–	–	–
Ishiyama *et al.* (2024) [13]	Y	Y	Y	Y	Y	U	Y	N/A	N/A	N/A	Y
Park *et al.* (2025) [22]	Y	Y	Y	Y	N	Y	Y	Y	N/A	N/A	Y
Mukai *et al.* (2020) [24]	Y	Y	Y	Y	Y	Y	Y	N/A	N/A	N/A	Y

N/A: not applicable; N: no; U: unclear; Y: yes

Q1-Q11 represent the items of the Joanna Briggs Institute (JBI) critical appraisal checklists. For case reports: Q1, patient's demographic characteristics; Q2, patient's history; Q3, current clinical condition of the patient; Q4, diagnostic tests or assessment methods; Q5, intervention(s) or treatment procedure(s); Q6, post-intervention clinical condition; Q7, adverse events or unanticipated events; Q8, takeaway lessons. For case series: Q1, criteria for inclusion; Q2, standard/reliable condition measurement; Q3, valid condition identification; Q4, consecutive inclusion; Q5, complete inclusion; Q6, participant demographics; Q7, clinical information; Q8, outcome/follow-up reporting; Q9, presenting site/clinic information; Q10, statistical analysis. For cross-sectional: Q1, inclusion criteria; Q2, study subjects and setting; Q3, valid and reliable exposure measurement; Q4, objective and standard condition measurement; Q5, confounding factors; Q6, strategies for addressing confounding; Q7, valid and reliable outcome measurement; Q8, statistical analysis. For cohort: Q1, group similarity and recruitment; Q2, similar exposure measurement for exposed and unexposed group; Q3, valid and reliable exposure measurement; Q4, identification of confounding factors; Q5, strategies for addressing confounding; Q6, absence of the outcome at study entry/exposure; Q7, valid and reliable outcome measurement; Q8, follow-up duration and adequacy; Q9, completeness/reasons for loss to follow-up; Q10, strategies for incomplete follow-up; Q11, statistical analysis.

Discussion

This systematic synthesis of 19 studies, encompassing p.R75P variant carriers from East Asian cohorts (primarily Japan and South Korea, with a smaller representation from China), illustrates that the p.R75P variant replicates the core clinical, neuroimaging, and genetic characteristics traditionally associated with CADASIL. The compiled dataset indicates a mid-adult onset (weighted mean age ≈57 years), a balanced sex distribution, and a strong familial aggregation (52% of carriers), which aligns with the autosomal-dominant transmission observed in canonical *NOTCH3* disease [4,38,39]. Vascular risk factors, particularly hypertension and dyslipidemia, were prevalent across cohorts, whereas diabetes and smoking were inconsistently reported. This mixed risk-factor profile reflects observations in other CADASIL series and East Asian clinical registries, where vascular comorbidity influences phenotype expression [5,8,40].

In the present synthesis, individuals carrying the *NOTCH3* p.R75P variant most frequently had ischemic stroke, with cognitive impairment, psychiatric symptoms, and migraine also reported, as well as occasional bulbar manifestations. Overall, this clinical profile broadly resembles the symptom spectrum described for classical cysteine-altering *NOTCH3* mutations in

established CADASIL cohorts [4,6,9,38]. The reported neuroimaging profile was also characterized by frequent involvement of the EC, along with frequent reports of lacunar infarcts and CMBs, recapitulating several core radiological features of CADASIL [4,6,41-43]. However, consistent with emerging East Asian data, ATL involvement appears to be less frequently reported in p.R75P than in conventional cysteine-altering mutations, suggesting a potentially distinct neuroimaging pattern [6,13].

Notably, CMBs and symptomatic ICH were frequently reported. In addition, in the comparative analysis, p.R75P was also associated with higher frequencies of symptomatic ICH than typical cysteine-altering variants. Although the wide confidence interval warrants cautious interpretation, these findings are consistent with the previously proposed "pro-hemorrhagic CADASIL" phenotype associated with p.R75P, a phenotype particularly emphasized in recent regional studies [13]. Together, these findings position p.R75P as a CADASIL-related arteriopathy with both shared and variant-specific features relative to canonical cysteine-altering *NOTCH3* mutations.

Comparison with cysteine-altering *NOTCH3* mutations shows that the p.R75P variant shares many core CADASIL features yet also displays notable clinical and imaging differences. Western CADASIL cohorts, largely composed of cysteine-altering missense variants concentrated in EGFr domains encoded by exons 3–6, typically present with earlier symptom onset, marked ATL and EC involvement, and widespread cognitive and psychiatric disturbances. These characteristic patterns underpin the high diagnostic accuracy of MRI-based criteria used in European and North American settings [7,44,45]. By contrast, East Asian studies examining the recurrent cysteine-sparing p.R75P substitution describe a somewhat modified phenotype. Although p.R75P carriers manifest the fundamental hallmarks of CADASIL, particularly ischemic events, cognitive decline, psychiatric symptoms, and nearly universal EC lesions, ATL involvement is less common, resulting in lower sensitivity of traditional temporopolar-focused diagnostic markers in Japanese and Korean cohorts [6,11,13]. Consistent with broader Asian CADASIL registries, p.R75P has been associated with a high frequency of CMBs and an increased risk of ICH compared with Western cohorts [4,6,22,26]. Overall, these data indicate that p.R75P is associated with the fundamental CADASIL phenotype while also displaying region-specific radiological features and notable hemorrhagic manifestations in East Asian populations.

Multiple lines of genetic and pathological evidence provide substantial support for the pathogenic relevance of p.R75P. Predominantly, East Asian studies have utilized comprehensive (33-exon) *NOTCH3* sequencing to exclude canonical cysteine-altering variants and have documented the familial co-segregation of p.R75P with the phenotype across several pedigrees [4,38,39]. Notably, several independent studies have demonstrated the deposition of the vascular N3ECD and GOM on electron microscopy in skin or vessel biopsies from p.R75P carriers, findings that are characteristic of the disease and have been employed as pathological evidence in the diagnosis of CADASIL [1,4,38,39,46]. The convergence of the absence (or extreme rarity) of p.R75P in population databases, its co-segregation, and GOM/N3ECD positivity provides substantial evidence supporting its pathogenic relevance and warrants formal reassessment of its current classification [7,9,38].

The mechanistic plausibility of the pathogenicity associated with a cysteine-sparing substitution, such as p.R75P, can be considered in the context of contemporary molecular models of *NOTCH3* dysfunction. The pathogenesis of CADASIL is characterized by the abnormal accumulation of N3ECD and the subsequent formation of GOM in mural cells, which leads to the degeneration of small-vessel walls and microcirculatory dysfunction [39,41,46]. In the case of p.R75P, the variant retains a normal cysteine count but is located within an EGFr region prone to aggregation. Given the structural properties of proline, substitution at position 75 could plausibly induce local backbone distortion in the EGFr, potentially disrupting folding, domain stability, and proteolytic processing. Consequently, the receptor may misfold and aggregate extracellularly, despite unchanged cysteine counts, because the adjacent residue (position 76) is a cysteine. This is similar to that seen with classical pathogenic variants [41,47]. However, these mechanistic consequences have not been directly demonstrated experimentally for p.R75P and should therefore be regarded as a mechanistic hypothesis rather than an established mechanism. Similar cysteine-sparing hypomorphic mutations (e.g., p.A1604T) have been experimentally

demonstrated to disrupt receptor trafficking and function [42,43,48], providing indirect support for the possibility that cysteine-sparing substitutions can disrupt *NOTCH3* function through mechanisms beyond cysteine alteration. These findings support the hypothesis that p.R75P may induce a toxic gain-of-aggregation and/or altered receptor biology, culminating in CADASIL-type arteriopathy [41,46,47].

The pronounced geographic concentration of the p.R75P variant in Japan and Korea indicates a potential population-specific enrichment or founder effect. Several cohort studies have consistently identified the Arg75Pro mutation in East Asian lineages, while it remains relatively absent in non-Asian cohorts and population controls, as previously documented in regional *NOTCH3* genotype surveys [4,13,38,40]. This epidemiological pattern has significant diagnostic implications, suggesting that targeted testing should prioritize p.R75P screening in East Asian patients presenting with CADASIL-compatible MRI findings. However, comprehensive sequencing is still required to rule out trans-acting or compound variants [6,9,11].

The results of this synthesis hold both immediate clinical significance and broader implications for the genetic interpretation of *NOTCH3* variants. Clinically, the near-universal presence of EC, lacunes, and CMBs coupled with the notable prevalence of clinical manifestations provides substantial evidence that *NOTCH3* p.R75P is unlikely to represent a benign polymorphism and supports its pathogenic relevance within the CADASIL spectrum. According to the ACMG/ClinGen framework, p.R75P is supported by multiple evidence categories relevant to pathogenicity, including PS4 (prevalence in affected individuals), PM2 (absence from population databases), PP1 (co-segregation with disease), and PP4 (phenotypic specificity), providing a basis for formal reassessment of its current classification in East Asian populations.

In clinical practice, genetic counseling should regard p.R75P as an autosomal-dominant CADASIL mutation characterized by variable expressivity. Diagnostic protocols in Asia should incorporate comprehensive *NOTCH3* sequencing, with a focus on exon coverage, and consider supplementary skin biopsy or electron microscopy in instances of genotype–phenotype discordance. From a research standpoint, further functional studies are necessary to elucidate the mechanisms by which the Arg75Pro substitution disrupts *NOTCH3* receptor folding or signaling while maintaining cysteine parity. Establishing population-based registries that integrate genomic, imaging, and longitudinal cognitive assessments would facilitate the refinement of penetrance estimates, the exploration of vascular risk modifiers, and a more profound understanding of disease heterogeneity and progression.

This review acknowledges several limitations. There was considerable heterogeneity among the included studies concerning design, case ascertainment, MRI acquisition parameters, and reporting detail. The relatively small sample size of the included studies limited the ability to explore potential sources of heterogeneity. While most recent studies employed full 33-exon *NOTCH3* sequencing, a minority utilized targeted exon testing, which raises the possibility of undetected coexisting variants. Not all studies conducted skin biopsy or electron microscopy, leading to variable confirmation of GOM and inconsistent pathological validation across cohorts [7,9,11]. Migraine with aura is considered a characteristic clinical manifestation of CADASIL, whereas migraine without aura occurs at a frequency comparable to that in the general population and lacks disease specificity [1-3]. However, separate analysis of migraine subtypes could not be performed because migraine with and without aura were not consistently reported separately across the included studies. In addition, the evidence base comprised primarily isolated case reports and small observational cohorts, resulting in variability in methodological quality and data completeness. However, given the potential limitations associated with case reports, sensitivity analyses restricted to observational studies were performed to assess the robustness of the present findings. As most included studies were cross-sectional or retrospective, information regarding the long-term evolution and progression of symptoms among carriers remains limited. Reporting bias is also a concern, as cases with prominent symptoms and MRI findings are more likely to be published, potentially inflating the perceived penetrance. Given the lack of widely endorsed guidelines for assessing publication bias in meta-analyses of proportions [21], potential methodological limitations were addressed through the application of the double arcsine transformation to stabilize extreme proportions and sensitivity analyses restricted to observational studies.

Conclusion

This extensive review of the *NOTCH3* p.R75P variant provides evidence that this mutation mirrors the typical CADASIL phenotype, while also exhibiting distinct region-specific characteristics. Compared with cysteine-altering variants, p.R75P is characterized by a similar clinical profile but shows a typical neuroimaging pattern, including prominent external capsule involvement, frequent lacunar infarcts and cerebral microbleeds, and a notably lower frequency of anterior temporal lobe lesions. The alignment of clinical, neuroimaging, genetic, and pathological data provides substantial evidence supporting the pathogenic relevance of p.R75P and justifies formal reassessment of its current classification according to current ACMG/ClinGen frameworks. Despite limitations due to inter-study variability and regional focus, the overall findings support consideration of *NOTCH3* p.R75P for reclassification from a variant of uncertain significance to a pathogenic or likely pathogenic mutation. Future research involving multicenter, genotype-confirmed cohorts and mechanistic studies will be crucial to confirm these results, elucidate functional impacts, and enhance diagnostic and counseling approaches for CADASIL in various populations.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools to support manuscript preparation. AI-based language models, including ChatGPT, were employed for language refinement, including improvements in grammar, sentence structure, and readability. All AI-assisted outputs were critically reviewed, verified, and revised by the authors. The scientific content, data interpretation, conclusions, and all final decisions presented in this manuscript were solely made by the authors, who take full responsibility for the integrity and accuracy of the work.

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