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RESEARCH ARTICLE

Input of exome sequencing in early-onset cerebral amyloid angiopathy

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Abstract

INTRODUCTION: Genetics of cerebral amyloid angiopathy (CAA) remains understudied.

METHODS: We assessed variants in Alzheimer's disease (AD) risk factor genes and differential diagnosis genes by performing exome sequencing among 78 patients with early-onset definite or probable CAA, after negative screening for APP mutation or duplication.

RESULTS: Among 14 genes involved in non-Aβ CAA, or vascular leukoencephalopathies, we detected pathogenic NOTCH3 variants in two patients, who exhibited lobar hematomas at the ages of 58 and 65, leading to a diagnosis redirection toward CADASIL. Of the remaining 76 patients, 23.1% carried at least one apolipoprotein E (APOE) ε2 allele and 43.6% carried at least one APOE ε4 allele, known as CAA risk factors. A total of 15 out of 76 (19.7%) carried either a loss-of-function or a rare predicted damaging missense or known AD risk variant in SORL1, TREM2, ABCA7, ABCA1, and ATP8B4.

DISCUSSION: Exome sequencing allowed the redirection toward CADASIL in two patients and suggested shared genetic factors between AD and CAA, beyond the APOE gene.

KEYWORDS
Alzheimer disease, CADASIL, cerebral angiopathy amyloid, genetic risk factors, intracerebral hemorrhage

Highlights

- The genetic component of cerebral amyloid angiopathy (CAA) remains understudied.

Gael Nicolas and David Wallon contributed equally to this study and are co-last authors.

- Rare differential diagnoses such as CADASIL should be considered, even in cases of cerebral hemorrhage.
- Our study suggests shared genetic factors between AD and CAA, beyond the *APOE* gene.
- Rare variants in *SORL1*, *TREM2*, *ABCA7*, *ABCA1* and *ATP8B4* might be susceptibility factors in early-onset CAA.,

1 | INTRODUCTION

Aβ-Cerebral amyloid angiopathy (CAA) is a severe disease characterized by deposits of Aβ peptide in the walls of cortical and leptomeningeal vessels, which lead to intracerebral hemorrhages (ICH) or cognitive decline. Aβ-CAA is often associated with Alzheimer's disease (AD), as Aβ peptide aggregation is central in the pathophysiology of both diseases.[1] The definite diagnosis of Aβ-CAA relies on neuropathology. A probable diagnosis can still be proposed in vivo, mainly based on imaging showing lobar hematomas, microbleeds, meningeal bleedings, or superficial siderosis in blood-sensitive magnetic resonance imaging (MRI) sequences, following revised v2.0 Boston criteria.[2]

Despite the high diagnostic performance of these criteria, the diagnosis remains probabilistic, and various differential diagnoses may explain the presence of spontaneous ICH, or microbleeds. For example, pathogenic variants in *NOTCH3*, *COL4A1*, or *COL4A2* genes have been reported in young patients with ICH, including apparently sporadic cases.[3,4] Furthermore, peptides other than Aβ can aggregate in cerebral blood vessels and cause a different form of CAA. Aggregation of gelsolin, transthyretin, prion, BRI2 protein, or cystatin may cause CAA in rare cases with a pathogenic variant in the corresponding gene. Such cases frequently associate CAA with extra-neurological features.[5,6] However, the distribution of these genetic differential diagnoses in a clinically diagnosed probable CAA cohort remains unclear in the absence of systematic genetic screening.

Most cases of Aβ-CAA occur sporadically with aging as the primary risk factor, but some patients experience an early onset (age at first symptoms set arbitrarily at < 66 years, similarly to early-onset AD [EOAD]), suggesting the contribution of genetic factors. Despite the exclusion of patients with disease onset before 50 in the revised Boston criteria v2.0, neuropathologically-proven autosomal dominant forms may be associated with a younger age of onset.[7] A small minority of patients (< 5%) exhibit monogenic forms of Aβ-CAA related either to a duplication[8] (or even a triplication[9]), or a single nucleotide variant of the *APP* gene. The genetic component of Aβ-CAA etiology remains largely understudied and unknown genetic risk factors may contribute to the disease beyond monogenic forms. *APOE* is the primary genetic risk factor gene, shared by Aβ-CAA and AD, with *APOE4* alleles increasing the risk of both conditions, and *APOE2* alleles decreasing the risk of AD but increasing the risk of CAA.[10–12] In AD, five major genes have been identified as moderate-to-strong risk factors through rare protein-damaging variants[13,14] namely *SORL1*,[15,16] *TREM2*,[17] *ABCA7*,[18,19] and recently *ABCA1* and *ATP8B4*.[20] Rare variants in these genes are reported with a moderate to strong effect for AD with odds ratios > 1.5,[14] while all the other (mainly common) AD risk alleles identified in genome-wide association studies have odds ratios (ORs) below 1.5.[13] We hypothesized that those susceptibility factors with a moderate to high effect on AD risk might also play a role in early-onset Aβ-CAA.

We performed the first study based on exome sequencing in probable or definite early-onset CAA patients following a nationwide recruitment. The first aim was to assess the possible presence of monogenic differential diagnoses (such as *NOTCH3*, *COL4A1*, *or COL4A2*). The second aim was the identification of putative genetic risk factors for Aβ-CAA through the screening of rare variants in *SORL1*, *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4* genes.

2 | METHODS

2.1 | CAA participants

Blood samples from unrelated cases with early-onset CAA (age at onset ≤ 65 years) were initially referred by hospitals throughout France, to two centers (either the CNR-MAJ, Rouen or the Department of Genetics, Lariboisière Hospital, Paris, France) for *APP* gene screening in a clinical setting (Figure 1). In the absence of *APP* pathogenic variants or duplications, we selected patients for exome sequencing among those fulfilling the revised Boston criteria[2] for probable CAA, probable CAA with supporting pathology, or definite CAA. With respect to the current version of the revised Boston 2.0 criteria, only a few exceptions were admitted: (i) the upper age limit was not retained here, and (ii) the presence of limited vascular impairment in the deep brain regions did not preclude the inclusion in this study if the other criteria were met and the patients exhibited a family history of CAA, cerebrospinal fluid (CSF) biomarkers showing low Aβ 42 levels, a medical history strongly suggestive of CAA associated with the presence of comorbid high blood pressure, which was thought to be responsible for deep microbleeds. Overall, four patients were included in the study using the latter exception (all of them showing numerous lobar hemorrhages and high blood pressure history).

Data collection included personal medical and family history assessment, neurological examination, CSF biomarkers if available, and

neuroimaging by MRI with blood-sensitive sequences (T2 GRE, SWI, or SWAN). Cognitive decline was defined as a combination of a cognitive impairment or complaint from the patient or his informant associated with an objective cognitive assessment (either bedside mental status examination or neuropsychological testing). A positive family history was defined by the presence of at least one relative with EOAD, CAA, or spontaneous lobar ICH among first-degree relatives. Patients were recruited regardless of family history. Only patients born to European-born parents were included to allow a comparison with the Alzheimer's Disease European Sequencing (ADES) consortium data.[20]

2.2 | Exome sequencing

Exome sequencing procedures are detailed in the Supplement Information. For variant interpretation, we established a list of 14 Mendelian genes known to be involved in AD, non-Aβ CAA,[6] familial cerebral cavernomatosis or vascular leukoencephalopathies (Table 1), and we applied the ACMG-AMP recommendations.[21]

In patients not carrying pathogenic or likely pathogenic variants in Mendelian genes, we assessed the presence of rare (allele frequency [AF] < 0.01) non-synonymous variants in the *TREM2*, *SORL1*, *ABCA7*, *ABCA1*, and *ATP8B4* genes. We extracted probable loss-of-function (LOF) variants (including nonsense, canonical splice site variants, and frameshift indels), and missense variants were annotated using REVEL. REVEL was used rather than other predictive scores of missense variant pathogenicity because of its well-validated performance.[22] The REVEL score is designed to help sort variants by gathering the information of multiple bioinformatics predictors of in silico variant effect predictions for missense variants, which was used in Holstege et al. with available thresholds.[20,22] The higher the score, the more likely the variant is to affect protein function.

Cumulative minor allele frequencies of different variant categories in these genes (LOF, missense with REVEL score > 0.75 for *ABCA1*, 0.50 for *SORL1*, or 0.25 for *TREM2*, *ABCA7* and *ATP8B4*)[20] were compared to that of EOAD, late-onset AD (LOAD) patients, and non-demented controls from the ADES consortium large dataset gathering multiple international studies and recently reported in Holstege et al.[20] We also listed patients with a definite AD-risk factor in these genes, defined as following: LOF variant in *TREM2*, *ABCA7*, *ABCA1*, or *SORL1*, or missense variant with demonstrated LOF effect or missense variant with nominal genome-wide significant association, following a recently published framework for risk variant interpretation in AD.[14]

2.3 | Statistical analyses

Quantitative variables are summarized as mean ± standard deviation (SD) unless otherwise specified. Carrier proportions are accompanied by 95% confidence intervals based on the binomial distribution due to the rarity of carriers.

Following ancestry comparison to 1000 Genomes data, none of the CAA patients overlapped with either the African (AFR) or Asian (SAS or EAS) super-populations in principal component analysis; therefore, all were kept for further analyses (see detailed method for principal component analysis in Supplemental Information).

Fisher exact tests were used to establish correlations between rare non-synonymous variants in *TREM2*, *SORL1*, *ABCA7*, *ABCA1*, and *ATP8B4* and clinical characteristics, CSF biomarkers, or APOE genotype. Linear regression was used to fit ages of onset and sex to APOE4 and APOE2 allele counts. Statistical significance was set at a threshold of 5%.

RESEARCH IN CONTEXT

1. **Systematic review**: The genetic component of cerebral amyloid angiopathy (CAA) etiology remains understudied. The authors conducted an exome sequencing analysis among 78 patients with early-onset definite or probable CAA, based on modified Boston criteria, after negative screening for APP mutation or duplication.
2. **Interpretation**: Our findings led to identifying two pathogenic *NOTCH3* variants in two probable CAA patients, suggesting that this rare differential diagnosis should be considered, even in cases of lobar cerebral hemorrhage. We established that 23.1% of CAA patients carried at least one apolipoprotein E (*APOE*) ε2 allele 43.6% carried at least one *APOE* ε4 allele, a known CAA risk factor, and 19.7% carried either a loss-of-function or a rare predicted damaging missense or known Alzheimer's disease (AD risk variant in *SORL1*, *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4*
3. **Future directions**: Our study suggests shared genetic factors between AD and CAA. Given their roles in the amyloid pathway, these data will be essential for the development of future therapeutic strategies targeting Aβ.

3 | RESULTS

3.1 | Inclusion of 78 definite or probable early-onset CAA patients

We included 70 probable and 4 definite CAA patients (Table 2). Four other patients were included despite few deep microbleeds given their family history of CAA or EOAD, or CSF biomarkers with low Aβ 42 levels, associated with neuroimaging features highly suggestive of CAA and a medical history of high blood pressure, which was thought to be responsible for the deep microbleeds. Fifteen (19.2%) patients had a positive family history, 9 with spontaneous lobar hemorrhage in first degree relatives, and 6 with EOAD in first degree relatives. The mean age at first neurological event was 58.2 ± 8.7 years, with ICH being

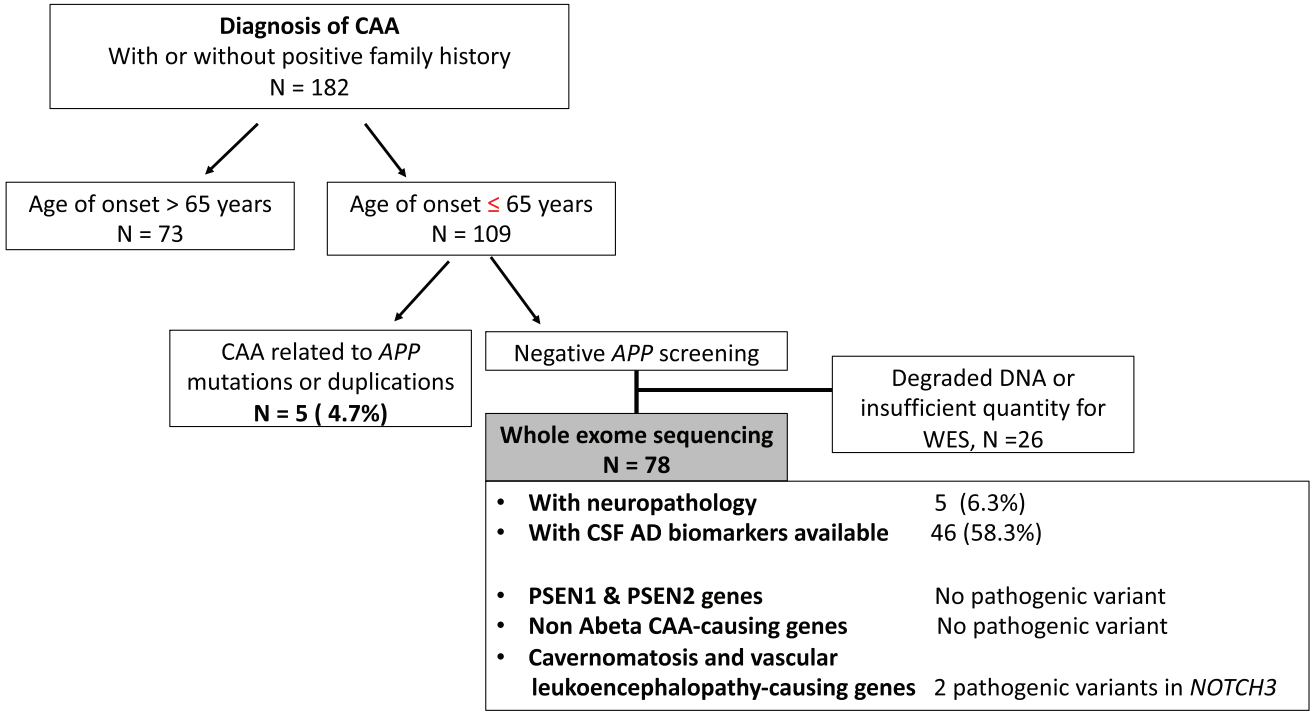

FIGURE 1 Summary of the study: CAA patients selected for WES and count of causative variants. CAA, cerebral amyloid angiopathy.

TABLE 1 List of 14 screened genes involved in Alzheimer's disease, non-Aβ CAA, cavernomatosis, or vascular leukoencephalopathy.

Disease categories	Gene
Alzheimer's disease and CAA	*PSEN1 (AD)*
	PSEN2 (AD)
Non Aβ -CAA	*TTR (AD)*
	CST3 (AD)
	GSN (AD)
	BRI2 (AD)
	PRNP (AD)
Familial cerebral cavernomatosis	*KRIT1 (CCM1) (AD)*
	MGC4607 (CCM2) (AD)
	PDCD10 (CCM3) (AD)
Vascular leukoencephalopathy	*NOTCH3 (CADASIL) (AD)*
	HTRA1 (CARASIL) (AR, AD)
	COL4A1 (AD)
	COL4A2 (AD)
	TREX1 (AD)
	COLGALT1 (AR)

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CAA, cerebral amyloid angiopathy.

the most common clinical presentation in 47.4% of patients, followed by cognitive decline in 33.3% and transient focal neurological episodes (TFNE) in 7.7%. Five patients presented with seizures, acute cognitive decline, and behavioral changes with an aspect of CAA-related inflammation on MRI according to current criteria.[23] Other symptoms included ischemic stroke (n = 1), dizziness, or nonspecific headache (n = 2) leading to cerebral imaging. During the course of the disease after diagnosis, 61.5% of patients presented at least one symptomatic ICH and 56.4% showed secondary progressive cognitive decline.

Among patients with CSF biomarkers available (n = 45), all but three patients showed decreased Aβ42 levels. Phospho-Tau levels were above the threshold in 24 patients (53.3%) and total Tau protein levels were abnormal in 14 (31.1%) (Figure 2). Overall, 22 patients (48.8%) presented with isolated decreased Aβ42 levels and 20 (44.4%) combined increased Tau or phospho-Tau with decreased Aβ42 levels, thus similar to the typical AD biomarker CSF profile.

3.2 | Contribution of monogenic differential diagnosis genes

No pathogenic or likely pathogenic variant was found in *PSEN1* and *PSEN2* genes nor non-Aβ CAA genes (*CST3*, *GSN*, *BRI2*, *TTR*, *PRNP*). Two patients carried distinct pathogenic variants in *NOTCH3* (Table 3; Figure 3), suggesting they were affected by CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Patient ROU-1149 carried p.(Arg728Cys) and experienced two lobar ICHs (first event: 65 years of age). Cerebral imaging showed lobar hemorrhage without lacunes in deep grey matter, and mild white matter hyperintensities, without prominent anterior temporal lobe involvement. The second patient, EXT-1182, carried the variant p.(Arg592Cys) and presented with a right thalamic stroke in the context of severe high blood pressure at age 58.

TABLE 2 Clinical characteristics of 78 definite or probable CAA French patients.

Age at onset, years, mean ± SD	58.2 ± 8.7 [32–65]
Sex, male *n* (%)	51 (65.3%)
First neurological event	
ICH, *n* patients (%)	37 (47.4%)
Cognitive decline, *n* patients (%)	26 (33.3%)
Ischemic stroke, *n* patients (%)	1
AAC-ri, *n* patients (%)	5 (6.3%)
TFNE revealing CSS, *n* patients (%)	6 (7.7%)
Vertigo or headache, *n* patients (%)	2 (2.5%)
Symptomatic lobar ICH	49 (62.8%)
Seizure during follow-up, *n* patients (%)	21 (26.9%)
Cognitive disorder during follow-up, *n* patients (%)	44 (56.4%)
CAA diagnosis following Boston's revised criteria	
Definite	4
Probable	70
Probable mixed angiopathy (1 or 2 deep microbleeds)	4
Familial history of ICH or EOAD in first-degree relative	19 (24.3%)
APOE genotype	
APOE2 carriers *n* (%)	18 (23.1%) with 3 homozygous
APOE4 carriers *n* (%)	34 (43.6%) with 12 homozygous
CSF biomarkers available, *n*(%)	45 (57.6%)
Aβ-42 (ng/mL), mean (normal > 635)	415.1
Aβ-40 (ng/mL), mean (normal > 12644)	7778.7 (20/45)
Tau (ng/mL), mean (normal < 466)	468.3
Phospho-Tau (ng/mL), mean (normal < 55)	72.9

Abbreviations: CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; CSS, cortical superficial siderosis; EOAD, early-onset Alzheimer's disease; ICH, intracerebral hemorrhage; SD, standard deviation; TFNE, transient focal neurological episodes.

Brain MRI revealed a left temporal sequela of ICH (without symptoms), more than 10 posterior lobar CMB but also CMB in the basal ganglia (initially thought to be in the context of severe hypertension), and white matter hyperintensities affecting temporal lobes. None of those two *NOTCH3* pathogenic variant carriers underwent LP. Overall, we retained CADASIL as the final diagnosis for those two patients with atypical lobar hemorrhages.

3.3 | *APOE* genotypes

After the exclusion of the two pathogenic *NOTCH3* variant carriers, 18 of the 76 remaining patients (23.1%) patients carried at least one *APOE2* allele and 34 (43.6%) carried at least one *APOE4* allele, including 3 homozygous *APOE2-2* and 12 *APOE4-4*. No significant association was found between *APOE*4 ($p = 0.32$ and $p = 0.29$) or *APOE*2 ($p = 0.75$ and $p = 0.77$) allele counts and age of onset or gender, respectively.

3.4 | Identification of suspected genetic risk factors: Rare non-synonymous variants in *SORL1*, *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4* genes

All rare non-synonymous variants identified in the five candidate genes were observed at the heterozygous state (Table 4). We observed 15/76 (19.7% [11.5%–30.5%]) carriers of either a LOF or a missense variant belonging to a category associated with AD.[20] See Supplemental Information for further details on *TREM2* and *SORL1* variants. The well-replicated AD-associated variants p.(Arg47His) and p.(Arg62His) in *TREM2* were found in two different patients, respectively. Two LOF *ABCA7* variants (one nonsense and one frameshift) were observed in CAA patients, along with five missense variants with a REVEL score > 0.25. Altogether, LOF and missense variants in *ABCA7* affected 9.2% [3.8%–18.1%] of cases compared to 6.2% in EOAD, 5.0% in LOAD and 3.9% in controls in ADES study.[20]

Interestingly, one LOF variant was found in a CAA patient (c.2115+1G > A splicing variant) in the recently reported *ABCA1* gene, despite the extreme rarity of LOF variants in controls (0.08%) and AD cases (0.28% in EOAD and 0.18% in LOAD).[20] *ABCA1* LOF and missense variants with a REVEL score > 0.75 affected 1.3% [0.0%–7.1%] of cases compared to 1.91% in EOAD, 1.5% in LOAD and 1.13% in controls in ADES study.

In the *SORL1* gene, no LOF variant was found. Finally, three distinct missense variants were seen in *ATP8B4* in two CAA patients. The cumulative MAF of variant carriers in *ATP8B4* in early-onset CAA patients (LOF + Missense Revel > 0.25 = 2.6% [0.3%–9.2%] was similar to that of EOAD (3.56%) and LOAD patients (3.08%) but higher than non-demented controls (2.09%) from ADES. Overall, only one individual carried more than one rare variant of interest: ROU-5131 carried two missense variants in *ATP8B4*,

TABLE 3 Characteristics of patients carrying *NOTCH3* pathogenic variants.

ID	APOE	AOO	Clinical presentation	Family history	MRI	Gene	Variant	Protein	REVEL score	gnomAD	Already reported in CADASIL
EXT- 1182-001	24	58	Medical history of depression Symptomatic right thalamic stroke at 58 in the context of severe high blood pressure No CSF available	Chronic migraine in his sister (no further details available regarding the presence of aura or MRI data) « Micro hemorrhages » on MRI in his brother but not available to us Father died from a heart attack around the age of 60 without any history of neurological disease Mother deceased in the context of dementia at 92 years of age	left temporal sequela of ICH (without symptom), more than 10 posterior lobar CMB but also CMBs in the basal ganglia (initially thought to be in the context of severe hypertension), and white matter hyperintensities affecting temporal lobes	NOTCH3	NM_000435.2: c.1774C > T	p.(Arg592Cys)	0.69	1/125, 189 carrier	[29]
ROU-1149-001	33	65	Two symptomatic lobar ICHs, the first on the left occipital lobe followed 9 years after by a second, right parietal ICH. No cognitive decline History of high blood pressure, No CSF available	No family history of stroke, dementia, or migraine with aura but no DNA or cerebral imaging from relatives was available	lobar hemorrhage without lacunes in deep grey matter, and mild white matter hyperintensities, without marked anterior temporal lobe involvement	NOTCH3	NM_000435.2: c.2182C > T	p.(Arg728Cys)	0.52	2/121, 945 carriers	[28]

Abbreviation: AOO, age of onset; APOE, apolipoprotein E; CSF, cerebrospinal fluid, ICH: intracerebral hemorrhage; MRI, magnetic resonance imaging.

although we could not determine if the variants were in trans or cis.

3.5 | Correlation between variants in *SORL1*, *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4* and clinical characteristics

No significant association was found between being a carrier of a prioritized rare variant in *TREM2*, *SORL1*, *ABCA7*, *ABCA1*, or *ATP8B4* and (i) *APOE2* alleles (3/14 *APOE2* allele in carriers of damaging variant versus 15/62 *APOE2* in noncarriers, OR = 0.86 [0.14–3.87], $p = 1$); (ii) *APOE4* alleles (5/14 *APOE4* alleles in carriers of rare variants versus 28/62 *APOE2* in noncarriers, OR = 0.67 [0.16–2.57], $p = 0.566$; (iii) showing isolated cognitive decline upon presentation (4/14 with initial cognitive decline in carriers of a rare variant versus 23/62 in noncarriers, OR = 0.56 [0.11–2.20], $p = 0.549$); (iv) symptomatic ICH (7/14 in carriers versus 39/62 in noncarriers, OR = 0.59 [0.15–2.26], $p = 0.384$); or (v) typical CSF profile of AD (n = 5/9 in carriers versus 18/39 in noncarriers, OR = 1.45 [0.27–8.48], $p = 0.720$).

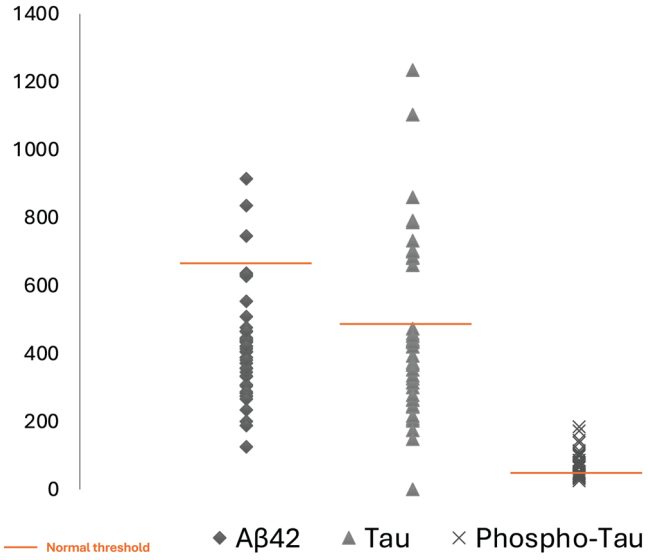

FIGURE 2 Scatterplots of CSF biomarkers (Aβ42, Aβ40, Tau, and phosphorylated Tau) levels in CAA patients ($n = 45$). CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid.

4 | DISCUSSION

For the first time to our knowledge, a whole exome sequencing (WES) study was performed in early-onset APP-negative CAA patients. We assessed rare monogenic forms of CAA, differential diagnoses and novel AD-associated rare variants in patients diagnosed with CAA, 94% of whom met the Boston 2.0 diagnostic criteria for probable or definite CAA (except the age criterion).[2]

4.1 | Contribution of monogenic differential diagnosis genes

Two patients eventually carried a *NOTCH3* pathogenic variant, leading us to reconsider the diagnosis of CADASIL. Both patients exhibited ICHs, which are rare in CADASIL, as reported in only 8% of 127 Taiwanese patients with CADASIL, 11 being strictly lobar[24] and between 0.5% and 2% in Caucasian CADASIL patients.[25,26] While CSS was absent in 364 French–German CADASIL patients, 47% presented with lobar CMB, and only 10% had strictly lobar CMB.[27] The current study illustrates that CADASIL might be considered in the case of lobar ICH or microbleeds, especially if associated with deep microbleeds or lacunes. Coexisting AD, CAA, and CADASIL neuropathological hallmarks have been reported, [28–30] but it remains difficult to speculate on a pathophysiological link. Of note, one of the patients with a pathogenic *NOTCH3* variant here also carried one rare missense variant Gly395Ser in *ATP8B4*, which is the main variant in that gene driving AD genetic risk,[20] and an *APOE2-4* genotype. Unfortunately, no histological study was available to evaluate co-existing CAA in this patient.

In this selected CAA population, we also searched for monogenic non-Aβ CAA causes,[6] but found no pathogenic variant. However, ICH in non-Aβ CAA is commonly associated with extra-neurological features,[31] The phenotype of the patients included here was not highly suggestive of non-Aβ CAA. Nevertheless, systematic genetic testing for TTR, CST3, BRI2, PRNP, or GSN in early-onset CAA cases with negative APP screening remains relevant. To our knowledge, no study has performed such systematic non-Aβ CAA genetic screening in a population of patients fulfilling the revised Boston criteria, regardless of familial history or extra-neurological features.

4.2 | Identification of known or suspected genetic risk factors: *APOE* and rare non-synonymous variants in *SORL1*, *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4* genes

Next, we examined *APOE* genotypes and genes recently associated with AD through rare variants. A significant percentage of patients had one APOE4 allele (43%) or at least one APOE2 allele (23%), which are higher than frequencies in Caucasian populations (*APOE4* around 21%–24% [32] and *APOE2* around 5% in Caucasian populations [11]). *APOE4* allele is a known risk factor for both CAA and AD,[33,34] while the role of *APOE2* in CAA is debated. It has been linked to hemorrhagic outcomes in CAA, but also to CAA severity and as a risk factor for CAA by itself.[35,36] We did not identify any association between the *APOE2* status and any specific hemorrhagic features, despite limited power. Overall, our series is however consistent with an enrichment of *APOE4* and *APOE2* carriers in CAA.

Then, we assessed variants within five genes of interest, recently discovered as AD risk factors with moderate to high effect.[14,20] Considering only fully validated AD-risk factors using a recently reported framework for AD risk variant interpretation,[14] five CAA patients (6.6%) carried either a LOF variant in *ABCA7* or *ABCA1*, one was a carrier of the R47H *TREM2* variant and one carried the R62H *TREM2*

TABLE 4 Rare variants identified in *TREM2, SORL1, ABCA1, ABCA7, and ATP8B4* in CAA patients and carrier characteristics.

ID	APOE	AOO	Clinical presentation	Chr coordinates	Type	Gene	cDNA	Protein	REVEL score[a]	GNOMAD
EXT- 2260-001	33	63	Symptomatic occipital ICH with lobar microbleeds on MRI/No cognitive decline	chr6:41129252	missense	TREM2	c.140G > A	p.(Arg47His)	0.33	0.00152857
ROU-5259-001	23	57	Cognitive decline/Walking disorder/Absence/Lobar microbleeds and CSS on MRI/CSF indicative of AD	chr6:41129207	missense	TREM2	c.185G > A	p.(Arg62His)		0.00762961
ROU-0367-001	23	<65	3 symptomatic lobar ICH/No cognitive decline	chr11:121495816[b]	missense	SORL1	c.6194A > T	p.(Asp2065Val)	0.57	0.00238854
EXT- 2376-001	33	43	Symptomatic parietal ICH with lobar microbleeds on MRI and small left deep infarct	chr11:121495816[b]	missense	SORL1	c.6194A > T	p.(Asp2065Val)	0.57	0.00238854
EXT- 0166-001	34	59	Rapidly progressive cognitive decline and behavioral disorders with early seizures. Autopsy showed severe CAA with coexisting AD lesions. No family history	chr11:121495816[b]	missense	SORL1	c.6194A > T	p.(Asp2065Val)	0.57	0.00238854
ROU-5131-001	23	48	2 symptomatic occipital ICH with lobar microbleeds on MRI, no cognitive decline. Of note, one treated aneurysm of the anterior cerebral artery	chr15:50223353 chr15:50366353	missense missense	ATP8B4 ATP8B4	c.1605G > T c.58C > G	p.(Leu535Phe) p.(Arg20Gly)	0.66 0.33	0.00207907 0.00277123
EXT- 0774-001	44	61	Behavioral disorders revealing CAA-related inflammation on MRI, biopsy confirming vascular Aβ deposits	chr15:50366373	missense	ATP8B4	c.38G > A	p.(Arg13Gln)	0.45	0.00264314
EXT- 2041-001	34	<65	Cognitive decline with lobar microbleeds and CSS on MRI, CSF biomarkers profile indicative of AD, family history of ICH in his mother at 54	chr19:1043091	LOF (non-sense)	ABCA7	c.631C > T	p.(Arg211[a])	NA	3.18776e-5
EXT- 1447-001	23	46	Occipital ICH followed 3 months after by new frontal ICH, biopsy confirmed CAA diagnosis	chr19:1055907	LOF (frameshift)	ABCA7	c.4208delT	p.(Leu1403fs)	NA	0.0005417
ROU-5270-001	34	61	Seizures and headaches revealing CAA-related inflammation	chr19:1045206	missense	ABCA7	c.1421C > T	p.(Thr474Met)	0.40	NA
EFA- 0620-001	44	55	Cognitive decline and early seizures, CSF profile with isolated decreased Aβ-42 level, family history of EOAD	chr19:1045170	missense	ABCA7	c.1385T > C	p.(Val462Ala)	0.49	NA
EXT- 1654-001	44	32	Atypical imaging suggestive of CAA-related inflammation with CSF indicative of AD	chr19:1047497	missense	ABCA7	c.2113G > A	p.(Ala705Thr)	0.38	NA

(Continues)

TABLE 4 (Continued)

ID	APOE	AOO	Clinical presentation	Chr coordinates	Type	Gene	cDNA	Protein	REVEL score[a]	GNOMAD
ROU-5187-001	33	45	Initially isolated cognitive decline followed by symptomatic ICH, lobar microbleeds showed on MRI, CSF profile with isolated decreased Aβ-42 level	chr19:1051006	missense	ABCA7	c.2639G > A	p.(Arg880Gln)	0.72	0.00137442
EXT-1704-001	33	39	Transient neurological episodes revealing disseminated CSS with lobar microbleeds on MRI	chr19:1059079	missense	ABCA7	c.5458G > A	p.(Gly1820Ser)	0.91	0.00025523
EXT-0636-001	33	50	Symptomatic occipital ICH, one deep lacunar infarct, numerous lobar microbleeds, CSF indicative of AD	chr9:107591196	LOF (splice)	ABCA1	c.2115+1G > A		NA	NA

Abbreviations: AD, Alzheimer's disease; AOO, age of onset; APOE, apolipoprotein E; CAA, cerebral amyloid angiopathy; CMB, cerebral microbleeds; CSF, cerebrospinal fluid; CSS, cortical superficial siderosis; EOAD, early-onset Alzheimer's disease; ICH, intra cerebral hemorrhage; LOF, loss-of-function.

[a]The same REVEL thresholds that of Holstege et al were used i.e > 0.25 for ABCA7 (NM_019112.3), TREM2(NM_018965.3), and ATP8B4 (NM_024837.3); > 0.50 for SORL1 (NM_003105.5) and > 0.75 for ABCA1 (NM_005502.3).

[b]This variant was excluded from Holstege et al. analyses and did not seem to be associated with AD in other studies.

variant. Among them, 3/5 presented spontaneous lobar ICH without cognitive decline suggestive of AD. In AD, moderate-to-high variant effect sizes were observed for LOF variants in *SORL1*, *TREM2*, and *ABCA1* (albeit not significant by itself for *ABCA1* probably because of the extreme rarity of LOF variants), while less strong variant effects were observed in *ABCA7* and for selected missense variants. Here, the small size of our series did not allow us to perform a burden test. We thus compared our results to published gene-based burdens from 16,036 AD cases and 16,522 controls.[20] Rare variants seemed to be in similar frequency ranges in *ABCA7* and *ATP8B4* in early-onset CAA as in EOAD, both compared to LOAD and non-demented controls from the ADES dataset, among the categories of variants associated with AD. However, additional biases did not allow us to directly compare gene burdens. The diverse quality of sequencing in ADES (gathering multiple studies with diverse capture and sequencing methods, leading to missing some variants in ADES, for example, the p.(Asp2065Val) recurrent variant in *SORL1*) might modify burdens of rare variants. Overall, our data suggest that *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4* might be involved in early-onset CAA with 12/76 (15.7% [8.4%–26.0%]) of cases carrying at least one rare predicted damaging or validated risk factor variant after exclusion of the *SORL1* p.(Asp2065Val) variant. Further larger studies are required to confirm these results and better understand the contribution of these genes to CAA.

Preliminary evidence already suggested a role for *SORL1*, *TREM2*, *ABCA7*, and *ABCA1* genes in CAA. For instance, severe levels of CAA next to typical AD neuropathology were found in AD patients carrying rare LOF and missense variants in *ABCA7* [37] and *SORL1*.[38] CAA-ri was already described in a patient carrying a *SORL1* variant.[39] In mouse models, the lack of *ABCA1* and *TREM2* considerably increased the level of CAA and lobar microbleeds.[40–42] Given their pathophysiological consequences (for more details in the Supplemental Information), if further confirmed, these promising candidates in CAA genetic determinism would underline the impaired Aβ-aggregation and secretion and lipid metabolism as a mainstay in CAA pathophysiology. As for *APP* mutations or duplications,[43] a same variant in these genes could drive AD risk in some patients and prominent CAA in others.

4.3 | Consideration of coexisting AD in our CAA cohort and study limits

CAA was the main driver of the phenotype in our patients, with more than 64% in total presenting specific CAA neuroimaging features (ICH, TFNE revealing CSS, CAA-related inflammation). Nevertheless, given the high frequency of coexistence of AD and CAA, the possibility that some patients have an underlying AD associated with CAA cannot be ruled out. They share common pathways and potentially shared genetic risk factors, while their clinical expression does not overlap in many aspects. This highlights the relevance of dedicated evaluation of genes validated in AD, in the field of early-onset CAA. We investigated whether the presence of these risk factors in our study population was independent of underlying AD. To this end, we examined potential correlations between carrying one of these rare variants and two key

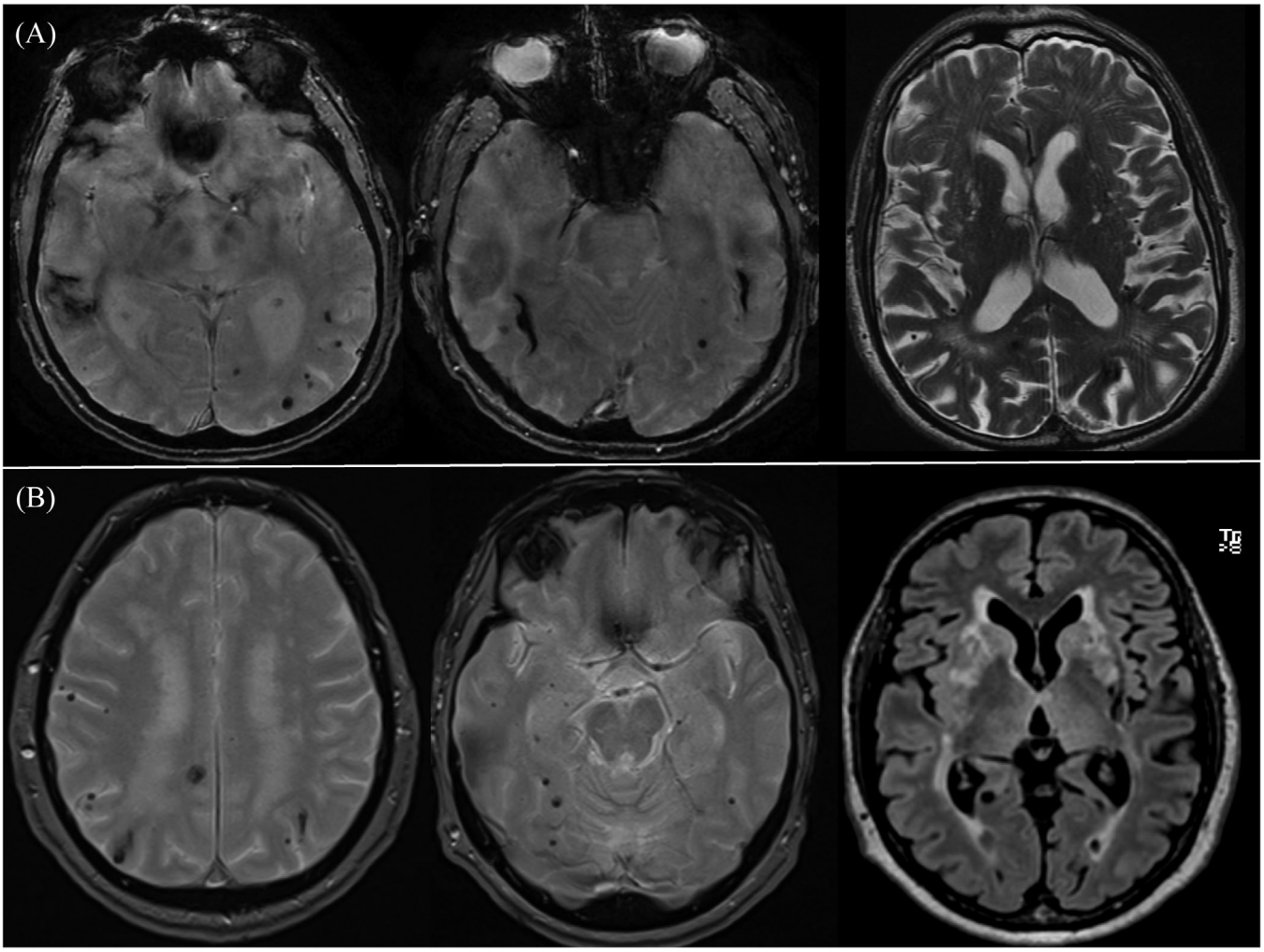

FIGURE 3 MRI imaging of the two carriers ROU-1149 (panel A) and EXT-1182 (panel B) of a pathogenic NOTCH3 variant presenting with a history initially compatible with CAA. Panel A: Left and middle axial T2-GRE weighted sequence showing several posterior lobar microbleeds and bilateral temporal ICH sequela; right: axial T2 weighted sequence showing mild periventricular white matter lesions of posterior topography and dilated perivascular spaces. Panel B: Left and middle axial T2-GRE weighted sequence showing several posterior lobar microbleeds; right: axial FLAIR weighted sequence showing mild periventricular white matter lesions and previous thalamic stroke. CAA, cerebral amyloid angiopathy; ICH, intra-cerebral hemorrhage; MRI, magnetic resonance imaging.

indicators: (1) CSF biomarkers suggestive of AD pathophysiology and (2) a cognitive (non-hemorrhagic) profile. No significant associations were found suggesting that the rare variants identified in the five AD-related genes are not specifically associated with patients suspected of having both CAA and AD. Instead, these variants may play a role in the development of CAA independently of AD pathology.

Our study suffered from two main limits: the small number of patients, which precludes any gene-based burden tests, and its retrospective nature although this is the most common strategy for genetic screening in a defined patient population. Some patients were enrolled before the publication of the v2.0 Boston criteria and the heterogeneity in terms of cerebral imaging such as 1.5 versus 3 Tesla MRI, T2* versus SWI weighted sequences, or some missing data due to the limited number of T2 weighted-sequence allowing the assessment of dilated perivascular spaces in semi-ovale centers for instance, precludes correlations between imaging features and genetics results. We therefore did not evaluate possible changes in perivascular spaces or microbleed count according to the presence of genetic risk factors. A prospective study with homogeneous neuroimaging data should be performed to better answer these questions.

In conclusion, by performing WES in 78 early-onset probable or definite CAA patients, we found two patients with CADASIL, suggesting that this rare differential diagnosis should be considered, even in cases of lobar hemorrhage. Rare predicted damaging or known risk variants in AD-associated genes *TREM2*, *ABCA7*, *ABCA1*, and *ATP8B4* were found in up to 15.7% of CAA patients, with 6.6% carrying at least one well-validated AD risk factor. In addition to *APOE* genotypes, some of these variants might thus have contributed partially to the development of CAA in the carriers. This suggests a putative shared genetic determinism between AD and CAA. Larger studies of early-onset CAA patients are required to confirm these results and to better understand the role of rare variants in AD-associated genes. Given their respective

roles in the amyloid pathway, these data will be essential for the development of future therapeutic strategies targeting Aβ in terms of both indication and safety.[44]

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest or competing interest. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

All patients or legal guardians provided informed, written consent for genetic and research analyses. Our local ethics committee (CERDE E2021-92) approved this protocol.

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REFERENCES

1. Greenberg SM, Bacskai BJ, Hernandez-Guillamon M, Pruzin J, Sperling R, van Veluw SJ. Cerebral amyloid angiopathy and Alzheimer disease—one peptide, two pathways. *Nat Rev Neurol*. 2020;16:30-42.
2. Charidimou A, Boulouis G, Frosch MP, et al. The Boston criteria version 2.0 for cerebral amyloid angiopathy: a multicentre, retrospective, MRI–neuropathology diagnostic accuracy study. *Lancet Neurol*. 2022;21:714-725.
3. Weng Y-C, Sonni A, Labelle-Dumais C, et al. COL4A1 mutations in patients with sporadic late-onset intracerebral hemorrhage. *Ann Neurol*. 2012;71:470-477.
4. Zhang C, Li W, Li S, et al. CADASIL: two new cases with intracerebral hemorrhage. *Ann Clin Transl Neurol*. 2017;4:266-271.
5. Yamada M. Cerebral amyloid angiopathy: emerging concepts. *J Stroke*. 2015;17:17-30.
6. Revesz T, Holton JL, Lashley T, et al. Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. *Acta Neuropathol*. 2009;118:115-130.
7. Kamp JA, Moursel LG, Haan J, et al. Amyloid β in hereditary cerebral hemorrhage with amyloidosis-Dutch type. *Rev Neurosci*. 2014;25:641-651.
8. Rovelet-Lecrux A, Hannequin D, Raux G, et al. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat Genet*. 2006;38:24-26.
9. Grangeon L, Cassinari K, Rousseau S, et al. Early-onset cerebral amyloid angiopathy and Alzheimer disease related to an APP locus triplication. *Neurol Genet*. 2021;7:e609.
10. Wu L, Zhao L. ApoE2 and Alzheimer's disease: time to take a closer look. *Neural Regen Res*. 2016;11:412-413.
11. Genin E, Hannequin D, Wallon D, et al. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. *Mol Psychiatry*. 2011;16:903-907.
12. Yu L, Boyle PA, Nag S, et al. APOE and cerebral amyloid angiopathy in community-dwelling older persons. *Neurobiol Aging*. 2015;36:2946-2953.
13. Bellenguez C, Charbonnier C, Grenier-Boley B, et al. Contribution to Alzheimer's disease risk of rare variants in TREM2, SORL1, and ABCA7 in 1779 cases and 1273 controls. *Neurobiol Aging*. 2017;59:220.e1-220.e9.
14. Nicolas G, Zaréa A, Lacour M, et al. Assessment of Mendelian and risk-factor genes in Alzheimer disease: a prospective nationwide clinical utility study and recommendations for genetic screening. *Genet Med*. 2024;26(5):101082. doi:10.1016/j.gim.2024.101082
15. Campion D, Charbonnier C, Nicolas G. SORL1 genetic variants and Alzheimer disease risk: a literature review and meta-analysis of sequencing data. *Acta Neuropathol*. 2019;138:173-186.
16. Nicolas G, Charbonnier C, Wallon D, et al. SORL1 rare variants: a major risk factor for familial early-onset Alzheimer's disease. *Mol Psychiatry*. 2016;21:831-836.
17. Jonsson T, Stefansson H, Steinberg S, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med*. 2013;368:107-116.
18. Cuyvers E, De Roeck A, Van den Bossche T, et al. Mutations in ABCA7 in a Belgian cohort of Alzheimer's disease patients: a targeted resequencing study. *Lancet Neurol*. 2015;14:814-822.
19. Le Guennec K, Nicolas G, Quenez O, et al. ABCA7 rare variants and Alzheimer disease risk. *Neurology*. 2016;86:2134-2137.
20. Holstege H, Hulsman M, Charbonnier C, et al. Exome sequencing identifies rare damaging variants in ATP8B4 and ABCA1 as risk factors for Alzheimer's disease. *Nat Genet*. 2022;54(12):1786-1794.
21. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med Off J Am Coll Med Genet*. 2015;17:405-424.
22. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016;99:877-885.
23. Auriel E, Charidimou A, Gurol ME, et al. Validation of clinicoradiological criteria for the diagnosis of cerebral amyloid angiopathy-related inflammation. *JAMA Neurol*. 2016;73:197-202.
24. Liao Y-C, Hu Y-C, Chung C-P, et al. Intracerebral hemorrhage in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: prevalence, clinical and neuroimaging features and risk factors. *Stroke*. 2021;52:985-993.
25. Puy L, De Guio F, Godin O, et al. Cerebral microbleeds and the risk of incident ischemic stroke in CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). *Stroke*. 2017;48:2699-2703.
26. Nannucci S, Rinnoci V, Pracucci G, et al. Location, number and factors associated with cerebral microbleeds in an Italian-British cohort of CADASIL patients. *PloS One*. 2018;13:e0190878.
27. Wollenweber FA, Baykara E, Zedde M, et al. Cortical superficial siderosis in different types of cerebral small vessel disease. *Stroke*. 2017;48(5):1404-1407.
28. Paquet C, Jouvent E, Mine M, et al. A cortical form of CADASIL with cerebral Aβ amyloidosis. *Acta Neuropathol*. 2010;120:813-820.
29. Gray F, Robert F, Labrecque R, et al. Autosomal dominant arteriopathic leuko-encephalopathy and Alzheimer's disease. *Neuropathol Appl Neurobiol*. 1994;20:22-30.
30. Thijs V, Robberecht W, De Vos R, Sciot R. Coexistence of CADASIL and Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2003;74:790-792.
31. Revesz T, Holton JL, Lashley T, et al. Sporadic and familial cerebral amyloid angiopathies. *Brain Pathol*. 2002;12:343-357.
32. Corbo RM, Scacchi R. Apolipoprotein E (APOE) allele distribution in the world. Is APOE* 4 a "thrifty" allele?. *Ann Hum Genet*. 1999;63:301-310.

33. Greenberg SM, Briggs ME, Hyman BT, et al. Apolipoprotein E epsilon 4 is associated with the presence and earlier onset of hemorrhage in cerebral amyloid angiopathy. *Stroke*. 1996;27:1333-1337.
34. Liu Y, Yu J-T, Wang H-F, et al. APOE genotype and neuroimaging markers of Alzheimer's disease: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry*. 2015;86:127-134.
35. Goldberg TE, Huey ED, Devanand DP. Associations of APOE e2 genotype with cerebrovascular pathology: a postmortem study of 1275 brains. *J Neurol Neurosurg Psychiatry*. 2020:323746.
36. Nicoll JA, Burnett C, Love S, et al. High frequency of apolipoprotein E epsilon 2 allele in hemorrhage due to cerebral amyloid angiopathy. *Ann Neurol*. 1997;41:716-721.
37. Van den Bossche T, Sleegers K, Cuyvers E, et al. Phenotypic characteristics of Alzheimer patients carrying an ABCA7 mutation. *Neurology*. 2016;86:2126-2133.
38. Alvarez-Mora MI, Blanco-Palmero VA, Quesada-Espinosa JF, et al. Heterozygous and homozygous variants in SORL1 gene in Alzheimer's disease patients: clinical, neuroimaging and neuropathological findings. *Int J Mol Sci*. 2022;23:4230.
39. Du Y, Liu C, Ma C, et al. Cerebral amyloid angiopathy-related inflammation: a case report presenting with a rare variant in SORL1 gene. *BMC Neurol*. 2019;19:97.
40. Yang J, Kou J, Lalonde R, Fukuchi K-I. Intracranial IL-17A overexpression decreases cerebral amyloid angiopathy by upregulation of ABCA1 in an animal model of Alzheimer's disease. *Brain Behav Immun*. 2017;65:262-273.
41. Wahrle SE, Jiang H, Parsadanian M, et al. Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. *J Biol Chem*. 2005;280:43236-43242.
42. Koldamova R, Staufenbiel M, Lefterov I. Lack of ABCA1 considerably decreases brain ApoE level and increases amyloid deposition in APP23 mice. *J Biol Chem*. 2005;280:43224-43235.
43. Grangeon L, Charbonnier C, Zarea A, et al. Phenotype and imaging features associated with APP duplications. *Alzheimers Res Ther*. 2023;15(1):93.
44. Perneczky R, Jessen F, Grimmer T, et al. Anti-amyloid antibody therapies in Alzheimer's disease. *Brain J Neurol*. 2023;146(3):842-849.

SUPPORTING INFORMATION

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

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APPENDIX 1: COLLABORATORS

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