TREX1 p.A129fs and p.Y305C variants in a large multi-ethnic cohort of CADASIL-like unrelated patients

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ABSTRACT

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and retinal vasculopathy with cerebral leukodystrophy and systemic manifestations (RVCL-S) are the most common forms of rare monogenic early-onset cerebral small vessel disease and share clinical, and, to different extents, neuroradiological and neuropathological features. However, whether CADASIL and RVCL-S overlapping phenotype may be explained by shared genetic risk or causative factors such as *TREX1* coding variants remains poorly understood. To investigate this intriguing hypothesis, we used exome sequencing to screen *TREX1* protein-coding variability in a large multi-ethnic cohort of 180 early-onset independent familial and apparently sporadic CADASIL-like Caucasian patients from the USA, Portugal, Finland, Serbia

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and Turkey. We report 2 very rare and likely pathogenic *TREX1* mutations: a loss of function mutation (p.Ala129fs) clustering in the catalytic domain, in an apparently sporadic 46-year-old patient from the USA and a missense mutation (p.Tyr305Cys) in the well conserved C-terminal region, in a 57-year-old patient with positive family history from Serbia. In concert with recent findings, our study expands the clinical spectrum of diseases associated with *TREX1* mutations.

1. Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and retinal vasculopathy with cerebral leukodystrophy and systemic manifestations (RVCL-S) represent 2 very rare pathologies with an incidence of 2-5/100,000 to only 13 families reported worldwide, respectively (Okada et al., 2020) (Razvi et al., 2005), (Richards et al., 2007)· (DiFrancesco et al., 2015)· (Monroy-Jaramillo et al., 2018). These monogenic vascular disorders share clinical features, including migraine, seizures and progressive subcortical dementia (https://www.omim.org/). To a different extent they also share the classical cerebral small vessel disease (cSVD) neuroradiological hallmarks (periventricular hyperintensities, superficial and deep white matter hyperintensities, status cribrosus, cortical small-microinfarcts, lacunar infarcts, cerebral microbleeds and cortical atrophy (Soong et al., 2013)· (Macaron et al., 2021)· (Dhamija et al., 2015)· (Raynowska et al., 2018)· (Saito et al., 2019)· (Schoemaker et al., 2019)· (Pantoni, 2010)), and neuropathological (capillaries with thickened walls (Saito et al., 2019)· (Brennan-Krohn et al., 2010)· (Lewandowska et al., 2010)· (Lesnik Oberstein et al., 2001)) features (Table 1).

Recently, *TREX1* p.Arg174Gly, has been detected in a patient with a classical CADASIL-like phenotype (Amico et al., 2022), in concert with the genetic reports of Pelzer and colleagues, describing significant contributions of *TREX1* mutations in CADASIL and CADASIL-like patients (Pelzer et al., 2013). However, whether CADASIL and RVCL-S overlapping phenotype may be influenced by shared genetic causative factors remains poorly investigated.

In this study, we hypothesized that *TREX1* mutations may play a critical role in CADASIL-like patients. To investigate this hypothesis, we performed exome sequencing in a multi-ethnic cohort of 180 early-onset apparently sporadic, CADASIL-like Caucasian patients from USA, Portugal, Finland, Serbia and Turkey, who did not carry any *NOTCH3* causative mutations.

2. Materials and methods

2.1. Patient cohort

The cohort was composed of 180 apparently sporadic and early-onset, CADASIL-like Caucasian patients from USA, Finland, Portugal, Serbia and Turkey (Table 2). Inclusion criteria for the USA cohort included cerebral small vessel disease (cSVD) diagnosis based on TOAST classification, early age at onset (<65 years [only 2 cases, whose age-at onset was 68 and 71 years old have been included in the study because they had a family history of the disease]) and no enrichment for vascular risk factors except for hypertension or smoking, which generally play a critical role in elderly people (Abraham et al., 2016).

The collection of samples included in this study has been approved by the ethics committee of the Faculty of Medicine, University of Coimbra, Coimbra, Portugal; the Ethics Committee of the School of Medicine, University of Belgrade (Serbia); the Ethics Committee of Istanbul Faculty of Medicine, Istanbul University and the Ethics Committee of the Hospital District of Southwest Finland. All NINDS Repository samples were collected only after an IRB-approved of the respective institutions, signed informed consent was secured by the submitter, as previously described (Messerschmidt et al., 2021, p. 1).

The diagnostic criteria for the CADASIL-like cohort from USA, Finland, Portugal, Serbia and Turkey were met by combining clinical symptoms, imaging data, positive medical history (at least one first-degree relative with small vessel disease) in the absence of known pathogenic mutations in *NOTCH3* and based on the previous literature (Pantoni et al., 2010). Ninety-six patients (53.3%) were from the USA (NINDS Repository), 34 (18.9%) from Portugal, 33 (18.3%) from Finland, 15 (8.3%) from Serbia and 2 (1.1%) from Turkey.

The US cohort has been already described in a previous genetic screening of APP and Aβ metabolism genes (Blumenau et al., 2020) and part of the Finnish cohort has been already included in a previous genetic screening focused on Mendelian genes causative for vascular dementia (Mönkäre et al., 2022). The mean age at disease onset was 52 years (SD=10.9) and 76 cases (42.2%) had a positive family history. Among the comorbidities and possible risk factors, hypertension was reported in 44.4% of the patients, diabetes type 2 in 18.3%, cardio-vascular comorbidities (myocardial infarction, atrial fibrillation) in 12.2%, migraine in 10.0% and hypercholesterolemia in 20%. Given the prevalent role of hypertension and diabetes mellitus 2 in cSVD in elderly people (Abraham et al., 2016) and the young age at onset of the cohort, these patients were considered enriched for genetic risk factors (Table 2). Finally, 478 controls >60 years of age were selected from the 'HEALTHY EXOMES', HEX database, a publicly available database, which collects exome sequencing data from elderly, neuropathologically assessed controls (https://www.alzforum.org/exomes/hex) (Guerreiro et al., 2018).

2.2. Exome sequencing

We performed whole exome sequencing (WES) on a cohort of 180 independent familial and early-onset sporadic cSVD and CADASIL-like cases. DNA was extracted from blood using standard protocols. Library preparation for next generation sequencing used 50 ng DNA. Exome libraries were prepared using Nextera Rapid Capture Exome Illumina) or SureSelect Exome Capture Kit v4 (Agilent). WES libraries were sequenced using paired-end reads of 75 or 100bp on either Illumina's NextSeq550, HiSeq2500 or HiSeq 4000.

2.2.1. Bioinformatics

For samples from the USA, reads were aligned using BWA-MEM v0.7.15 (Li, 2013) to the reference GRCh37 (hs37d5.fa), separate read groups were assigned for all reads from 1 lane, and duplicates

Table 1
Clinical, Neuroradiological and Neuropathological Features of CADASIL and RVCL-S

Feature	CADASIL	RVCL-S	References
Genetics	NOTCH3	TREX1	
Pathogenic mechanism	Impaired vascular smooth muscle cell relaxation	Vascular endothelial dysfunction	
Age at onset	Adult onset: 35 to 40 years Death usually in sixth decade	Onset in adulthood Death occurs 5 to 10 years after onset	https://www.omim.org/
Clinic	Central Nervous System	Central Nervous System	https://www.omim.org/
	Recurrent subcortical lacunar strokes Progressive subcortical dementia (6% of patients) Migraine (40% of patients) Seizures (2%-10% of patients) Pseudobulbar palsy Gait abnormalities	Subcortical lacunar strokes Progressive subcortical dementia Migraine Dysarthria Seizures Hemiparesis Apraxia	
	Eyes	Eyes	
	- Acute vision loss due to optic nerve infarction (rare) - Nonarteritic anterior ischemic optic neuropathy (NAION)	progressive decreased visual acuity Retinal vasculopathy Retinal exudates Retinal hemorrhage Macular cotton wool spots	
Neuroimaging (MRI/CT)	MRI (T2-Flair): lacunar infarcts and diffuse leukoencephalopathy with involvement of corona radiata, external capsules, and anterior temporal poles and cerebral microbleeds	CT: dominant frontal hypoattenuation with extensive vasogenic edema and several nonspecific calcifications MRI: ring-enhancing frontal mass lesion with associated edema., Multiple small T2 hyperintense lesions in the bilateral periventricular white matter.	https://www.omim.org/
Neuropathology	Vessels: degeneration of vascular smooth muscle cells and pericytes as well as the presence of granular osmiophilic material (GOM) within vessels. Central nervous system: diffuse myelin rarefaction, lacunes and cortical apoptosis.	Coalesced and partly necrotic lesions with calcification occupying the white matter of the left and right frontoparietal lobes, confluent foci of coagulation necrosis and focal calcifications surrounded by reactive gliosis in the white matter. In small vessels within the lesions, fibrous expansion of the adventitia with relative preservation of smooth muscle cells and thickening of the intimal layer	https://www.omim.org/

were masked using Samblaster v0.1.24 (Faust and Hall, 2014). Standard QC was performed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). The variants were then called using GATK UnifiedGenotyper v3.7 (DePristo et al., 2011) and annotated using Jannovar v0.24 (Jäger et al., 2014) using RefSeq v105 exons.

For non-USA samples, data processing followed GATK's Best Practices v4 as implemented in the WARP pipeline "Exome Germline Single Sample v3.0.0". Samples were joint called using GATK's GenotypeGVCFs with quality control procedures as described in the tool's Best Practices v4. Variants were annotated with snpEff v4.3t (http://snpeff.sourceforge.net/) and dbNSFP v4.0a (http://database.liulab.science/dbNSFP).

2.3. APOE genotyping

APOE ε2 allele has been reported to increase the susceptibility for cerebral small vessel disease (Groot et al., 2018) (Gesierich et al., 2016). Therefore, to exclude additional potential cerebral small vessel disease risk factors such as an enrichment for APOE ε2 allele we performed APOE genotyping in our CADASIL-like cohort.

APOE genotypes comprising the APOE ε2, ε3 and ε4 alleles, were assayed using LightCycler 480 Instrument II (Roche), as previously described (Blumenau et al., 2020). Briefly, SNP-specific primers and probes were designed by Thermo Fisher (TaqMan genotyping as-

says). The polymorphisms distinguish the ε2 allele from the ε3 and ε4 alleles at amino acid position 158 (rs7412) and the ε4 allele from the ε2 and ε3 alleles at amino acid position 112 (rs429358). TaqMan real-time polymerase chain reaction assays (PCR) consisted in 10 ul of Taqman Universal PCR Master Mix (Thermo Fisher), 0.5 ul of assay, 8.5 ul of water and 1ul of DNA at 20ng/ul. The 20 μl total volume reaction was loaded in 96-well plates and PCR was performed in a LightCycler 480 Instrument II (Roche), using a cycling program of: 95°C for 10 min; 40 cycles of 95°C for 15 sec and 60°C for 1 min. Each sample was run in duplicates. Twelve negative controls (water) were included in each plate and were consistently called correctly.

2.4. Statistical analysis and methods to prevent bias

The study had at least 80% power for the detection of common variants, MAF >5%, with strong protective (OR <0.6) or risk effect (OR >2), with a significance value of two-sided α= 0.05, as previously described (Messerschmidt et al., 2021, p. 1). Low frequency and rare variants were defined as having a 1%<MAF<5% and MAF<1%, respectively, either in cases or controls. Minor allele frequency was based either on HEX database for elderly controls >70 years of age or ExAC database version 0.3.1 (http://exac.broadinstitute.org/).

All methods were performed in accordance with the relevant guidelines and regulations.

Table 2
cSVD and CADASIL-like Exome Sequencing Cohort.

Country of Origin	N	Disease	AAO (SD)	M:F	N Cases With Family History (%)	Hypertension (%)	Diabetes (%)	N cases With Migraine (%)	N Cases With Heart Comorbidities (MI, AF) (%)	N Cases With Hypercholesterolemia (%)
US	96	cSVD	51.5 (8.1)	0.82	43 (44.7)	58 (60.4)	29 (30.2)	NA	11 (11.4)	2 (2)
Portugal	34	CADASIL-like	44.5 (12.3)	0.36	9 (26.5)	NA	NA	9 (26.5)	1 (3)	9 (26)
Finland	33	CADASIL-like	60.4 (11.9)	0.84	16 (48.5)	22 (66.6)	4 (12.1)	9 (27.3)	10 (30.3)	17 (51.5)
Serbia	15	CADASIL-like	NA	0.63	7 (46.6)	NA	NA	NA	NA	7 (47)
Turkey	2	CADASIL-like	NA	1.0	1 (50)	NA	NA	NA	NA	1 (50)
Total or average	180 (96+ 84)		52 (10.9)	0.71	76 (42.2)	80 (44.4)	33 (18.3)	18 (10)	22 (12.2)	36 (20)

Key: AAO, age at onset; AF, atrial fibrillation; CSVD, cerebral small vessel disease; F, female; M, male; MI, myocardial infarction; SD, standard deviation.

3. Results

We performed exome sequencing in a large multi-ethnic cohort of 180 early-onset apparently sporadic CADASIL-like Caucasian patients from USA, Portugal, Finland, Serbia and Turkey.

None of the patients carried any pathogenic mutations in *NOTCH3* clustering in epidermal growth factor like repeat domains, mapping to exons 3 and 4 or consisting in the gain or loss of cysteines.

We hypothesized that part of the genetic causative factors leading to CADASIL-like syndrome could be explained by genetic variability of protein coding regions and splicing sites in *TREX1*.

We screened *TREX1* in our cohort and identified 2 very rare coding variants: a loss of function variant (p.Ala129fs) located in the catalytic domain of the protein, in an apparently sporadic 46-year-old patient from the USA, and a missense variant (p.Tyr305Cys) in the well-conserved C-terminal region of the protein, in a 57-year-old patient with a positive family history from Serbia (Table 3).

3.1. Patient A, TREX1 p.Ala129fs

This 46-year-old female patient from the USA presented a very early-onset and apparently sporadic cerebral small vessel disease. Besides being a former smoker, she had no cardiovascular risk factors and there was no positive family history for cardiovascular diseases. She carried an heterozygous frameshift variant, p.Ala129fs, resulting in a premature translational stop signal in the *TREX1* gene and mapping to the *TREX1* well conserved catalytic domain (Fig. 1 A, B), harboring *TREX1* p.Arg174Gly, recently reported in a patient with CADASIL-like syndrom (Amico et al., 2022). This variant is expected to disrupt the last 176 amino acids of the TREX1 protein, therefore likely affecting the C-terminal region, where the majority of RVCL-S mutations have been reported. This variant is predicted as pathogenic/likely pathogenic, like 22/24 (92%) of frameshift mutations that have been reported in the literature in *TREX1* (Fig. 2) (https://simple-clinvar.broadinstitute.org/). In addition, this patient carried the *APOE* ε4 allele in heterozygosity that, as reported in previous studies, may not critically influence the susceptibility to cSVD (Groot et al., 2018), (Gesierich et al., 2016) (Table 3).

3.2. Patient B, TREX1 p.Tyr305Cys

This female patient displayed the first symptoms at a very early-age, 57 years old, and originated from Serbia. She presented hyperlipidemia, mild cognitive impairment, had a positive family history and her mother had migraine. She was heterozygous for a variant, p.Tyr305Cys, classified as variant of uncertain significance, based on the American College of Medical Genetics and Genomics (ACMG) criteria (Richards et al., 2015), located in the C-Terminal domain of TREX1. This domain of the protein is known to harbor loss of function mutations causative for RVCL-S (Richards et al., 2007). This variant is very rare (MAF 1.4e-04) and clusters in a very well conserved domain among different species (Fig. 1C, Table 3). Importantly, p.Tyr305Cys has already been described as causative for CADASIL-like disease in 2 patients: a 53-year old Dutch patient who displayed presenile dementia, basal ganglia and pontine lacunar infarcts and bilateral confluent white matter lesions (Pelzer et al., 2013) and a 39-year old Finnish patient presenting migrane without aura, severe and pervasive cognitive impairment, hypertensive retinopathy and severe amyloid angiopathy and whose MRI scans displayed lacunar infarcts and several microhemorrhages (Mönkäre et al., 2022).
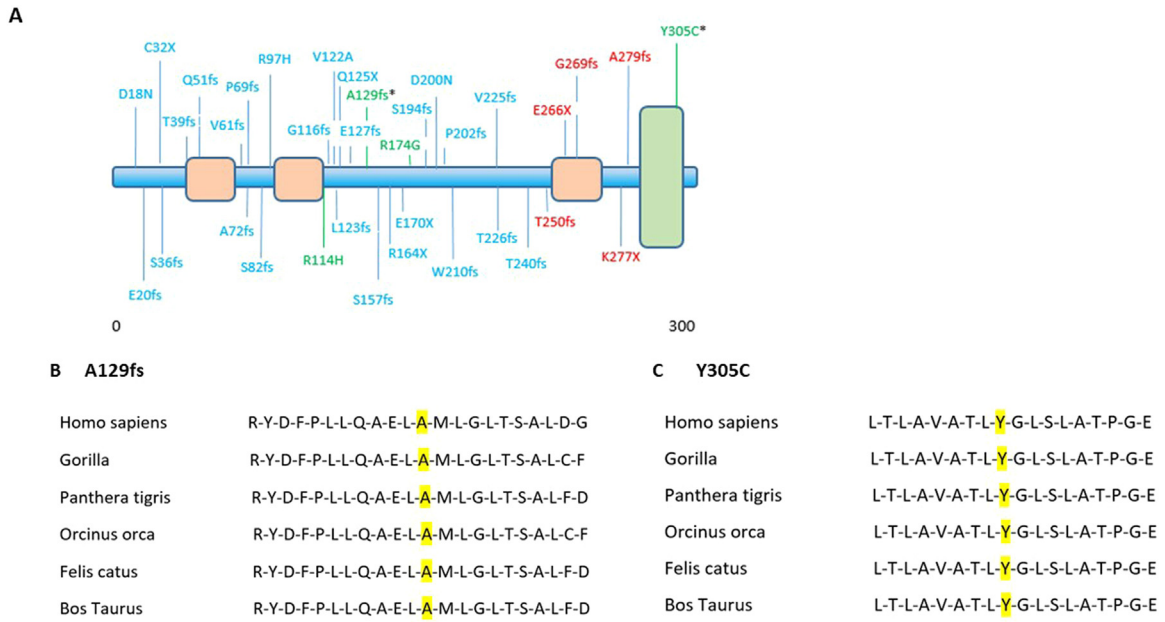
Fig. 1. (A) Pathogenic and likely pathogenic mutations detected in *TREX1*, based on Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar). In blue, variants in the N-terminal catalytic domain (1-242) containing the exonuclease activity. In red, variants in the C-terminal region (243-314) that facilitates the perinuclear localization of the enzyme. In green, pathogenic variants associated to CADASIL-like phenotypes, * variants detected in our cohort. (B–C). *TREX1* p.A129 and p.Y305 are within well conserved domains across different species. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.
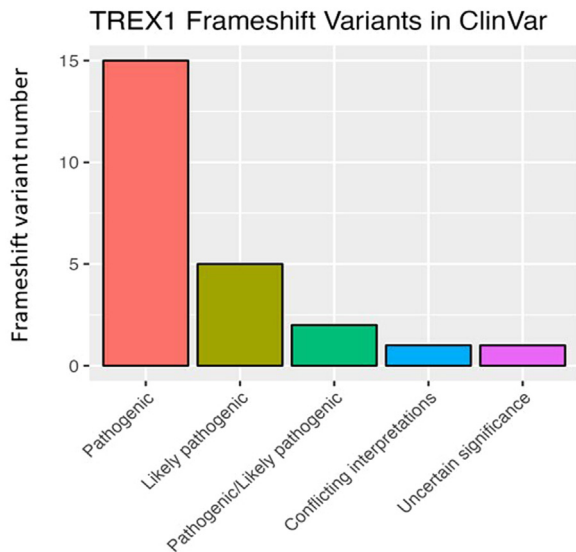
Fig. 2. Frameshift mutations detected in *TREX1* with respective effect, reported in ClinVar (https://simple-clinvar.broadinstitute.org). The vast majority (22/24 [92%]) of *TREX1* frameshift mutations, irrespective of the protein domain, are pathogenic or likely pathogenic.

Finally, *TREX1* p.Tyr305Cys has been previously reported in patients with systemic lupus erythematosus (SLE) (Namjou et al., 2011).

Therefore, although classified as variant of uncertain significance, based on ClinVar database and on the previous literature, we hypothesize that *TREX1* p.Tyr305Cys may be pathogenic or likely pathogenic.

Importantly, both Patient A and Patient B did not display severe clinical and neuroradiological hallmark features of RVCL-S (or SLE and AGS) such as retinal vasculopathy, Raynaud's phenomenon, and mild liver micronodular cirrhosis, cardiomyopathy and renal insufficiency, and subcortical calcifications and tumor-like cerebral lesions (DiFrancesco et al., 2015).

In addition, these 2 variants were not found in a cohort of 478 controls from the UK (https://www.alzforum.org/exomes/hex), where we report 3 rare heterozygous missense and likely benign mutations (p.Arg169His, p.Cys263Ser, p.Glu321Gly, Table 3).

Finally, these samples were collected before 2019, excluding a possible COVID-19 infection as vascular risk factor for some of these cases (Cruciani et al., 2021)·(Rajendran et al., 2021)·(Trifan et al., 2020).

4. Discussion

In this study we tested the hypothesis that part of the genetic susceptibility or inheritability of a large cohort of early-onset, unrelated CADASIL-like patients, not carrying any *NOTCH3* pathological mutations, may have been explained by protein coding genetic variability in *TREX1*.

We report 2 very rare variants in *TREX1*, p.Ala129fs and p.Tyr305Cys in 2 apparently sporadic and familial cases from the USA and Serbia, respectively. Importantly, both of these do not represent the canonical *TREX1* mutations leading to RVCL-S. p.Ala129fs locates in the conserved catalytic domain, harboring different loss of function (LoF) mutations leading to Aicardi-Goutières-Syndrome (AGS) or systemic lupus erythematosus (SLE). In line with our findings, a recently described *TREX1* variant, mapping to the catalytic domain, p.Arg174Gly, has been reported in a patient with a classical CADASIL-like phenotype (Amico et al., 2022). Moreover, it is plausible that p.Ala129fs may activate a nonsense mediated decay, likely leading to TREX1 reduced function on endothelial cells with subsequent increased susceptibility to small vessel disease. p.Tyr305Cys maps to the C-Terminal domain of the protein, where frameshift mutations are causative for RVCL-S and has been already reported in a 2 early-onset CADASIL-like patients from Nederland and Finland displaying overlapping features like progressive cognitive impairment to dementia, lacunar infarcts confluent and mild to severe retinopathy (Pelzer et al., 2013)·

Table 3
TREX1 Coding Variants Detected in the cSVD-CADASIL-Like Cohort and in the HEX Database Cohort of Elderly Controls (> 60 years old). TREX1 transcript ID NM_033629.6, GRCh37.

Patient ID	Gene	Position	rsID	cDNA	change	Aa	ClinVar Classification	ExAC	Domain	Phenotype	Origin	Gender	AAO	APOE	Family History	Vascular Risk Factors
Patient A	TREX1	3:48508470	rs763229085	c.416del	LOF	Ala129fs	Pathogenic/ Likely pathogenic	4e-05	N-terminal catalytic domain	cSVD	USA	Female	46	34	absent	Former smoker (0.7 y)
Patient B	TREX1	3:48508968	rs370504038	c.1079A>G	missense	Tyr305Cys	Uncertain significance	1.4e-04	C-terminal domain	CADASIL-like	Serbia	Female	57	33	Positive (mother with migraine)	hyperlipidemia
CTRLS_HEX	TREX1	3:48508395	rs72556554	c.341G>A	missense	p.Arg114His	Conflicting interpretations of pathogenicity	1.5e-04	N-terminal catalytic domain	HEX controls	UK	NA	>60	NA	NA	NA
CTRLS_HEX	TREX1	3:48508677	rs146524913	c.623G>C	missense	p.Cys208Ser	Uncertain significance	0.9e-04	C-terminal domain	HEX controls	UK	NA	>60	NA	NA	NA
CTRLS_HEX	TREX1	3:48508851	rs55999987	c.797A>G	missense	p.Glu266Gly	Conflicting interpretations of pathogenicity	1.6e-03	C-terminal domain	HEX controls	UK	NA	>60	NA	NA	NA

Key: Aa, amino-acid; AAO, age at onset; CTRLS, controls; CTRLS_HEX, ... cSVD, cerebral small vessel ischemic disease; LOF, loss of function; Neg, negative.

(Mönkäre et al., 2022) as well as distinctive signs such as migraine without aura, cerebral amyloid angiopathy and microhemorrhages (Mönkäre et al., 2022) . The carrier in our cohort presented a positive familial history for migraine however did not display severe dementia or retinal vasculopathy, suggesting that the same TREX1 mutation may give rise to heterogeneous cardiovascular phenotypes, as already described for other TREX1 coding variants such p. V235fs, reported in a Mexican family with RVCL-S phenotypic spectrum (Monroy-Jaramillo et al., 2018) and p.Arg114His, found in a CADASIL-like and SLE patient and reported in 6 controls (Pelzer et al., 2013)ꞏ (Lee-Kirsch et al., 2007)ꞏ (Namjou et al., 2011).

Moreover, none of the carriers in our cohort presented severe clinical and neuroradiological hallmark features of RVCL-S (or SLE and AGS) such as retinopathy, Raynaud's phenomenon, and mild liver micronodular cirrhosis, renal failure and cardiomyopathy, cerebral pseudotumoral lesions and subcortical calcifications (DiFrancesco et al., 2015). This depicts TREX1 as a pleiotropic gene, where the same mutation or different mutations in the same domain lead to a spectrum of cardiovascular phenotypes, further expanding TREX1 broad range of genotype–phenotype correlations (Table 4). This is in line with a well consolidated genetic paradigm and exemplified by several well conserved monogenic Mendelian genes, such as CSF1R, SORL1 and TREM2, whose rare coding pathogenic mutations have been reported to cause a wide spectrum of dementing phenotypes (Sassi et al., 2018, p. 3)ꞏ (Xiromerisiou et al., 2021)ꞏ (Ibanez et al., 2018)ꞏ (Carmona et al., 2018).

Importantly, although TREX1 is mostly expressed in endothelial cells (Sassi et al., 2018, p. 3) and TREX1 mutations have been linked to endothelial dysfunction leading to small vessel disease and CADASIL to impaired smooth muscle cell relaxation(de Boer et al., 2018), we hypothesize that endothelial impairment may play a pathophysiological role and influence also smooth muscle cells. In addition, there is evidence for endothelial dysfunction in the microvasculature of CADASIL patients (Stenborg et al., 2007). It is therefore plausible that TREX1 p.Ala129fs and p.Tyr305Cys through direct endothelial damage or, alternatively, through a endothelial-dependent smooth muscle cell degeneration, may cause a CADASIL-like phenotype.

Importantly, this is the first study investigating the role of TREX1 mutations in a large multi-ethnic cohort of unrelated CADASIL-like patients, who did not carry causative mutations in NOTCH3. Previous literature investigated the role of TREX1 in multigenerational families or small groups of related patients from Asia (Taiwan, China, Japan), Europe (Italy, Nederland, Germany, Switzerland), USA (St. Louis, Los Angeles) and Mexico (Soong et al., 2013)ꞏ (Xie et al., 2021)ꞏ (Saito et al., 2019)ꞏ (DiFrancesco et al., 2015)ꞏ (Pelzer et al., 2013)ꞏ (Hedderich et al., 2020)ꞏ (Dhamija et al., 2015)ꞏ (Monroy-Jaramillo et al., 2018). Our study expands the clinical endophenotypes associated to TREX1 coding mutations and the genetic spectrum at the basis of CADASIL-like phenotype and warrants additional studies in larger cohorts. Finally, considering the likely endothelial-smooth muscle cell microvascular pathophysiological link between RVCL-S and CADASIL, and the striking clinical and neuroradiological variability of NOTCH3 pathogenic mutations (Opherk et al., 2006), it may be worth investigating the role of TREX1 variants as potential genetic modifiers in CADASIL patients.

Disclosure statement

Table 4
Phenotypic Spectrum Caused by *TREX1* Mutations.

Feature	AGS	SLE	RVCL	CADASIL-Like	References
Inheritability	AR, AD	AD	AD		https://www.omim.org/
Genetics	*TREX1* homozygous, compound heterozygous, or heterozygous mutations	*TREX1* heterozygous mutations	*TREX1* heterozygous C-terminal frameshift mutations	*TREX1* heterozygous mutations	
Pathogenic mechanism	Impaired TREX1 exonuclease activity with accumulation of intracellular DNA repair and replication intermediates, which then trigger an inappropriate viral-like innate immune response.	Impaired TREX1 exonuclease activity with accumulation of intracellular DNA repair and replication intermediates, which then trigger an inappropriate viral-like innate immune response.	Impaired TREX1 exonuclease activity with accumulation of intracellular DNA repair and replication intermediates, endothelial dysfunction	Impaired TREX1 exonuclease or catalytic activity with accumulation of intracellular DNA repair and replication intermediates, endothelial dysfunction, likely indirect impairment of smooth muscle cells	https://www.omim.org/
Age at onset	Onset within first year of life	Onset between ages 16-55,	Onset in adulthood	Onset in adulthood	https://www.omim.org/
Clinic	Neurologic Encephalopathy Developmental retardation, profound Truncal hypotonia Tetraplegic spasticity Dystonia Abnormal eye movements Seizures Systemic manifestations Microcephaly, progressive, Hepatomegaly, Splenomegaly, Chilblains, Purpura, Petechiae, Thrombocytopenia	Neurologic Seizures Psychosis Systemic manifestations Erythematous malar rash Photosensitivity iscoid rash, - Leukopenia Thrombocytopenia Hemolytic anemia	Neurologic Lacunar strokes; Progressive subcortical dementia (executive function, processing speed); Migraine; Dysarthria; Seizures; Hemiparesis; Apraxia Systemic manifestations Retinopathy Renal failure Hepatic failure	Neurologic Lacunar strokes; Progressive subcortical dementia (executive function, processing speed); Migraine; Dysarthria; Seizures; Hemiparesis; Apraxia	https://www.omim.org/
Neuroimaging (MRI/CT)	Progressive cerebral atrophy bilateral, symmetric intracerebral calcifications, especially in the basal ganglia and periventricular areas, deep white matter hypodensities, Leukoencephalopathy		MRI: mass lesion with calcifications and associated edema	MRI: lacunar subcortical strokes, confluent periventricular white matter hyperintensities	https://www.omim.org/

Key: AD, autosomal dominant; AGS, Aicardi-Goutieres Syndrome; AR, autosomal recessive; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CT, computer tomography; MRI, magneto-resonance imaging; RVCL-S, retinal vasculopathy with cerebral leukodystrophy and systemic manifestations; SLE, systemic lupus erythematosus.

Author contributions

Data availability

All data generated or analyzed during this study are included in this published article and are publicly available at the ClinVar repository (https://www.ncbi.nlm.nih.gov/clinvar/), accession numbers are SCV002571716 - SCV002571720.

Acknowledgements

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