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# Most common NOTCH3 mutations causing CADASIL or CADASIL-like cerebral small vessel disease: A systematic review

Georgina Boston [a], Dan Jobson [a], Toshiki Mizuno [b], Masafumi Ihara [c], Raj N Kalaria [a,*]

[a] Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK
[b] Department of Neurology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[c] Department of Neurology, National Cerebral and Cardiovascular Centre, Osaka, Japan

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ABSTRACT

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a monogenic disorder caused by mutations in the *NOTCH3* gene. The main aim of our survey was to determine if there is an association between phenotypes and genotypes across the most common *NOTCH3* mutations found in CADASIL patients. We systematically searched clinical studies and genomic databases from 1996 to 2023 to first identify the most common mutations responsible for CADASIL. We found the six most common *NOTCH3* missense mutations globally were the p.R75P, p.R133C, p.R141C, p.R169C, p.R182C, and p.R544C, of which p.R133C was described to occur most often. Focusing on studies with comprehensive clinical records, our analysis further suggested that the p.R75P, p.R141C, p.R182C and p.R544C genotypes were highly congruent with the presence of white matter hyperintensities on magnetic resonance imaging (MRI), which was the most common phenotypic characteristic across all four mutations. We found the p.R141C mutation was associated with increased severity of disease. We also found the average age of onset in p.R544C carriers was more than a decade later compared to the p.R141C carriers. However, statistical analysis showed there were no overall differences between the phenotypic characteristics of the two common mutations, p.R141C and p.R544C. Geographically, China and Japan were the only two countries to report all the four common mutations vis a vis p.R75P, p.R141C, p.R182C and p.R544C. There is a possibility that this is due to a combination of a founder effect, but there also could be sampling biases.

## Introduction

Cerebral autosomal dominant arteriopathy with sub-cortical infarcts and leukoencephalopathy or CADASIL, is a monogenic hereditary form of cerebrovascular small vessel disease (cSVD), and the leading genetic cause of mostly subcortical strokes in adults [3]. Typical CADASIL prevalence is estimated to be 1.3–5 per 100,000 [42,67], although CADASIL or CADASIL-like syndromes are likely underdiagnosed in the general global population [24]. Most of the disease is caused by heterozygous mutations in the *NOTCH3* receptor-encoding gene, which contains 7Kb shared by 33 exons and located at chromosome locus 19p13 [33]. Pathogenic mutations are mostly confined in exons 2–24, which code for 34 epidermal growth factor-like repeat (EGFr) domains [20]. Currently ~230 cysteine- ($NOTCH3^{cys}$) and ~140 arginine-altering mutations, which are pathogenic or likely pathogenic variants in the *NOTCH3* gene [30]. Most of these mutations are thought to influence the pathogenesis of CADASIL through one or more mechanisms [19]. There is strong clustering of the $NOTCH3^{cys}$ mutations, particularly in exons 3 and 4, corresponding with EGFr domain 1–6. These are associated with increased severity of disease although the mechanisms are not entirely clear [9,55]. Recent screening of large exome databases globally, has shown that the frequency of the archetypal cysteine altering $NOTCH3^{cys}$ mutations in the EGFr domains 7–34 is estimated to be more than 100-fold higher than expected from estimates of the typical CADASIL genotype [54]. Such discoveries have also changed the original tenet that mutations in the *NOTCH3* gene were fully penetrant to produce the disease phenotype.

The clinical onset of CADASIL typically involves migraines with aura within the second and third decades of life, followed by psychiatric disturbances including apathy, mood changes, depression, leading to cognitive impairment, subcortical dementia and premature death [3]. Diagnosis, however, mostly coincides with the onset of stroke, with

* Corresponding author: Translational and Clinical Research Institute, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, UK.
*E-mail address:* raj.kalaria@newcastle.ac.uk (R.N. Kalaria).

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motor disability and dementia between the fourth and sixth decades (Fig. 1). With the exception of some rare occurrences, CADASIL has previously been observed in pre-symptomatic mutation-carrying individuals as increased nodular T2-weighted magnetic resonance imaging (MRI) signal in perivascular areas, which later becomes increasingly diffuse extending to the external capsule and anterior temporal lobes as well as the thalamic regions as the disease progresses [3]. Less commonly, seizures are reported in 10 % of CADASIL cases, and in some as vascular Parkinsonism [10,13,63]. CADASIL has a wide phenotypic spectrum across different *NOTCH3* mutations, yet how the precise molecular mechanisms or associated influencing factors precipitate or modify the disease are not clear.

The NOTCH3 receptor is comprised of an extracellular domain (N3ECD), transmembrane domain (N3TMD), and intracellular domain (N3ICD), which is expressed in vascular smooth muscle cells (VSMC) and pericytes of small arteries [53]. The mechanism of the functioning NOTCH3 receptor is highly conserved from Drosophila, with studies reporting its involvement in vascular development and VSMC remodelling via proteolytic cleavage of the receptor, and subsequent release of the N3ECD and N3ICD [58]. Interestingly, using validated cSVD imaging markers and clinical outcomes, Hack et al. [15] have recently identified three clinically distinct NOTCH3-SVD EGFr risk categories and three high risk-EGFr domains located outside of the EGFr domains 1–6. It is expected that such EGFr risk classifications will provide important criteria for individualised disease prediction. In mutant *NOTCH3* carriers, N3ECD accumulates in the interstitial space, and is thought to have high affinity for molecules in alternative signalling pathways, disrupting vascular homeostasis, and leading to the production of granular osmiophilic material (GOM). GOM has been identified in a variety of systems and is widely found in skin biopsies of CADASIL patients, allowing it to be used as a diagnostic tool for CADASIL [61]. Currently, it is unclear whether GOM deposits are simply the result of vascular degeneration, or whether GOM itself exhibits pathological effects on vessel homeostasis. More recent studies investigating molecular mechanisms of CADASIL have pointed towards evidence of endoplasmic reticulum stress, nuclear abnormalities and inflammatory processes [8,49,68], as well as defective autophagosome-lysosome fusion in CADASIL patients [16]. Other studies have investigated the role of endothelial damage/repair markers and astrocyte damage within CADASIL, reflecting the increasingly complex nature of CADASIL pathophysiology [17,51].

We conducted a systematic review of the global literature and searched genomic databases from 1996 until 2023, to determine 1) the most common *NOTCH3* mutations given that genetically characterised CADASIL cases were also earlier described in France, Germany, Italy, Sweden and Finland, and 2) whether there are strong associations between the most common *NOTCH3* mutations and specific phenotypic and demographic characteristics of CADASIL patients.

### *Search methods and screening*

In the global published literature, there are numerous reports on CADASIL associated mutations in the *NOTCH3* gene. Using specific search terms (Table 1), we identified clinical studies from journals and databases such as PubMed, as well as through snowball sampling of relevant literature (Fig. 2). We followed a systematic approach to screen clinical databases and genomic databases from January 1996 to December 2023 to determine the most common mutations. From this

**Table 1**
Search terms used to define each characteristic investigated within the study.

| Characteristic | Alternative terms |
|---|---|
| Stroke | 'Ischaemic attack', 'transient ischaemic attack', 'TIA', 'mini stroke', 'cerebral haemorrhage', 'cerebral infarction'. |
| Migraine | 'Migraine with aura', 'migraine without aura', 'headache' unless specifically stated as not a migraine. |
| Dementia | 'Cognitive impairment', 'memory impairment', 'dementia'. |
| Psychiatric disturbance | 'Mental disorder' or any specific psychiatric disturbance listed in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[(2)]. |
| Bulbar symptoms | 'Bulbar palsy', 'pseudobulbar palsy', 'dysarthria', 'dysphagia', 'dysphonia'. |
| Gait disturbance | 'Difficulty walking'. |
| Motor abnormality | 'hemiparesis', 'hemiplegia', 'motor palsy', 'dyskinesia'. |
| White matter hyperintensities | 'WMH'. |
| Vascular risk factors | 'Hypertension', 'diabetes', 'diabetes mellitus', 'hyperlipidaemia', 'dyslipidaemia', 'smoking', 'alcohol consumption', 'hyperthyroidism', 'cerebral microbleeds'. |

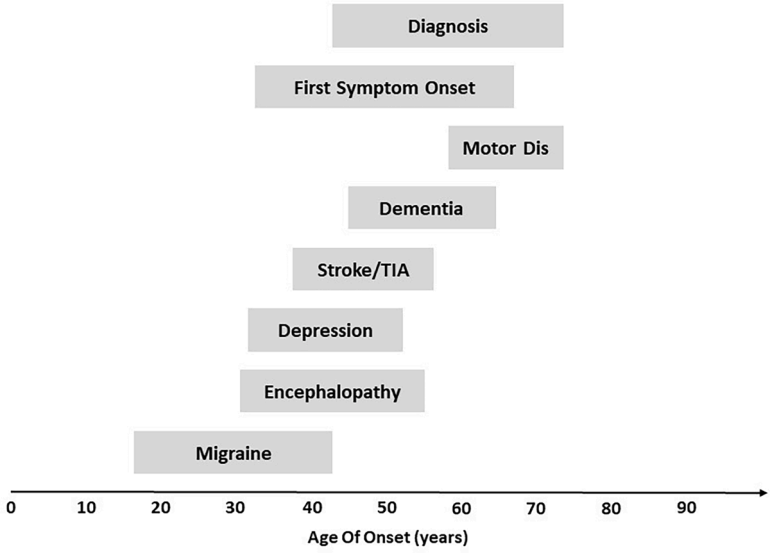


**Fig. 1.** Migraine is typically the first symptom associated with CADASIL, occurring at a mean age of 29 (±13.1), followed by depression and encephalopathy at a mean age of 41 (±10.2) [8,9]. Diagnosis of CADASIL occurs at a mean age of 57.8 (±14.7), with the onset of stroke/transient ischaemic attack (TIA) at a mean age of 47 (±9.5), dementia at a mean age of 54.6 (±9.7), and motor disability (MD) at a mean age of 60.5 (±2.5) [10,11]. Data derived from several references [1,2,13,46,60]. Plot adapted from Chabriat et al. [3].

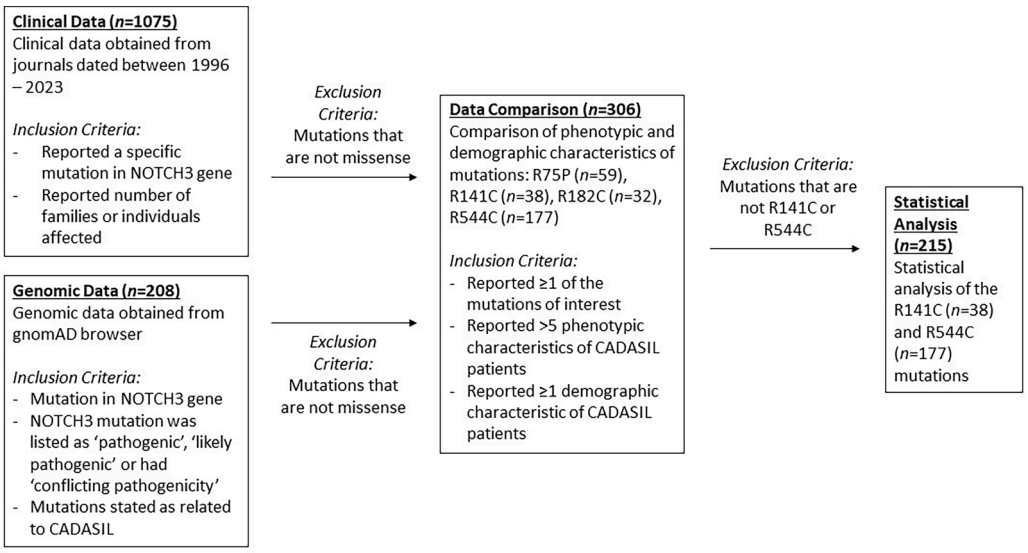


**Fig. 2.** Flow chart showing the numbers of cases evaluated within this report. Clinical data show the total number of publications and reports in public databases and the genomic data show the total number of reports found in the GnomAD database until December 2023. A total of 62 studies were reported on the mutations of interest: p.R75P, p.R141C, p.R182C and p.R544C. These all reported more than five desired phenotypic/demographic characteristics and clinical symptoms of mutation carriers.

original screening, we noted all the mutations mentioned across more than 60 relevant CADASIL clinical studies incorporating epidemiological or frequency data. Non-probability sampling was used due to CADASIL patients representing under-researched populations.

### *Type of NOTCH3 mutations*

*NOTCH3* mutations described in at least 20 families carrying one of any *NOTCH3* pathogenic variants were collected for the strict purpose of determining the most common missense mutations which result in CADASIL disease or pathology [30]. Although numerous variants and insertion, deletion and nonsense *NOTCH3* mutations are present in small proportions of CADASIL patients, these were excluded to focus purely on missense *NOTCH3* mutations we could find from clinical studies reported between 1996 and 2023 (Fig. 2). In addition, we searched genomic data from The Genome Aggregation Database, v2.1.1 (https://gnomad.broadinstitute.org/) to find such missense mutations.

For both above sources, data were included if a mutation in the *NOTCH3* gene was indicated to be 'pathogenic', 'likely pathogenic', or had 'conflicting pathogenicity', and there was evidence that they exhibited a relation to CADASIL. Studies were included if they indicated a mutation in the *NOTCH3* gene, as well as denoted the number of families or individuals affected. If the number of families were given in a study rather than the number of individuals, the number of individuals were assumed to be equal to the number of families given, as one family must contain at least one individual. This allowed us to formulate a running order of the 12 most frequently reported mutations in clinical studies (Table 2).

**Table 2**
The 12 most frequently reported *NOTCH3* mutations.

| EGFr Domain | Amino Acid Substitution (EGFr 1 → 34) | *NOTCH3* Nucleotide Substitution (DNA change) | No. Families | No. Patients | Rank No. (by cases) |
|---|---|---|---|---|---|
| **1** | **p.R75P** | **c.224G>C** | **45** | **58** | **5** |
| **2** | **p.R90C** | **c.268C>T** | **31** | **42** | **6** |
| 2 | p.R110C | c.328C>T | 24 | 23 | 10 |
| **3** | **p.R133C** | **c.397C>T** | **247** | **256** | **1** |
| **3** | **p.R141C** | **c.421C>T** | **94** | **131** | **2** |
| 3 | p.R153C | c.457C>T | 32 | 42 | 7 |
| **4** | **p.R169C** | **c.505C>T** | **64** | **72** | **4** |
| **4** | **p.R182C** | **c.544C>T** | **69** | **80** | **3** |
| 5 | p.R207C | c.619C>T | 21 | 13 | 12 |
| 8 | p.R332C | c.994C>T | 28 | 29 | 9 |
| 14 | p.R544C | c.1630C>T | 36 | 36 | 8 |
| 15 | p.607C | c.1819C>T | 22 | 19 | 11 |

Bold rows show the six most frequent mutations of which four were investigated further in relation to phenotypic characteristics or having more than five symptoms and vascular risk factors.

### *Clinical data collection and statistics*

Our aim was also to determine if there is an association between characteristics of CADASIL patients and most common *NOTCH3* gene mutations. Thus, we searched for studies with inclusion criteria incorporating complete information on the demographic and phenotypic characteristics of patients carrying specifically the topmost common mutations diagnosed with CADASIL. Three demographic characteristics: average age of onset (AAO), gender ( % male), and country, and nine phenotypic characteristics including apparent or overt stroke, migraine, cognitive impairment or dementia, psychiatric disturbance, bulbar symptoms, gait disturbance or motor abnormality, white matter hyperintensities (WMH) and vascular risk factors, were determined to be important. Using these as the strict inclusion criteria (Fig. 2) and searching for one of the four most common mutations (Table 3), we extracted details from a total of 33 studies (Tables 3 and 4). This meant that even though in some studies up to 200 p.R133C mutations were described [37], we did not include such studies in subsequent analysis because they were from national screening centres and reported incomplete information on the target demographic and phenotypic characteristics.

Due to variation across studies, a few assumptions were made: first, if data for a characteristic were not available from a study, the characteristic was listed as 'Not Reported' or 'NR'. Second, if a symptom was investigated within the study, but not reported in an individual, the individual was assumed to be negative for that symptom. Third, if the age of onset was not given in the study, age of onset was defined as the age at commencement of investigation. Similarly, due to the variation of terms used to describe characteristics across studies, broad symptom categories were formed. For the purpose of the study, phenotypic

**Table 3**
Percentage of individuals, carrying one of the four most common *NOTCH3* mutations in different countries.

| Mutation | Study | n | AAO (years) | Gender ( % Male) | Country | Stroke ( %) | Migraine ( %) | CI or Dementia ( %) | Psychiatric Disturbance ( %) | Bulbar Symptoms ( %) | Gait Disturbance ( %) | Motor Abnormality ( %) | WMH ( %) | Vascular Risk Factors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Arg 75 Pro (p. R75P) | Kim et al [26]. | 6 | 55.7 | 33.3 | Korea | 50 | 16.7 | 66.7 | 0 | NR | NR | NR | 83.3 | HT (33.3 %), DM (33.3 %), DL (16.7 %) |
| | Kim et al. [25] | 5 | 52.6 | 40 | Korea | 80 | 0 | NR | 0 | NR | NR | NR | 100 | DL (60 %), HT (40 %) |
| | Matsushima et al. [34] | 1 | 76.0 | 0 | Japan | 0 | 0 | 100 | 0 | 0 | NR | 0 | 100 | None |
| | Okada et al. [44] | 3 | 51.3 | NR | Japan | 0 | 33.3 | 33.3 | NR | 66.7 | NR | NR | 100 | HT (3.3 %), Cerebral Microbleeds (66.7), DL (100 %) |
| | Mizuno et al. [36] | 4 | 48.3 | 0 | Japan | 25 | 50 | 0 | 25 | 50 | 50 | 75 | 75 | HT (25 %), DM (25 %) |
| | Wang et al. [33] | 1 | 34.0 | 100 | China | 100 | 0 | 0 | 100 | NR | NR | NR | 100 | NR |
| | Mukai et al. [39, 40] | 14 | 53.6 | 42.9 | Japan | 42.9 | 42.9 | 53.8 | 28.6 | 35.7 | NR | 30.8 | 100 | Smoking (30.8 %), DL (23.0 %), DM (5.7 %) |
| | Takei et al. [59] | 24 | 55.1 | 84.6 | Japan | 79.2** | 26.1 | 45.8 | 8.3 | 39.1 | NR | 12.5 | 100 | HT (39.1 %), DL (39.1 %), DM (17.4 %) |
| | Jung et al. [23] | 1 | 55.0 | 100 | Korea | 100 | 100 | 0 | NR | NR | 100 | 100 | 100 | NR |
| Arg 141 Cys (p. R141C) | Gurumukhani et al. [14] | 1 | 46.0 | 0 | India | 0 | 100 | 100 | NR | 100 | 100 | 100 | 100 | None |
| | Stevens et al. [57] | 4 | 48.0 | 75 | UK | NR | 0 | 100 | 100 | 100 | NR | NR | 100 | NR |
| | Yi-Chung Lee et al. [29] | 1 | 41.0 | 100 | China | 100 | 0 | 0 | 0 | NR | NR | NR | 100 | NR |
| | Mukai et al. [40] | 18 | 49.5 | 55.6 | Japan | 83.3 | 50 | 38.9 | 27.8 | 16.7 | NR | 61.1 | 100 | Smoking (55.6 %), DM (5.6 %), DL (22.2 %) |
| | Yadav et al. [65]* | 4 | 54.5 | 100 | India | 25 | 100 | 100 | 75 | NR | 50 | 50 | 25 | HT 20 %) |
| | Murakami *et al.* [41] | 4 | 44.8 | 25 | Japan | 50 | 25 | 50 | 25 | 100 | 25 | 25 | 25 | NR |
| | Onder et al. [45] | 1 | 43.0 | 0 | Turkey | 0 | 100 | 100 | NR | 100 | NR | 100 | 100 | HT (100 %), Hyper-thyroidism (100 %) |
| | Lee et al. [28] | 1 | 41.0 | 100 | Taiwan | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | NR |
| | Matsuhima *et al.* [34] | 1 | 52.0 | 0 | Japan | 100 | 100 | 100 | 100 | 0 | NR | 0 | 100 | NR |
| | Sathe et al. [56] | 2 | 28.5 | 0 | USA | 50 | 100 | 50 | 100 | NR | NR | NR | 100 | NR |
| | Gorukmez et al. [11] | 1 | 47 | 0 | Turkey | 0 | 100 | 0 | 0 | NR | NR | NR | 100 | NR |
| Arg 182 Cys (p. R182C) | Wang et al. [64] | 5 | 41.8 | 60 | China | 20 | 20 | 60 | 20 | NR | NR | NR | 100 | NR |
| | Paraskevas *et al.* [50] | 2 | 18.0 | 0 | Greece | 0 | 100 | 0 | 100 | NR | NR | 50 | 50 | NR |
| | Guo et al. [12] | 1 | 50.0 | 0 | China | 0 | 100 | 100 | 0 | NR | 100 | NR | 100 | NR |
| | Gustavsen et al. (2003) [45] | 9 | 43.6 | 77.8 | Norway | 33.3 | 11.1 | 22.2 | 33.3 | 22.2 | 22.2 | 33.3 | NR | NR |
| | Mukai et al. [40] | 14 | 47.3 | 57.1 | Japan | 50 | 50 | 61.5 | 15.4 | 15.4 | NR | 38.5 | 100 | Smoking (46.2 %), DL (23.1) |
| | Joutel et al. [21] | 1 | 55.0 | 100 | USA | 100 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | NR |
| Arg 544 Cys (p. R544C) | Min et al. [35] | 29 | 55.1 | 51.7 | Korea | 55.2 | 17.2 | 65.5 | 20.7 | NR | NR | NR | 95 | HT (24.1 %), DM (13.8 %), Smoking (6.9 %), DL (13.8 %) |
| | Choi et al. [5] | 53 | 59.0 | 54.7 | Korea (Jeju Island) | 41.5 | 8 | 1.9 | NR | NR | NR | NR | 75 | HT (73.6 %), DM (13.2 %), Smoking (32.1 %), HC (24.55 %), Alcohol Consumption (24.5 %) |
| | Liao et al. [31] | 79 | 56.9 | NR | Taiwan | 73.4 | 3.8 | 48.1 | 15.2 | NR | 17.7 | NR | 83.6 | NR |
| | Kim et al. [26] | 7 | 57.1 | 14.3 | Korea | 85.7 | 14.3 | 28.6 | 14.3 | NR | NR | NR | 71.4 | HT (57.1 %), DM (14.3 %), DL (28.6 %) |
| | Mukai et al. [39] | 1 | 44 | 100 | Japan | 100 | NR | 0 | NR | NR | NR | 100 | 100 | DL (100 %) |
| | Guo et al. [12] | 7 | 65.1 | 71.4 | China | 14.3 | 14.3 | 100 | 100 | NR | 14.3 | NR | 80 | NR |
| | Pan et al. [48] | 1 | 54 | 0 | China | 100 | NR | 100 | 0 | NR | NR | 100 | 100 | NR |

Phenotypic characteristics of CADASIL assessed were stroke, migraine, cognitive impairment (CI)/dementia, psychiatric disturbance, bulbar symptoms, gait disturbance or motor abnormality and white matter hyper-intensity (WMH), as well as the demographic characteristics including average age of onset (AAO), gender ( % male) and country. n means number of cases reported. Vascular risk factors included hypertension (HT), dyslipidaemia (DL), diabetes (DM) and hypercholesterolaemia (HC). If a symptom was investigated in the study, but not reported (NR) in an individual, it was assumed the individual was negative for that symptom. If individual ages were reported, the mean is given; If age of onset was not given, age was defined as age at investigation. *No data were given for 1/5 patients, so the individual was excluded from statistical analysis.

**Table 4**
Average age of onset, gender and main features across four most common mutations.

| Mutation | p.R75P ($n = 59$) | p.R141C ($n = 38$) | p.R182C ($n = 32$) | p.R544C ($n = 177$) |
|---|---|---|---|---|
| Average age of onset, years (SE) | 54 (4) | 45 (2) | 43 (5) | 56 (2) |
| Gender, % Male | 50 (14) | 41 (13) | 49 (16) | 49 (15) |
| Country ( %) | Korea: 20<br>Japan: 78<br>China: 2 | India: 13<br>UK: 10<br>China: 3<br>Japan: 61<br>Turkey: 5<br>Taiwan: 3<br>USA: 5 | China: 19<br>Greece: 6<br>Norway: 28<br>Japan: 44<br>USA: 3 | Korea: 50<br>Taiwan: 45<br>Japan: 1<br>China: 4 |
| Stroke, % (SE) | 53 (13) | 51 (13) | 34 (15) | 67 (12) |
| Migraine % (SE) | 30 (11) | 61 (14) | 64 (17) | 12 (3) |
| Cognitive Impairment/ Dementia, % (SE) | 38 (13) | 58 (13) | 41 (16) | 49 (16) |
| Mood / Behavioural Disturbances, % (SE) | 23 (14) | 48 (15) | 28 (15) | 30 (18) |
| Bulbar Symptoms, % (SE) | 38 (11) | 59 (19) | 13 (6) | 0 (0) |
| Movement Disorder† (gait, motor abnormalities), % (SE) | 41 (18) | 56 (16) | 63 (15) | 58 (24) |
| White Matter Hyperintensities, % (SE) | 95 (3) | 86 (19) | 90 (10) | 86 (5) |

Numbers represent average or mean % (standard error, SE) of mutation carriers reported to exhibit symptoms or risk factors. †Gait disturbance and motor abnormality frequencies were combined to categorise as movement disorder (cf. Table 3).

characteristics were defined as appropriate in Table 1 and all of the following statistical analysis was carried out using Minitab Software (https://www.minitab.com/).

The mean (m) % of patients experiencing each characteristic ($m = \sum x/n$), and the sample standard deviation (s), SD, $\left(s = \sqrt{\sum (x_i - m)^2/N}\right)$ were determined for four of the most common *NOTCH3* mutations. An independent *t*-test was carried out to determine whether there was a statistical difference between the p.R141C ($n = 38$) and p.R544C ($n = 177$) mutations across the following characteristics: Average age of onset (AOO), gender and stroke (https://statistics.laerd.com/). Independent *t*-tests were selected for this normally distributed data, which had no significant outliers and homogeneity of variances were determined using Levene's test.

The Mann-Whitney U-Test was carried out for % of individuals experiencing migraine that did not display homogeneity of variances, as well as for the % of individuals experiencing dementia, psychiatric disturbances, and WMH data sets, which did not follow a normal distribution. An alpha value of 0.05 was selected as the threshold of significance and probability of a type I error for all statistical tests performed within this study.

### *Ethical considerations*

No ethical considerations were required for this study, as the data collected in this systematic review were previously published and available in the public domain. The data collected in this study were anonymised and ethically considered by the primary data collectors, therefore, there are no issues with confidentiality, anonymity, informed consent or potential for harm.

## Results

After applying strict inclusion criteria, we found 1075 clinical studies and 208 cases from the genomic databases (Fig. 2). Using this strategy, the top six frequently reported *NOTCH3* mutations responsible for the pathophysiology of CADASIL were found to be as follows: *Arg 133 Cys* (p.R133C), *Arg 141 Cys* (p.R141C), *Arg 182 Cys* (p.R182C), *Arg 169 Cys* (p.R169C), *Arg 75 Pro* (p.R75P) and *Arg 544 Cys* (p.R544C) (Fig. 3, Table 2). Amongst these the p.R133C mutation was found to be the most common mutation exhibited in CADASIL patients worldwide, reported in a total of 263 individuals, of which only 7 carriers were found in the genomic database GnomAD. This was followed by the p.R141C mutation, which was present in 131 patients, with 4 listed in the genomic database (Fig. 3). We found the p.R544C mutation as the third most common, apparent in at least 36 individuals described in the clinical studies but the majority ($n = 83$) in the genomic database (Fig. 3). The remaining mutations p.R75P, p.R169C and p.R182C were apparent in a total of 59, 78 and 84 carriers respectively, of which only a small number were reported in the GnomAD database – 1, 6 and 4, respectively.

### *Phenotypic characteristics of NOTCH3 mutations*

Next, we selected the four of the six most common mutations for further analysis on the basis that studies were selected where the phenotypic characteristics as well as more than 5 clinical symptoms including WMH were reported (Table 3 and Supplementary Fig. 1). These were the p.R75P, p.R141C, p.R182C and p.R544C identified in both clinical studies and genomic databases involving 33 studies and 306 cases (Table 3). The p.R75P was particularly explored due to its unique nature as a cysteine sparing, arginine involved *NOTCH3* mutation but still proved as a pathogenic variant. The most common phenotypic characteristic across all four of the investigated mutations was evidence of WMH, which was reported in 95 % of p.R75P, 86 % of p.R141C, 90 % of p.R182C and 86 % of p.R544C the CADASIL cases. This was an important characteristic but surprisingly not entirely classified even in studies with large cases such as the Finnish cohort, with clearly evident founder effect [37].

The p.R141C mutation was the only mutation investigated in which all the phenotypic characteristics were present in greater than 50 % of the p.R141C population (Table 4). In contrast, overt stroke was reported in only 34 % of the p.R182C carriers with the highest frequency in p.R544C genotype carriers.

An important characteristic of CADASIL at least in the early stages is migraine (Fig. 1). In our survey, we searched for CADASIL cases which presented with migraine with aura as well as those without aura. The incidence of migraine varied considerably within geographical regions and up to 100 % in some studies (Table 3). Compared to the p.R75P and p.R544C mutation carriers, reported frequencies of migraine were high in the p.R141C and p.R182C population i.e. 30 % and 12 % versus 61–64 %, respectively (Tables 3 and 4).

Table 3 further shows that as well as the six phenotypic characteristics (stroke, migraine, cognitive impairment/dementia, psychiatric disturbance and WMH, and the four vascular risk factors (Fig. 4), there were two other phenotypic characteristics that appeared in multiple CADASIL patients as gait disturbance and motor abnormalities, which overlap in some cases. As such, gait disturbance was reported in two p.R75P carriers, four p.R141C carriers, four p.R182C carriers and fifteen p.R544C carriers. Whereas motor abnormalities were reported in seven p.R75P, sixteen p.R141C, ten p.R182C and one p.R544C carrier. However, to obtain an overall picture of movement disorder in CADASIL, we combined these frequencies and found that at least 40 % of the four most common mutation carriers exhibited either gait or motor impediments (Table 4).

Regarding vascular risk factors, there was substantial variation found between mutation carriers and risk factors (Fig. 4). The p.R182C individuals were the only group not reporting hypertension or diabetes. Other vascular risk factors observed in clinical studies, but not included here were cerebral microbleeds in two p.R75P carriers, hyperthyroidism in one p.R141C carrier, and notable alcohol consumption in thirteen p.R544C carriers (Table 3). We also noted that carriers of the p.R75P mutation had a greater tendency to acquire thalamic microbleeds

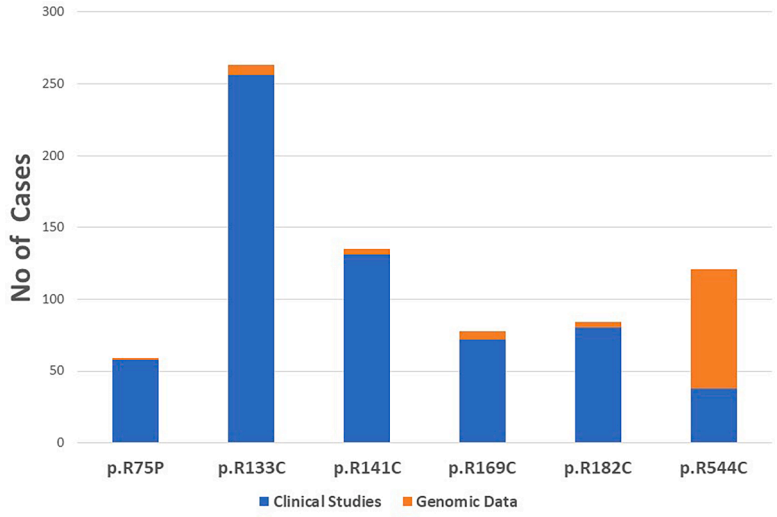


**Fig. 3.** Stacked histograms showing the number of cases of the most common six *NOTCH3* mutations reported in clinical studies and genomic databases.

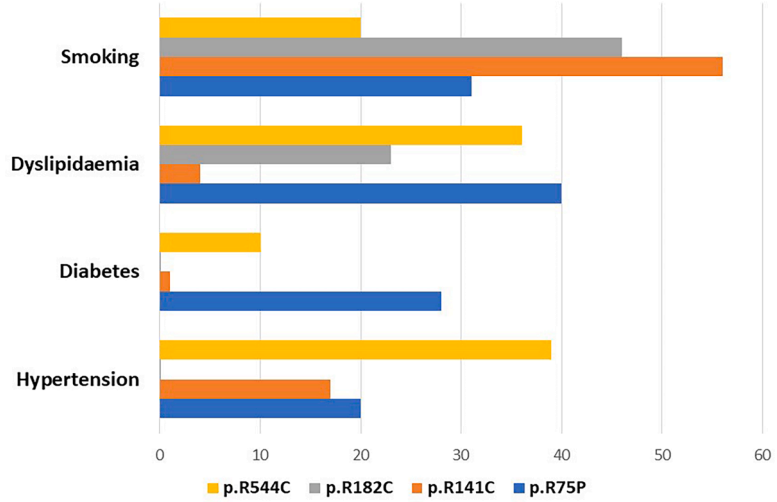


**Fig. 4.** Clustered column showing the percentage of CADASIL patients with vascular risk factors across the four common *NOTCH3* mutations e.g. p.R75P, p.R141C, p.R182C and p.R544C.

compared to those with other mutations [59].

### *Demographic characteristics and geographical distribution of NOTCH3 mutations*

The average age of onset was found to be later in life in those carrying the p.R75P and p.R544C mutations (Table 4). This was manifest almost a decade earlier on average in the p.R141C and p.R182C mutation carriers (Table 4). Both men and women almost equally exhibit these mutations although the % male figure was slightly lower in those carrying the p.R141C.

Across all top four mutations investigated, China and Japan were the only two countries reporting all four most common mutations; Japan reported the largest number of the p.R75P, p.R141C and p.R182C mutations, with 78 %, 61 % and 44 % respectively (Table 4; Fig. 5). The p.R75P mutation carriers seem to mainly reside in Okinawa and Kyushu, the southwestern part of Japan [36,62]. China, on the other hand, had a consistently low number of cases of the p.R75P, p.R141C and p.R544C mutations, with 2 %, 3 % and 4 % respectively. Given the criteria required information on ≥5 phenotypic characteristics, we found overall that three countries reported the p.R75P mutation, seven reported the p.R141C mutation, five reported the p.R182C mutation and four reported the p.R544C mutation. The p.R141C and p.R182C mutations included reports from countries that did not report any of the other *NOTCH3* mutations, including Turkey, India and the UK in the p.R141C group, and Greece and Norway in the p.R182C group.

Korea reported many cases of the p.R75P and p.R544C mutations, with 32 % and 50 % frequencies; however, there were no reported cases of the p.R141C or p.R182C mutations. USA on the other hand, reported only a small number of cases of both the p.R141C and p.R182C mutations, with 5 % and 3 %. Whereas Taiwan reported a small number of cases of the p.R141C mutation, but a significantly larger number of the p.R544C mutation at 45 % (Table 4). Also, a surprising finding was that 0.9 % of the Taiwanese population carries the *NOTCH3* p.R544C mutation. Turkey, India, UK, Greece and Norway reported cases with at least one of the four mutations (Fig. 5). Besides Japan, there also appears to be a relatively a high frequency of the p.R141C mutation in India and the UK.

### *Percentages of p.R141C and p.R544C carriers with CADASIL phenotypes*

Of the four mutations investigated, the p.R141C and p.R544C

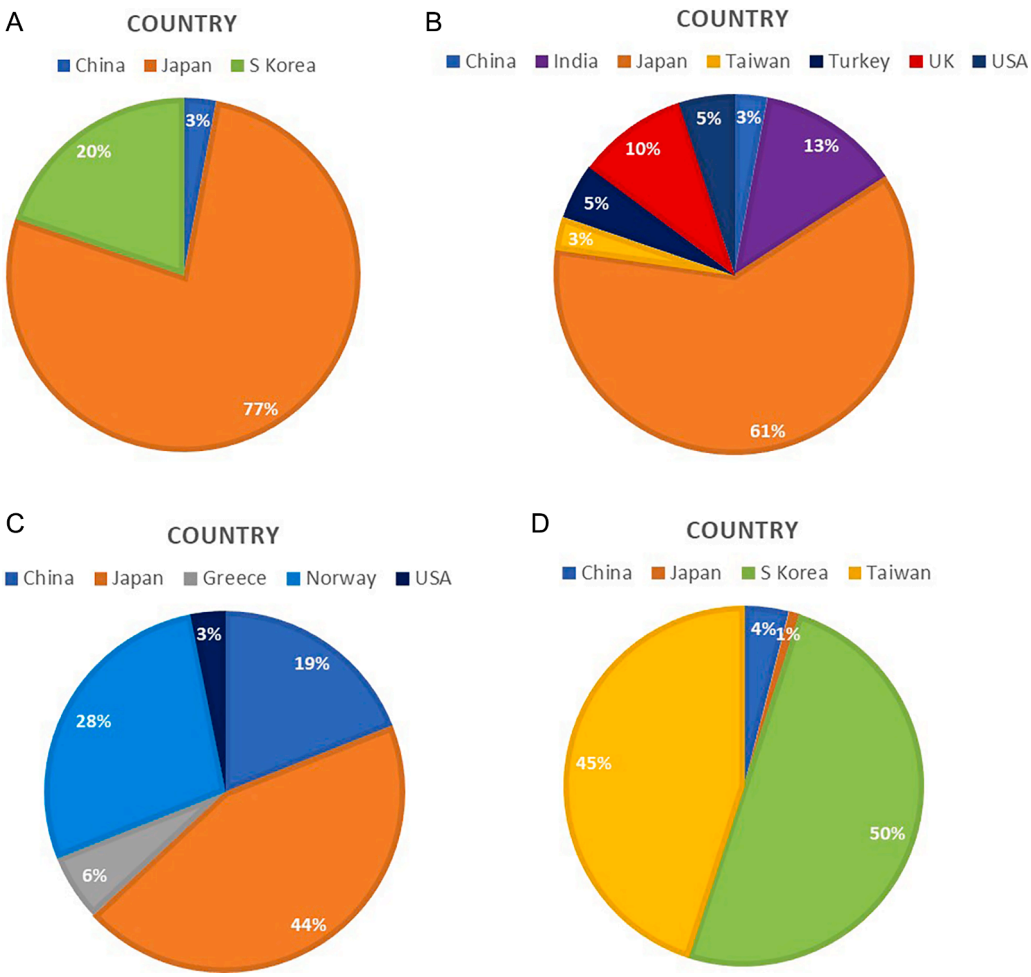


**Fig. 5.** A-D: Pie charts showing geographical distributions of the p.R75P (A), p.R141C (B), p.R182C (C) and p.R544C (D) mutations.

mutations were selected for additional statistical analyses as they were the most common in the clinical studies and genomic database. Using several statistical tests for variance and mean differences, we found that the p.R141C mutation had significantly earlier age of onset than p.R544C carriers ($p < 0.01$) (Table 4). However, independent *t*-tests showed that the percentage of male carriers or the percentage of individuals with stroke between the p.R141C and p.R544C carriers were not different ($p > 0.05$). Whilst the following characteristics including WMH, migraine, cognitive impairment or dementia and psychiatric disturbance also did not display respective statistical differences between p.R141C and p.R544C mutation carriers ($p > 0.05$). However, it seems there was less incidence of migraine in p.R544C carriers (Table 4).

## Discussion

Our systematic review provides a global view regarding *NOTCH3* mutations and their links with the phenotypic and demographic characteristics of CADASIL. First, the results showed that the most common *NOTCH3*$^{cys}$ mutations worldwide in our CADASIL patient survey are the p.R133C, p.R141C. p.R182C and p.R544C mutations according to five or more analysable phenotypic charactersitics. Using rather stringent criteria, however, we found there were a total of 135 of the p.R141C and 119 p.R544C mutation carriers, compared to only 84 carriers of the fourth most common *NOTCH3* mutation, p.R182C. Not surprisingly, majority of the most common mutations determined in this report were located within the EGFr 1–6 domain [22], where strong disease-associated variant clustering occurs and the phenotypes of mutation carriers are characterised by more aggressive disease [55].

Second, we found that the p.R544C mutation is very common in Asians although it also occurs in Europe [43]. This is compatible with the recent finding in the recent STROMICS genome study involving whole genome sequencing of 10,241 Chinese stroke patients [4]. In this analysis, the p.R544C mutation was found in 15 carriers in total all who had a stroke. We also found the p.R544C mutation carriers exhibited greater age of onset at 56 years compared to those with *NOTCH3*$^{cys}$ mutations within the EGFr 1–6 domain. A study by Liao et al. [31], reported the average age of onset in p.R544C carriers to be 57 years, in comparison to an average of 48 years in those with *NOTCH3*$^{cys}$ mutations in EGFr 1–6 domain. Similarly, Lee et al. [27] reported an average age of onset of 62 years. There is a possibility of sample bias, as these populations [27,31] were largely limited to clinics held at the Taipei Veterans General Hospital and the Jeju National University Hospital. However, as this mutation is located outside downstream from EGFr 1–6 region, in the EGFr 14 domain of exon 11 these observations collectively suggest disease progression is different and less aggressive in p.R544C carriers [55].

The average age of onset in p.R75P genotype carriers was also markedly later than those with the p.R141C and p.R182C mutations, with differences of 9 and 11 years respectively. This trend was reported by others including Mukai et al. [39], who found the average age of onset was 54 years in the p.R75P carriers compared to those with p.R141C and p.R182C carriers. Ueda et al. [62] focusing largely on clinical aspects, determined the average age of onset to be 54 years in p.R75P carriers in comparison to 44 years in *NOTCH3*$^{cys}$ mutations, such as the p.R133C mutation. This is consistent with a mean age at onset of disease at 46 years in p.R133C carriers in the Finnish population [37].

In our analysis, patients carrying mutations in the EGFr 1–6 region were more likely to experience four of the six phenotypic characteristics: migraine, cognitive impairment or dementia, bulbar symptoms and WMH, than carriers of mutations in EGFr 7–34. On the other hand, there was an indication that those who had mutations in the EGFr 7–34 domain exhibited more stroke episodes than those with changes in EGFr 1–6 region. Irrespective, we found the p.R141C mutation in particular was associated with a more severe phenotype compared to the other mutations. Previously, Rutten et al. [55] determined that mutations in the EGFr 1–6 region were susceptible to earlier onset stroke by ~8.2 years, lower survival rates by ~10.1 %, and increased WMH volume. This is also consistent with the study by Mukai et al. [39] suggesting that the p.R75P and p.R544C mutations were associated with a milder phenotype as opposed to the p.R141C and p.R182C mutations. Collectively, these observations again imply that the type and location of mutations in the *NOTCH3* gene contribute to disparities in the phenotypic characteristics of CADASIL. The milder phenotype in p.R544C carriers is possibly because the mutation is not an authentic cysteine-altering change per se because the 544th amino acid of NOTCH3 is located between the EGFr domain 13–14 and does not change the number of cysteine residues. Although remarkably the presence of GOM in skin has been confirmed in a patient homozygous for the p.R544C mutation [39]. While consanguinity was demonstrated in the parents of the p.R544C patient neither had an overt stroke episode until they died. The onset of stroke in the homozygous patient was at 63 years, who experienced a series of recurrent strokes, finally developing dementia and became bedridden [39]. Thus, the p.R544C carriers appears to exhibit milder effects at earlier stages but then they continue to progress similar to those with typical *NOTCH3*$^{cys}$ mutations [31]. Compared to typical *NOTCH3*$^{cys}$ mutations, patients with p.R544C mutation are also difficult to diagnose even after a stroke episode because onset age of stroke does not appear distinctly different from sporadic stroke patients. The p.R544C patients also frequently have several vascular risk factors suggesting that the genetic effect of p.R544C although mild, may be enhanced by co-existing vascular disease [39].

Our analysis confirms that the p.R75P mutation although not cysteine altering is pathogenic. It appears East Asian specific, particularly found in Japanese and Korean individuals diagnosed with CADASIL, exhibiting all the phenotypic characteristics including high burden of WMH. In our systematic search, we also noted that intracerebral haemorrhages and cerebral microbleeds are more common in East Asians than in Caucasians [6,47]. Taken together, is it plausible that structural differences in the manner in which p.R75P aggregates could promote haemorrhages? Most recently, Ishiyama et al. [18] have shown that amongst both Japanese and Korean patients the p.R75P mutation was strongly associated with intracerebral haemorrhages and cerebral microbleeds in the general absence of temporopolar lesions. Structural analysis showed that unlike cysteine thiols in conventional mutations, proline residues in p.R75P lowers correct disulfide bond formation probability, indirectly causing aggregation. Thus, although the p.R75P mutation resulted in less vascular N3ECD accumulation it apparently causes more haemorrhagic presentations.

In all four mutations, the most frequent phenotypic characteristic was WMH, which was reported in >80 % of CADASIL patients. Association between WMH and p.R544C was also found in the recent STROMICS study from China but there were no cases with the p.R75P mutation[4]. This was significantly higher than the variety of other symptoms investigated in this analysis. We also observed that the p.R75P mutation carriers had the highest frequency of WMH within their population but lacking typical white matter changes in the anterior temporal lobe [38]. While the volumes could potentially be different between even p.R75P and p.R544C carriers, this opens the field to further exploration of differences in white matter pathologies in relation to different types of mutations [7,52,66].

Chabriat et al. [3] reported migraine with aura is the typical first symptom in CADASIL, but frequency of migraine without aura in CADASIL patients is similar as in the general population. On the other hand, Liem et al. [32] reported that most studies showed that some CADASIL patients complained of migraine without aura and other studies showed low frequency of migraine with aura. Therefore, we utilised CADASIL cases which showed both migraine with aura as well as those without aura in this study.

The geographical distribution of CADASIL patients with identified mutations was variable, with only Japan and China reporting cases of all mutations investigated. Unsurprisingly, the most common mutation, p.R141C was found in more countries compared to the other three mutations. Of the p.R75P, p.R141C and p.R182C mutations, Chinese and Japanese studies typically investigated larger sample sizes in comparison to the other countries. For example, in the p.R75P data set, Mukai et al. [39] conducted a study in a total of 200 patients whereas the study from Korea [25] had only 34 CADASIL patients. A similar trend was evident for the p.R141C and p.R182C mutations. With such drastic differences in sample sizes between studies and the rather low prevalence of typical CADASIL the overall validity of our findings could be questioned. It is difficult to gather large as well as similar size samples to decrease the risk of type II errors in statistical analyses. However, the differences in geographical distribution could be due to the founder effect, in which due to population separation, there is decreased genetic variability. This appears especially likely when considering that almost 60 % of Korean p.R544C carriers originated from Jeju Island, which is separated from mainland South Korea.

## Limitations

Given our study design, one of the main limitations of our systematic survey was the incomplete availability of comprehensive data. We relied primarily on published literature for sources of data, especially regarding phenotypic and demographic characteristics. This could have led to publication and sampling biases possibly showing that certain mutations particularly in the EGFr 1–6 region were even more common. It would also have been ideal to extract primary clinical data. As in most cases within this study, the clinical studies we screened did not report on all the characteristics in the inclusion criteria. It could have been that in some cases, the patient was or was not presenting the characteristic. Thus, if the characteristic was not reported in the clinical study, the report had to be excluded and may have therefore reduced the overall sample size for each characteristic.

More investigation into cysteine sparing mutations, such as that of p.R75P and those outside the EGFr 7–34 domain are needed. It could have been useful to determine the statistical significance of cysteine sparing and other mutations with milder phenotypes. It would also be intriguing to utilise reports of cysteine-sparing mutations to 3D model the mutated NOTCH3 receptor and thereby determine the validity of such statements.

## Conclusions

Currently, there are still gaps in our knowledge of CADASIL, and with no effective therapeutic interventions to date, research into this disease is becoming increasingly valuable. There are clearly some associations prevalent between the respective phenotypes and genotypes observed in CADASIL patients. The p.R133C, p.R141C, p.R182C and p.R544C *NOTCH3*$^{cys}$ mutations were found to be most common globally. The cysteine sparing mutation p.R75P is commonly found in Asia. There were differences in the average age of onset in p.R141C and p.R544C carriers but in none of the other features of cSVD. In general, mutations present in the EGFr 1–6 domains were associated with more severe disease than the p.R544C mutations located outside of this region. Future recommendations for research would include the use of primary data collection, further comparisons between cysteine-altering and cysteine-sparing mutations and determining the statistical significance of phenotypical differences across other pathogenic mutations.

## CRediT authorship contribution statement

**Georgina Boston:** Writing – original draft, Methodology, Formal analysis, Data curation. **Dan Jobson:** Writing – review & editing, Data curation. **Toshiki Mizuno:** Writing – review & editing, Methodology. **Masafumi Ihara:** Writing – review & editing, Data curation. **Raj N Kalaria:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare no competing financial interests.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cccb.2024.100227.

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