Broad phenotype of cysteine-altering \textit{NOTCH3} variants in UK Biobank
CADASIL to nonpenetration

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Abstract

Objective

To determine the small vessel disease spectrum associated with cysteine-altering \textit{NOTCH3} variants in community-dwelling individuals by analyzing the clinical and neuroimaging features of UK Biobank participants harboring such variants.

Methods

The exome and genome sequencing datasets of the UK Biobank (\(n = 50,000\)) and cohorts of cognitively healthy elderly (\(n = 751\)) were queried for cysteine-altering \textit{NOTCH3} variants. Brain MRIs of individuals harboring such variants were scored according to Standards for Reporting Vascular Changes on Neuroimaging criteria, and clinical information was extracted with ICD-10 codes. Clinical and neuroimaging data were compared to age- and sex-matched UK Biobank controls and clinically diagnosed patients from the Dutch cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) registry.

Results

We identified 108 individuals harboring a cysteine-altering \textit{NOTCH3} variant (2.2 of 1,000), of whom 75\% have a variant that has previously been reported in CADASIL pedigrees. Almost all variants were located in 1 of the \textit{NOTCH3} protein epidermal growth factor–like repeat domains 7 to 34. White matter hyperintensity lesion load was higher in individuals with \textit{NOTCH3} variants than in controls (\(p = 0.006\)) but lower than in patients with CADASIL with the same variants (\(p < 0.001\)). Almost half of the 24 individuals with brain MRI had a Fazekas score of 0 or 1 up to age 70 years. There was no increased risk of stroke.

Conclusions

Although community-dwelling individuals harboring a cysteine-altering \textit{NOTCH3} variant have a higher small vessel disease MRI burden than controls, almost half have no MRI abnormalities up to age 70 years. This shows that \textit{NOTCH3} cysteine altering variants are associated with an extremely broad phenotypic spectrum, ranging from CADASIL to nonpenetration.
Glossary

ADNI = Alzheimer’s Disease Neuroimaging Initiative; ADNI GO = ADNI Grand Opportunities; CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; EGFr = epidermal growth factor–like repeat; ICD-10 = International Classification of Disease, 10th edition; LLS = Leiden Longevity Study; UKB = UK Biobank; WMH = white matter hyperintensity.

Cysteine-altering NOTCH3 variants cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), the most common hereditary cerebral small vessel disease, characterized by stroke and dementia.1 CADASIL disease severity is variable, ranging from first stroke in the third decade to a stroke-free survival up to the eighth decade. On brain MRI, however, all individuals with such NOTCH3 variants have been described to have at least a high white matter hyperintensity (WMH) burden, even those who are not, or not yet, clinically manifest.2–4

Recently, cysteine-altering NOTCH3 variants identical to those associated with CADASIL were shown to be surprisingly frequent in the Genome Aggregation Database.2,5 This suggests that patients diagnosed with CADASIL represent only the small minority of individuals with such variants, namely those at the very severe end of the small vessel disease spectrum. The neuroimaging and clinical features of community-dwelling individuals with cysteine-altering NOTCH3 variants could not be studied until now because large population exome and genome databases did not include health records or neuroimaging data. In 2019, the UK Biobank (UKB) released whole-exome sequences of 50,000 UKB participants, providing the unique opportunity to link genetic information of a large community-dwelling cohort to individual health records and neuroimaging data.

The aim of this study was to determine the small vessel disease phenotype associated with cysteine-altering NOTCH3 variants in community-dwelling individuals. We analyzed the clinical and neuroimaging features of individuals with such a variant in UKB and compared this to UKB controls and to patients from the Dutch CADASIL registry.

Methods

Standard protocol approvals, registrations, and patient consents

For all UKB and Alzheimer’s Disease Neuroimaging Initiative (ADNI) participants, signed consent was obtained according to the Declaration of Helsinki. All CADASIL studies performed at the Leiden University Medical Center have been approved by the local ethics committee. The 100-Plus Study and Leiden Longevity Study (LLS) have been approved by their respective local ethics committees.

Ascertainment of NOTCH3 cysteine-altering variants and clinical and neuroimaging analysis in UKB

Details on the UKB study have been described previously.6 In short, UKB is a prospective biobank study in the United Kingdom including ≈500,000 individuals 40 to 69 years of age at initial enrollment in 2006. Approximately 9.2 million individuals who lived within 25 miles of one of the assessment centers were invited to enter the cohort, of whom 5.5% participated in the baseline assessment.7 The ethnic background of UKB participants is White in 95.9%, Asian in 1.9%, Black in 1.6%, and mixed or other in 0.6%. Extensive phenotypic information was collected through touchscreen questionnaires and physical examinations. A subset of ≈20,000 individuals also underwent 3T brain MRI as a part of the UKB Imaging Study. Inclusion in the Imaging Study was based on traveling distance to the imaging center; there was no selection based on clinical information. At the start of 2019, the first 50,000 exomes were released. Methods used for exome sequencing are detailed elsewhere.8 We queried these exomes for CADASIL-associated NOTCH3 variants, i.e., missense variants that lead to a cysteine amino acid alteration in 1 of the 34 epidermal growth factor–like repeat (EGFr) domains of the NOTCH3 protein (amino acid position 40–1373) (uniprot.org/uniprot/Q9UM47). Variants were classified according to their position along the EGFr domains (EGFr 1–6 or 7–34) according to a previous study showing that variants in EGFr 7 to 34 are frequent in the population cohorts and are associated with a broader and milder phenotype than EGFr 1 to 6 variants, which are most frequent in CADASIL cohorts.9 Given the small number of individuals with an EGFr 1 to 6 variant in UKB (n = 3), further analyses included only the individuals with an EGFr 7 to 34 variant. The following clinical information was extracted: main ICD-10 codes, reaction time measured by the mean time to correctly identify matches in the card game Snap, history of smoking, smoking pack-years, blood pressure at intake, diagnosis of diabetes mellitus, cholesterol levels (total, low-density lipoprotein, high-density lipoprotein), and medication. Hypertension was defined as a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg. Hypercholesterolemia was defined as total cholesterol >6.5 mmol/L or low-density lipoprotein >3.5 mmol/L. History of ischemic stroke was determined from self-report, hospital records, and death records. If a brain MRI was available, it was scored according to the Standards for Reporting Vascular Changes on Neuroimaging guidelines.9 The WMH lesion load in the deep white matter and periventricular white matter was assessed semiquantitatively with the Fazekas scale.10 The following
Cysteine-altering NOTCH3 variants in UKB

In the 50,000 UKB exomes, we identified 108 individuals (59 male, 49 female) with a cysteine-altering NOTCH3 variant, corresponding to a frequency of 2.2 in 1,000. There were 35 distinct cysteine-altering NOTCH3 variants, and 75% of individuals had a variant that has been previously reported in CADASIL pedigrees (table 1). The mean age of the individuals with a cysteine-altering NOTCH3 variant was 64.9 years (range 49–81 years, SD 8.2 years). In the vast majority of individuals (97.2%), the NOTCH3 variant was located in one of the EGFr domains 7 to 34 (UKBNOTCH3 7-34); only 3 individuals had a NOTCH3 variant located in one of the EGFr domains 1 to 6 (figure 1A). Most individuals (86.1%) with a NOTCH3 variant were White; 9.3% were Asian; and 4.6% had other ethnicities. NOTCH3 variants were relatively enriched in Asians, which represent only 1.9% of the UKB ($p < 0.001$, Fisher exact test).

Stroke and dementia in individuals with a cysteine-altering NOTCH3 variant in UKB

UKBNOTCH3 7-34 cases had a lower stroke frequency and a later onset of stroke than patients with CADASIL with an EGFr 7 to 34 variant (CADASILNOTCH3 7-34 cases) (1.9% vs 30.6% up to age 75 years, hazard ratio 0.03, 95% confidence interval 0.01–0.07, log-rank test) (figure 1B). Stroke frequency in UKBNOTCH3 7-34 cases was not significantly higher than stroke frequency in the whole UKB population (1.9% vs 1.2%, $p = 0.375$, Fisher exact test). None of the UKBNOTCH3 7-34 cases had an ICD-10 code consistent with dementia, mild cognitive impairment, or migraine with aura.

Neuroimaging phenotype in individuals with a cysteine-altering NOTCH3 variant in UKB

The presence of small vessel disease MRI markers in the 24 UKBNOTCH3 7-34 cases for whom brain MRI was available ranged from an MRI consistent with mid-adult–onset CADASIL to practically nonpenetrance up to age 70 years (figure 2A). The neuroimaging phenotype in UKBNOTCH3 7-34 cases was much milder than in CADASILNOTCH3 7-34 cases (figure 2B), with a lower WMH lesion load and a much lower frequency of lacunes, microbleeds, and brain atrophy (figure 2, C–E and table 2). However, UKBNOTCH3 7-34 cases did have a significantly higher WMH lesion load compared to UKB controls (table 2). There was no difference in WMH lesion load between cases with a previously unreported cysteine-altering variant and those with a variant that has previously been described in CADASIL pedigrees. 14 UKBNOTCH3 7-34 cases with a Fazekas score of 2 or 3 for both deep white matter and periventricular white matter had a significantly longer reaction time than UKBNOTCH3 7-34 cases with a Fazekas score of 0 or 1 ($553 \pm 107$ vs $477 \pm 55$ milliseconds, $p = 0.03$, independent-samples $t$ test).
| NOTCH3 variant | Exon | EGFr domain | Frequency in UKB | Frequency in gnomAD | Previously reported in CADASIL literature |
|----------------|------|-------------|------------------|---------------------|------------------------------------------|
| p.Arg110Cys    | 3    | 2           | 1                | 0                   | Yes                                      |
| p.Arg182Cys    | 4    | 4           | 1                | 1                   | Yes                                      |
| p.Arg207Cys    | 4    | 5           | 1                | 2                   | Yes                                      |
| p.Cys360Tyr    | 7    | 9           | 1                | 0                   | No                                       |
| p.Ser476Cys    | 9    | 13          | 1                | 0                   | No                                       |
| p.Cys516Phe    | 10   | 13          | 2                | 0                   | No                                       |
| p.Arg544Cys    | 11   | 14          | 1                | 77                  | Yes                                      |
| p.Arg578Cys    | 11   | 14          | 2                | 10                  | Yes                                      |
| p.Arg607Cys    | 11   | 15          | 1                | 0                   | Yes                                      |
| p.Arg640Cys    | 12   | 16          | 4                | 9                   | Yes                                      |
| p.Arg654Cys    | 13   | 16          | 1                | 0                   | No                                       |
| p.Ser671Cys    | 13   | 17          | 1                | 0                   | No                                       |
| p.Arg728Cys    | 14   | 18          | 1                | 2                   | Yes                                      |
| p.Arg785Cys    | 15   | 20          | 2                | 1                   | Yes                                      |
| p.Trp802Cys    | 15   | 20          | 1                | 0                   | No                                       |
| p.Cys873Arg    | 17   | 22          | 1                | 0                   | No                                       |
| p.Gly994Cys    | 18   | 25          | 1                | 0                   | No                                       |
| p.Arg1031Cys   | 19   | 26          | 2                | 0                   | Yes                                      |
| p.Arg1076Cys   | 20   | 27          | 1                | 1                   | Yes                                      |
| p.Cys1108Arg   | 20   | 28          | 1                | 0                   | No                                       |
| p.Cys1110Arg   | 21   | 28          | 2                | 0                   | No                                       |
| p.Cys119Tyr    | 21   | 28          | 1                | 2                   | No                                       |
| p.Cys1137Arg   | 21   | 29          | 1                | 0                   | No                                       |
| p.Arg1143Cys   | 21   | 29          | 18               | 5                   | Yes                                      |
| p.Arg1190Cys   | 22   | 30          | 2                | 16                  | No                                       |
| p.Arg1201Cys   | 22   | 30          | 3                | 9                   | No                                       |
| p.Arg1210Cys   | 22   | 31          | 1                | 2                   | No                                       |
| p.Cys1222Gly   | 22   | 31          | 13               | 30                  | Yes                                      |
| p.Arg1231Cys   | 23   | 31          | 33               | 221                 | Yes                                      |
| p.Arg1242Cys   | 23   | 31          | 2                | 2                   | No                                       |
| p.Cys1275Ser   | 23   | 32          | 1                | 0                   | No                                       |
| p.Gly1283Cys   | 24   | 32          | 1                | 0                   | No                                       |
| p.Cys1315Trp   | 24   | 33          | 1                | 0                   | No                                       |
| p.Cys1315Phe   | 24   | 33          | 1                | 0                   | No                                       |
| p.Cys1324Ser   | 24   | 33          | 1                | 0                   | No                                       |

Abbreviations: CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; EGFr = epidermal growth factor–like repeat; gnomAD = Genome Aggregation Database; UKB = UK Biobank.
altering NOTCH3 variant, which was located in EGFr domain 31. He had been enrolled in ADNI as a cognitively healthy control. At the time of the last study site visit, he was 84 years old and did not have a history of stroke or other neurologic symptoms. His score on the Mini-Mental State Examination was 30 of 30. Brain MRI showed subtle WMH in the external capsules and frontal subcortical areas, dilated perivascular spaces, brain atrophy, and 2 small cerebellar infarcts (figure 3). No cysteine-altering NOTCH3 variants were present in individuals with Alzheimer dementia or cases with mild cognitive impairment from ADNI.

Discussion

Our investigation of the neuroimaging and clinical features of UKB participants harboring CADASIL-associated cysteine-altering NOTCH3 variants reveals that patients with CADASIL represent only the very severe and rare end of the NOTCH3-associated disease spectrum. The vast majority of community-dwelling individuals harboring such NOTCH3 variants turn out to have a significantly milder small vessel disease phenotype. In fact, in almost half of individuals for whom MRI was available, there were no neuroimaging abnormalities up to age 70 years.

Almost all cysteine-altering NOTCH3 variants found in UKB participants were located in EGFr domains 7 to 34 of the NOTCH3 protein. Roughly one-third of patients diagnosed with CADASIL harbor variants in EGFr domains 7 to 34, which shows that the small vessel disease phenotype associated with NOTCH3 EGFr 7 to 34 variants is very broad, ranging from a severe, mid-adult–onset CADASIL phenotype to non-penetrance. This suggests an important role for so far unknown genetic modifiers, the identification of which will likely be key for future individualized disease prediction in individuals harboring a cysteine-altering NOTCH3 variant. Until these factors have been elucidated, a positive family history compatible with CADASIL could be used as a proxy for the presence of these exacerbating genetic risk factors. From our findings in the UKB, it seems that individuals with a negative family history and a chance finding of a cysteine-altering NOTCH3 variant, when located in EGFr domains 7 to 34, likely have a very low risk of developing the classic severe CADASIL phenotype. A brain MRI in mid-adulthood could be done to determine whether signs of small vessel disease are present. Awareness of the broad clinical spectrum associated with cysteine-altering NOTCH3 variants is important because whole-exome sequencing is increasingly being implemented in clinical practice, increasing the risk of encountering such a variant as a secondary finding.

In contrast to NOTCH3 variants located in EGFr domains 7 to 34, those located in the first 6 EGFr domains are rarely present in population-based cohorts but are found in approximately two-thirds of CADASIL pedigrees. Cysteine-altering NOTCH3 variants in one of the EGFr domains 1 to 6, therefore, seem to be highly penetrant, predisposing to a typical CADASIL disease course in the vast majority of cases. Variant location proximal or distal to EGFr domain 6 therefore seems to be a key determinant for disease severity. The molecular mechanisms underlying the difference in disease severity between EGFr 1 to 6 variants and 7 to 34 variants are unknown. Regardless of EGFr location, all these cysteine-altering NOTCH3 variants lead to an unpaired cysteine residue and disrupted disulfide bridge formation. We hypothesize that there may be a difference in the established proaggregatory properties of the mutant NOTCH3 proteins. Taken together, these findings suggest...
Figure 2 Neuroimaging in cases with a cysteine-altering NOTCH3 variant in UKB

(A) Brain MRI T2-fluid-attenuated inversion recovery (FLAIR) images of 4 representative cases with a cysteine-altering NOTCH3 variant in the UK Biobank (UKB). From left to right: a 50-year-old woman with a normal brain MRI; a 52-year-old woman with periventricular and subcortical white matter hyperintensities (WMH) (Fazekas deep white matter [DWM] score 2 and periventricular white matter [PVWM] score 3) and a lacune; a 70-year-old man with only minimal WMH in the external capsules (Fazekas DWM score 1 and PVWM score 1); and a 72-year-old man with subcortical and basal ganglia WMH (Fazekas DWM score 3 and PVWM score 3). (B) Brain MRI T2-FLAIR images of 4 representative cases with a cysteine altering NOTCH3 variant in epidermal growth factor–like repeat (EGFr) 7 to 34 from cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) pedigrees. From left to right: a 50-year-old woman with extensive WMH (Fazekas DWM score 3 and PVWM score 3); a 58-year-old man with only minimal WMH (Fazekas DWM score 1 and PVWM score 1); a 68-year-old woman with extensive WMH (Fazekas DWM score 3 and PVWM score 3) and lacunes; and a 77-year-old woman with extensive WMH (Fazekas DWM score 3 and PVWM score 3) and lacunes. (C and D) Violin plots showing Fazekas DWM and PVWM scores of UKB controls, UKB NOTCH3 7-34 cases, and CADASIL NOTCH3 7-34 cases. WMH lesion load in UKB NOTCH3 7-34 cases was significantly lower than in CADASIL NOTCH3 7-34 cases but significantly higher than in UKB controls (for statistical analyses, see table 2). Almost half (10 of 24) of UKB NOTCH3 7-34 cases had a Fazekas score of 0 or 1 in both DWM and PVWM. In contrast, almost all (21 of 24) CADASIL NOTCH3 7-34 cases had a Fazekas score of ≥2 in both DWM and PVWM. (E) Bar charts showing the frequency of lacunes, microbleeds, and brain atrophy in UKB controls, UKB NOTCH3 7-34 cases, and CADASIL NOTCH3 7-34 cases. *A lacune.
Table 2 Characteristics of UKB NOTCH3 7–34 cases, UKB controls, and CADASIL NOTCH3 7–34 cases with brain MRI

|                                | UKB NOTCH3 7–34 (n = 24) | UKB controls | CADASIL NOTCH3 7–34 (n = 24) | p Value |
|--------------------------------|--------------------------|--------------|-----------------------------|---------|
| Mean (SD) age at brain MRI, y  | 60.9 (8.3)               | 61.5 (8.2)   | 60.4 (9.7)                  | NS      |
| Male, n (%)                    | 15 (62.5)                | 15 (62.5)    | 10 (41.6)                  | NS      |
| Hypertension, n (%)            | 9 (37.5)                 | 7 (29.1)     | 5 (20.8)                   | NS      |
| Smoking, n (%)                 | 6 (25.0)                 | 10 (41.7)    | 3 (12.5)                   | NS      |
| Diabetes mellitus, n (%)       | 0                        | 0            | 2 (8.3)                    | NS      |
| Hypercholesterolemia, n (%)    | 7 (29.1)                 | 9 (37.5)     | 11 (45.8)                  | NS      |

UKB NOTCH3 7–34 cases compared to UKB controls

|                                | UKB NOTCH3 7–34 | UKB Controls | p Value |
|--------------------------------|-----------------|--------------|---------|
| Fazekas DWM score, n           |                 |              |         |
| 0                              | 1               | 7            | 0.006   |
| 1                              | 12              | 13           |         |
| 2                              | 8               | 4            |         |
| 3                              | 3               | 0            |         |
| Fazekas PVWM score, n          |                 |              |         |
| 0                              | 2               | 7            | 0.002   |
| 1                              | 8               | 13           |         |
| 2                              | 8               | 3            |         |
| 3                              | 6               | 1            |         |
| Lacunes, n (%)                 | 2 (8.3)         | 2 (8.3)      | 1.000   |
| Microbleeds, n (%)             | 0               | 0            | 1.000   |
| Atrophy, n (%)                 | 0               | 0            | 1.000   |

UKB NOTCH3 7–34 cases compared to CADASIL NOTCH3 7–34 cases

|                                | UKB NOTCH3 7–34 | UKB Controls | p Value |
|--------------------------------|-----------------|--------------|---------|
| Fazekas DWM score, n           |                 |              |         |
| 0                              | 1               | 0            | <0.001  |
| 1                              | 12              | 3            |         |
| 2                              | 8               | 6            |         |
| 3                              | 3               | 15           |         |
| Fazekas PVWM score, n          |                 |              |         |
| 0                              | 2               | 1            | 0.002   |
| 1                              | 8               | 2            |         |
| 2                              | 8               | 4            |         |
| 3                              | 6               | 17           |         |
| Lacunes, n (%)                 | 2 (8.3)         | 16 (66.7)    | <0.001  |
| Microbleeds, n (%)             | 0               | 12 (50)      | <0.001  |
| Atrophy, n (%)                 | 0               | 13 (54.2)    | <0.001  |

Abbreviations: CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; DWM = deep white matter; PVWM = periventricular white matter; UKB = UK Biobank.
that NOTCH3-associated small vessel disease severity depends on at least 3 factors: the location of the cysteine-altering NOTCH3 variant in the 34 EGFr domains, unknown genetic modifiers, and the presence or absence of common vascular risk factors such as smoking and hypertension.17

Although we found that the phenotype associated with NOTCH3 EGFr 7 to 34 variants in UKB participants was significantly milder than in patients with CADASIL, there was an increased WMH burden compared to controls. Therefore, individuals with such mutations in the population are likely at increased risk for developing cognitive deficits because WMH are known to be associated with vascular cognitive impairment and dementia.18 Indeed, UKB participants with a NOTCH3 mutation and a high WMH lesion load had increased reaction times, a measure for early cognitive and behavioral alterations, compared to those with no or low WMH burden.19 In UKB and in the Genome Aggregation Database, NOTCH3 EGFr 7 to 34 mutations are especially frequent in Asians (1%),2 suggesting that these variants may contribute to the relatively high prevalence of small vessel disease in Asians.20,21 It is likely that individuals with cysteine-altering NOTCH3 variants are also at increased risk for small vessel stroke; this was shown to be the case for 2 highly frequent NOTCH3 variants (p.Arg544Cys and p.Arg1231Cys).4,22 The fact that we did not find this increased risk of stroke in individuals with a cysteine-altering NOTCH3 variant in UKB may be due to stochastic effects because of relatively low stroke frequencies in community-dwelling volunteer cohorts. Due to the healthy volunteer selection bias in UKB,7 individuals with disability or dementia are likely also underrepresented. Furthermore, mild cognitive impairment, dementia, and migraine with aura were captured by the use of ICD-10 codes, which likely also contributes to an underestimation of these features in the cohort. Finally, most individuals in UKB are White, so other ethnicities are underrepresented.

This study shows that CADASIL constitutes only the very severe and rare end of the NOTCH3-associated small vessel disease spectrum. The majority of individuals harboring cysteine-altering NOTCH3 variants have a significantly milder and later-onset small vessel disease, with a substantial number of individuals having no neuroimaging abnormalities up to age 70 years.

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Appendix Authors

| Name                     | Location                  | Contribution                                      |
|--------------------------|---------------------------|---------------------------------------------------|
| Julie W. Rutten, MD, PhD | Leiden University Medical Center | Designed and conceptualized the study, analyzed and interpreted the data, performed statistical analysis; drafted the manuscript for intellectual content |
## Appendix (continued)

| Name                        | Location                                      | Contribution                                                                 |
|-----------------------------|-----------------------------------------------|-------------------------------------------------------------------------------|
| Remco J. Hack, MD, MSc      | Leiden University Medical Center               | Major role in the acquisition of data, revised the manuscript for intellectual content |
| Marco Duering, MD           | Institute for Stroke and Dementia Research     | Major role in the acquisition of data, revised the manuscript for intellectual content |
| Gido Gravestejin, MD, MSc   | Leiden University Medical Center               | Analyzed and interpreted the data, revised the manuscript for intellectual content |
| Johannes G. Dauwerse        | Leiden University Medical Center               | Analyzed and interpreted the data, revised the manuscript for intellectual content |
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| Martin Dichgans, MD, PhD    | Institute for Stroke and Dementia Research     | Revised the manuscript for intellectual content                               |
| Rainer Malik, PhD           | Institute for Stroke and Dementia Research     | Major role in the acquisition of data, revised the manuscript for intellectual content |
| Saskia Lesnik Oberstein MD, PhD | Leiden University Medical Center             | Designed and conceptualized the study, interpreted the data, drafted the manuscript for intellectual content |

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