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RESEARCH ARTICLE

TRIM25 mutation (p.C168*), coding for an E3 ubiquitin ligase, is a cause of early-onset autosomal dominant dementia with amyloid load and parkinsonism

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Abstract

Introduction: Patients with familial early-onset dementia (EOD) pose a unique opportunity for gene identification studies.

Methods: We present the phenotype and whole-exome sequencing (WES) study of an autosomal dominant EOD family. Candidate genes were examined in a set of dementia cases and controls (n = 3712). Western blotting was conducted of the wild-type and mutant protein of the final candidate.

Results: Age at disease onset was 60 years (range 56 to 63). The phenotype comprised mixed amnestic and behavioral features, and parkinsonism. Cerebrospinal fluid and plasma biomarkers, and a positron emission tomography amyloid study suggested Alzheimer's disease. WES and the segregation pattern pointed to a nonsense mutation in the *TRIM25* gene (p.C168*), coding for an E3 ubiquitin ligase, which was absent in the cohorts studied. Protein studies supported a loss-of-function mechanism.

Discussion: This study supports a new physiopathological mechanism for brain amyloidosis. Furthermore, it extends the role of E3 ubiquitin ligases dysfunction in the development of neurodegenerative diseases.

KEYWORDS
Alzheimer's disease, E3 ubiquitin ligase, early-onset autosomal dominant dementia, family-based whole-exome sequencing study, *TRIM25*

Highlights

- A *TRIM25* nonsense mutation (p.C168*) is associated with autosomal dominant early-onset dementia and parkinsonism with biomarkers suggestive of Alzheimer's disease.
- TRIM25 protein studies support that the mutation exerts its effect through loss of function.

- TRIM25, an E3 ubiquitin ligase, is known for its role in the innate immune response but this is the first report of association with neurodegeneration.
- The role of TRIM25 dysfunction in development of amyloidosis and neurodegeneration merits a new line of research.

1 | BACKGROUND

Continuous advancements in sequencing techniques have made time- and cost-effective detection of genetic variants a reality. While extensive genome-wide association studies allow for detection of risk loci for Alzheimer's disease (AD), whole-exome sequencing (WES) of families with inherited dementia is more likely to detect causal variants. Early-onset dementia (EOD), defined as onset prior to age 65 years, occurs in ≈0.098% of the population,[1,2] and patients with the disease often have a higher genetic burden than late-onset cases, with ≈10% showing an autosomal dominant inheritance pattern.[3] This subgroup of EOD families poses a unique opportunity for gene identification studies, which may pinpoint novel molecular pathways potentially relevant to all dementia patients.

We present a family-based WES study of a Spanish EOD family with autosomal dominant inheritance that was conducted in the context of an European Early-Onset Dementia (EU EOD) Consortium collaboration.[4] Biomarkers in cerebrospinal fluid (CSF) and plasma indicated the presence of amyloidosis and likely AD owing to elevated phosphorylated tau (p-tau)181, though the phenotype was also relevant for associated parkinsonism. The study revealed a nonsense mutation in the *TRIM25* gene (p.C168*) as the underlying gene defect. This mutation has not been reported in genetic databases and was not found in an analysis of extended patient–control cohorts, suggesting a rare cause of familial dementia.

TRIM25 codes for an RNA-binding protein (i.e., tripartite motif-containing protein 25) acting as an E3 ubiquitin ligase,[5] enzymes which are involved in the selective recognition and ubiquitination of proteins with multiple outcomes such as degradation through the proteasome system[6,7] or activation of signaling pathways.[8] Mutations in other E3 ubiquitin ligases are known to be associated with neurodegenerative diseases such as juvenile recessive Parkinson's disease (Parkin)[9] or Lafora disease (Malin).[10,11] The study of this family adds a new gene to the panel of genetic causes of neurodegenerative diseases and suggests a novel underlying mechanism for brain amyloidosis. Furthermore, it extends the role of E3 ubiquitin ligase dysfunction in the development of neurodegenerative diseases.

2 | MATERIAL AND METHODS

2.1 | Family

The EOD family was recruited from the Memory Clinic of Fundación Jiménez Díaz (Madrid, Spain) and selected for molecular genetic research in the context of an EU EOD Consortium collaboration (family tree in Figure 1). The kindred consisted of eight children of a woman (FH) with EOD at 60 years of age in whom the segregation pattern was consistent with autosomal dominant inheritance. Seven of the children have been clinically evaluated and provided DNA samples after informed consent. Five (P1 to P5) have developed EOD at an average age of 60 years (range 56 to 63 years), while two (III-6 and III-7) remain cognitively spared at 68 and 65 years of age, respectively. AD was the likely diagnosis, based on CSF biomarkers and positron emission tomography (PET) amyloid studies of P3, and plasma biomarkers of P1, P2, P4, and P5.

FH was a first and only child, as her mother died during childbirth. Her father, who died at 60 years of age, subsequently married his sister-in-law, with whom he had another seven children before she also died prematurely in an accident at age 55 years. This "second branch" of the pedigree was dementia-free, considering the large number of offspring with no reports of dementia ($n = 21$); four cognitively normal relatives from this branch (cases III-9 to III-12, ages 49 to 72) agreed to provide DNA samples for this study.

DNA of the index patient (P1) was found to be negative for mutations in *PSEN1*, *PSEN2*, *APP*, *MAPT*, *GRN*, *VCP*, *TARDBP*, *FUS*, *SNCA*, *TREM2*, *CSF1R*, *SQSTM1*, *Serpini1*, and *TYROBP* using a next-generation sequencing (NGS) dementia panel. Additionally, repeat-primed polymerase chain reaction (PCR) assay for *C9orf72* revealed no hexanucleotide expansion.

2.2 | Whole-exome sequencing, variant calling, and single nucleotide polymorphism genotyping

WES was performed on gDNA of patients (P1, P2, P3, P4) and one unaffected sibling (III-6) using the SeqCap EZ Exome Library v3.0 kit from Roche. Biotinylated oligonucleotide baits were included to cover all coding exons and untranslated regions (UTRs) in the genome to capture complementary single strand gDNA. Subsequently, sample libraries were amplified through bridge amplification, and DNA clusters were sequenced on an Illumina NextSeq500 Sequencing platform. The analysis was performed with a standardized pipeline integrated in the GenomeComb package (http://genomecomb.sourceforge.net/).[12] The pipeline used Fastq-Mcf for adapter clipping. Reads were then aligned using Burrows-Wheeler Aligner (BWA-MEM) (https://arxiv.org/abs/1303.3997) against the human reference GRCh37 (hg19). Bam files were sorted and duplicates were removed using biobambam bammarkduplicates2. Realignment in the vicinity of indels was performed with the Genome Analysis Toolkit (GATK).[13] Variants were called at

RESEARCH IN CONTEXT

1. **Systematic Review**: Whole exome sequencing of familial early-onset dementias (EOD) pose a unique opportunity for gene identification studies, which may provide new molecular insights into neurodegenerative diseases.
2. **Interpretation**: We report a family with autosomal dominant EOD and parkinsonism caused by a nonsense mutation in *TRIM25* (p.C168*), coding for an E3 ubiquitin ligase, through a loss-of-function mechanism. This gene has been previously related with the innate immune response but not with neurodegeneration.
3. **Future Directions**: This report opens a whole new line of research to address the relationship between *TRIM25* and neurodegeneration. As examples, to explore: (a) the probable link between amyloidosis/Alzheimer's disease pathology and TRIM25 dysfunction, (b) whether TRIM25 dysfunction could be associated with Lewy body formation or other ways of striatonigral degeneration, (c) the relationship between the role of TRIM25 in the innate immune response and in neurodegeneration, (d) the presence of *TRIM25* mutations in international cohorts.

all positions with a total coverage ≥ 5 using GATK (version 3.8 UnifiedGenotyper). At this stage, positions with a coverage < 5 or a quality score < 30 were considered unsequenced. The resulting variant sets of different individuals were combined and annotated using GenomeComb.

Several consecutive steps of variant prioritization were applied to extract candidate pathogenic mutations. Following the dominant inheritance model, variant selection was based on high-quality (coverage ≥15×), protein-altering coding variants shared by the four patients in heterozygous state, with minor allele frequency (MAF) ≤ 0.01% according to the Genome Aggregation Consortium_Non-Finnish European (GnomAD NFE) database. Combined Annotation Dependent Depletion (CADD) scores were generated to predict the impact of nonsynonymous variants (http://cadd.gs.washington.edu/score).[14] Selected variants were validated by Sanger sequencing.

In the unaffected relatives from the "second branch" sampled later (III-9, III-10, III-11, III-12), the 16 prioritized candidate variants obtained on WES analysis were genotyped by a custom-designed multi-amplicon single nucleotide polymorphism (SNP) panel (Agilent Technologies Niel, Multiplicom) followed by sequencing on a MiSeq sequencer (Illumina). Variants were validated by Sanger sequencing. Finally, DNA samples from siblings III-7 and P5 became available and were analyzed for the remaining candidate variants by Sanger sequencing.

2.3 | Haplotype sharing analysis

Allele sharing was investigated using polymorphic short tandem repeat (STR) markers surrounding *TRIM25* and *EEA1*. STRs were PCR-amplified using fluorescent-labeled primers and sized using GeneScan 500 Liz Size Standard (Applied Biosystems) on an ABI3730xl DNA Analyzer (Applied Biosystems).

2.4 | Patient–control cohorts

We performed full exonic resequencing of selected candidate genes (as previously described and validated by Sanger sequencing) in two sets of unrelated Spanish and Belgian patients as well as controls recruited from the EU EOD Consortium. We first included cohorts of frontotemporal dementia (FTD) cases, as the phenotype of two of the siblings comprised many behavioral features. Later, we extended the study to AD cases after findings from CSF biomarkers and amyloid PET of P3 supported an underlying AD pathology.

The Spanish set included the following: (a) 583 FTD patients (mean age at onset 63 ± 10 years), 37 with concomitant amyotrophic lateral

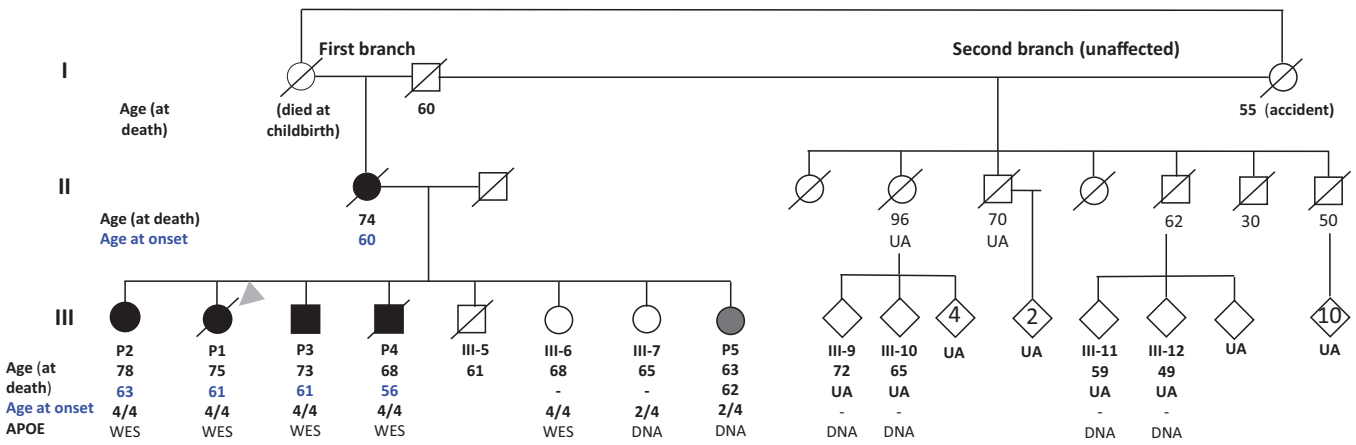

FIGURE 1 Pedigree of the family. Affected individuals in black (arrowhead in index patient P1). Age at last examination or age at death (top). WES indicates subjects who underwent whole-exome sequencing. *APOE*, apolipoprotein E; UA, unaffected; WES, whole exome sequencing

sclerosis (ALS), with clinical diagnosis based on established international diagnostic criteria;[15–17] (b) 496 control individuals (mean age 60 years ± 14), with no personal or family history of neurodegenerative disease and a Mini-Mental State Examination score > 25; and (c) 34 cases with EOD and a strong family history recruited from the same geographical area as the reported family and with the implication of other known genes ruled out by a NGS dementia panel. We also consulted data on the final candidate genes in the Collaborative Spanish Variant Server (CSVS, http://csvs.babelomics.org),[18] which currently stores genomic information on 2094 unrelated Spanish individuals from the normal population, patients with neurologic and psychiatric diseases, as well as all other kinds of conditions. Variants with MAF < 0.01 and CADD > 20 were listed.

We additionally consulted an in-house Belgian WES database of 269 FTD patients and 236 AD cases for the selected candidate genes. All had been screened for known disease-causing genes associated with the FTD-ALS spectrum, AD, prion disease, and Parkinson's disease.

All research participants or their legal representatives provided informed consent to participate in clinical and genetic research. Consent forms were approved by the local ethics committees at Fundación Jiménez Díaz (Madrid, Spain) and the University of Antwerp (Belgium).

2.5 | TRIM25 transcript and protein studies

We examined whether the mutant mRNA was degraded through nonsense-mediated mRNA decay (NMD) by Sanger sequencing of cDNA obtained from lymphoblast cells of P3, using primers mapping to exon 1 and 2 flanking the position of the p.C168* mutation. We performed co-immunoprecipitation to determine whether the C168* mutant TRIM25 was able to dimerize with wild-type (WT) TRIM25. Extracts prepared from HEK 293 TRIM25 knock-out cells transfected with plasmids expressing T7-tagged C168* TRIM25 and enhanced green fluorescent protein (eGFP)-tagged WT TRIM25 were incubated with protein A agarose with immunoglobulin G, anti-T7, or anti-eGFP antibody. The bound proteins were separated on a 4% to 12% sodium dodecyl sulfate polyacrylamide gel and analyzed by western blotting using anti-TRIM25 antibody (Abcamab167154).

To determine whether C168* TRIM25 had any effect on the ubiquitination activity of WT TRIM25, HEK 293 cells with TRIM25 knocked out were transfected with eGFP-TRIM25 on its own or co-expressed with C168* TRIM25. The extracts were analyzed by western blotting.

3 | RESULTS

3.1 | Clinical phenotypes

The main clinical features of the affected siblings are summarized in Table 1.

Initial clinical symptoms included cognitive decline in all affected siblings consisting of memory loss, anomia, and apraxia. P2 and P3 have developed an AD-type phenotype with predominant memory impairment, while another two (P1 and P4) had a mixed dementia syndrome with behavioral and neuropsychiatric features (compulsive behaviors, agitation, delusions, and hallucinations) associated with a prominent dysexecutive syndrome in the early stages. Both required treatment with neuroleptics and developed severe parkinsonism that resolved only partially after discontinuing risperidone. In the middle stages of the disease all siblings were aphasic, presenting multiple apraxias and visual agnosia. P3 has also developed a spontaneous right predominant parkinsonism. P2 and P3 have experienced a slower decline than P1 and P4, both of whom had substantial functional decline within 5 years of onset. P5 currently exhibits a behavioral phenotype with depression, apathy, and social withdrawal, together with anomia, reiterative conversations, and obsessive thoughts. The survival time in the three deceased siblings was 12 to 14 years.

Neuroimaging showed asymmetric left predominant atrophy or hypoperfusion (single photo emission computed tomography with Technetium-99m hexamethyl propylenamine oxime) in three siblings (P1, P3, and P4). A computed tomography scan of P1 obtained 1 year before death showed severe atrophy of the temporal lobes (Figure 2A). A brain magnetic resonance imaging scan of P3 revealed mild atrophy of the left temporal pole and parietal cortex (Figure 2B). Diffuse symmetric cortical atrophy with severe hippocampal atrophy was observed in P2 at 72 years of age.

CSF biomarkers were analyzed in P3 at 68 years of age with the following results: amyloid beta (Aβ)42 567 pg/ml (normal > 700), Aβ42/40 ratio 0.079 (> 0.068), p-tau181 99 pg/ml (< 59), and total tau (t-tau) 631 pg/ml (< 410). This is an A–T+N+ pattern if A is rated based on the Aβ42/40 ratio (preferable according to Delaby et al.[19]), but A+T+N+ if rated only by Aβ42 levels.[20,21] Although elevated p-tau181 is highly specific for AD, we further completed the study of P3 with an amyloid PET with 18 F-flutemetamol (Vizamyl, GE Healthcare), which was positive (Figure 2C). Plasma AD biomarkers (Aβ42/40 ratio, t-tau, and p-tau181) in stored samples of P1, P2, P4, and P5, analyzed through a Simoa assay kit (Quanterix), further suggested AD (Table 1). Apolipoprotein E (*APOE*) testing indicated that four affected patients (P1, P2, P3, and P4) and the unaffected sibling III-6 were homozygous for *APOE* ε4/ε4. Unaffected sibling III-7 and P5 were *APOE* ε2/ε4.

3.2 | Whole-exome sequencing

WES of four affected (P1 to P4) and one unaffected (III-6) siblings detected a total of 1315 high-quality, protein-altering coding variants shared in heterozygous state by all affected siblings. Filtering for rare, high-penetrant likely pathogenic mutations (MAF < 0.01%) prioritized 16 candidates (Table 2), including three loss-of-function (LOF) variants, two in-frame deletions, and 11 missense, with 10 of them having a CADD score > 20. Based on WES data from unaffected sibling III-6 and SNP genotyping of the 16 candidate variants in four unaffected offspring from the second branch (III-9, III-10, III-11, III-12), we excluded 12 variants that were present in one or more cognitively healthy relatives (Table 2). The list of candidate genes was narrowed down to three genes with CADD score > 20: *TRIM25*

TABLE 1 Summary of clinical phenotypes of affected siblings

Case/sex	Age at onset/ current age	Remarkable clinical features (staged)	Neuroimaging (CT/MRI)	Other studies
P1 / F	61 / 75*	**Mild**: delirium and hallucinations, obsessive thoughts. Anomia and visual agnosia. Dysexecutive, perseverative. Severe parkinsonism after risperidone **Moderate**: mutism. Persistent parkinsonism (without neuroleptics) **Severe**: global aphasia, frontal reflexes, axial and limb rigidity (> right), occasional myoclonus	Cortical atrophy, left predominant Severe bitemporal atrophy	**SPECT HMPAO**: bilateral frontotemporal hypoperfusion plus left parietal ^**Plasma biomarker** (64 years): p-tau 5.14
P2 / F	63 / 78	**Mild**: memory loss, anomia, disorientation **Moderate**: aphaso-apraxo-agnosic syndrome **Severe**: severe aphasia, sporadic myoclonus. No behavioral problems	Hippocampal and diffuse cortical atrophy	**Plasma biomarkers (77 years)**: Aβ42 7.1; ratio Aβ42/40 0.043; total tau 3.9
P3 / M	61 / 73	**Mild**: memory loss, anomia, apraxia. Obsessive thoughts. Secondary parkinsonism (reversible) **Moderate**: slow progression. Aphaso-apraxo-agnosic syndrome. No behavioral problems **Severe**: mild right parkinsonism	Atrophy of left temporal pole and biparietal, left predominant	**CSF biomarkers**: Aβ42 567 (> 700); ratio Aβ42/40 0.079 (> 0.068); total tau 631 (< 410); p-tau 99 (< 59); **PET 18F-flutemetamol**: positive
P4 / M	56 / 68*	**Mild**: loss of recent memory, anomia, apraxia. Significant behavioral phenotype: obsessive and psychotic thoughts, irritability, apathy, insomnia **Moderate**: aggressive, hyperphagia. Parkinsonism after risperidone **Severe**: myoclonus and seizures	Mild bifrontal atrophy, left predominant	**SPECT HMPAO**: left predominant frontotemporal hypoperfusion plus biparietal right predominant **Plasma biomarkers (64 years)**: Aβ42 7.9; ratio Aβ42/40 0.048; total tau 8.1; p-tau 6.21
P5 / F	61 / 63	**Mild**: depression, social withdrawal, repetitive conversations, memory loss	Not available	**Plasma biomarkers (62 years)**: Aβ42 4,0; ratio Aβ42/40 0.048; total tau 4,8; p-tau 1,64

*Deceased.

^Plasma biomarkers are supportive of AD when: Aβ42 < 18,1 pg/ml; Aβ42/40 ratio < 0.061; total tau > 3,4 pg/ml. For p-tau181 the reference levels in our cohorts are: cognitively healthy, 70- to 85 year-old individuals, mean 1.42 pg/ml (range 0.3 to 4.5); individuals with advanced dementia mean 3.14 pg/ml (range 0.6 to 8.0).

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; CT, computed tomography; MRI, magnetic resonance imaging; p-tau, phosphorylated tau; SPECT HMPAO, single photo emission computed tomography with Technetium-99m hexamethyl propylenamine oxime.

(p.C168*), *ABI3BP* (p.D1004Ifs*16), *DDX54* (p.M154V), and a missense *EEA1* variant (p.V693A) with CADD 18.2.

In a subsequent step, sibling III-7 was confirmed as unaffected at 65 years of age and her DNA analysis was positive for the *ABI3BP* and *DDX54* variants, reducing the candidate pathogenic variants with full co-segregation to the *TRIM25* and *EEA1* mutations. Finally, genetic analysis of the last affected sibling (P5) was positive for both the *TRIM25* and *EEA1* mutations.

3.3 | STR haplotyping analysis

STR-based haplotype analyses revealed full segregation of shared haplotypes in the four patients around the *TRIM25* and *EEA1* genes, with alternative haplotypes in the unaffected siblings (Table S1 in supporting information). Notably, the shared haplotypes around the *TRIM25* and *EEA1* genes were also absent from the second branch (III-9 to III-12).

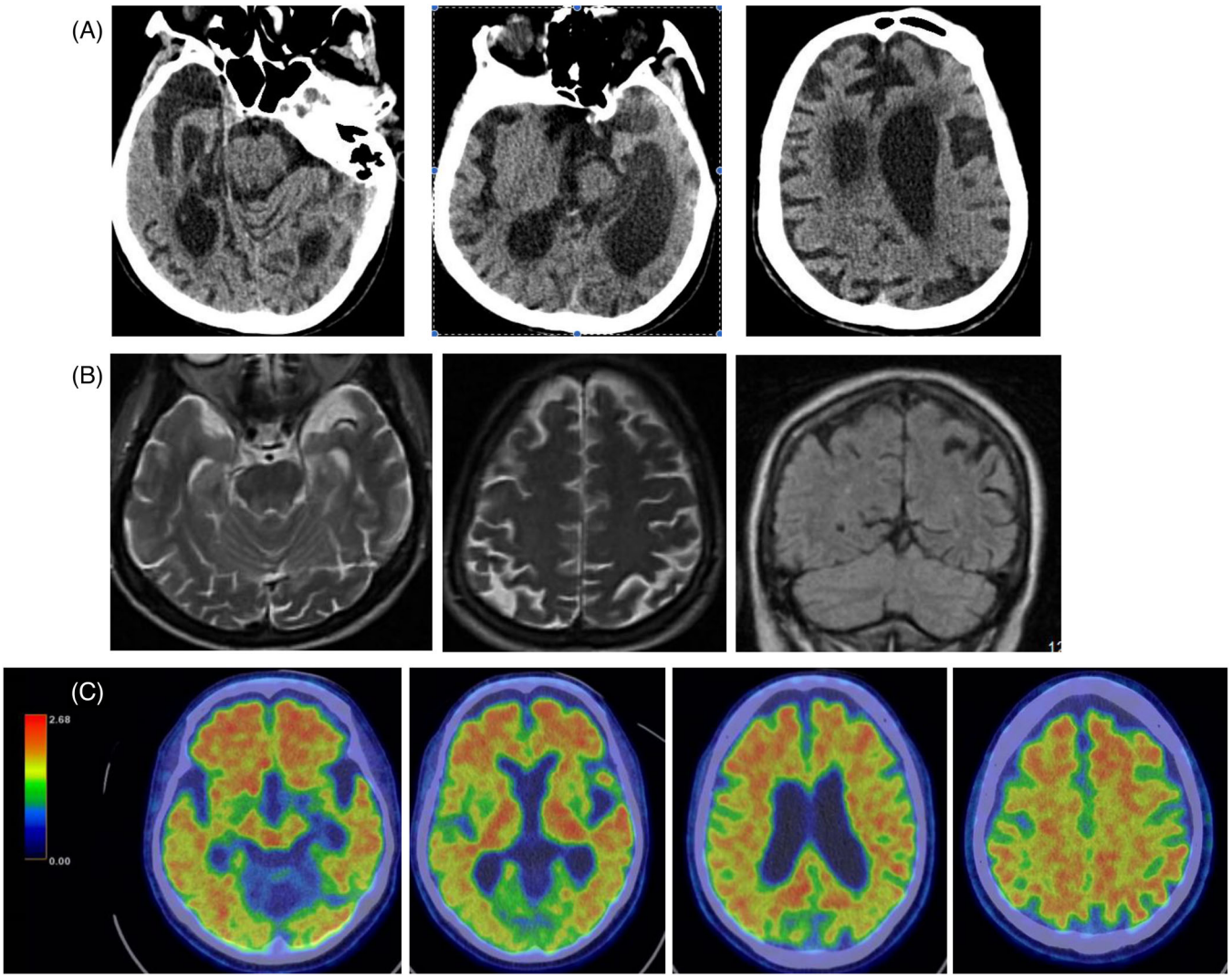

FIGURE 2 Neuroimaging of affected siblings P1 and P3: A, Computed tomography scan of P1 showing severe atrophy at 75 years of age; (B) magnetic resonance imaging scan of P3 at 68 years of age showing atrophy of left temporal pole and biparietal; (C) amyloid positron emission tomography with 18F-flutemetamol of P3 at 71 years of age

3.4 | Follow-up of candidate genes in patient–control cohorts

Full exonic resequencing of *TRIM25* in the Spanish cohort of FTD, EOD cases, and controls detected one missense variant with MAF < 0.01 and CADD > 20 in one control and none in patients (Table 3). The CSVS dataset included one missense variant in one patient and six in controls. In the Belgian population, one missense variant was found in one patient and a nonsense variant (p.E515*) was identified in a patient with a clinical diagnosis of probable FTD in 1996. The clinical significance of this mutation is unknown. CSF was sampled and banked at the time and later recovered to analyze AD biomarkers with negative results.

With regard to the *EEA1* variants, potentially pathogenic variants were found in 0.7% to 3.8% (Spanish–Belgian) of the cohorts. All were missense variants of unknown significance and were similarly distributed across the patient-control groups (Table 3). The CSVS dataset contained two nonsense variants (one in a patient and another in a control) plus missense variants in 5.2% of the individuals in the studied groups.

3.5 | TRIM25 transcript and protein studies

cDNA sequencing of *TRIM25* showed the presence of the mutant transcript, ruling out nonsense-mediated mRNA decay and suggesting the formation of a truncated protein. We examined whether the mutant TRIM25 p.C168* could have a dominant-negative effect on the function of WT TRIM25. To do that, we overexpressed TRIM25 p.C168* and analyzed auto-ubiquitination of WT TRIM25. WT TRIM25 was present

TABLE 2 Filtered variants from WES data analysis in the family (P1, P2, P3, P4, and III-6) and analysis of unaffected relatives (III-7, and III-9 to III-12) reduced the final variants segregating with the disease to TRIM25 and EEA1

Chr.	Genomic position	Gene	Predicted protein	GnomAD NFE MAF (%)	CADD score	dbSNP147	GTEx	Presence in unaffecteds
1	43.804.235	MPL	p.L79Efs*83	6/126610 (0.005)	0	rs587778514	N/A	III-6
1	52.385.700	RAB3B	p.K186Q	0/111708 (0.00)	24.1	–	no	III-6, III-9
2	167.330.808	SCN7A	p.A94E	0/56090 (0.00)	32	rs200874141	N/A	III-6
2	206.166.215	PARD3B	p.S807C	5/108616 (0.005)	26.3	rs753948335	yes	III-6
3	77.530.342	ROBO2	p.D213E	2/125208 (0.0016)	20.7	rs184080216	yes	III-9, III-10
3	100.470.497	ABI3BP	p.D1004Ifs*16	–	23.9	–	yes	III-7
3	159.482.285	IQCJ-SCHIP1	p.S116_S117del	–	28.3	–	yes	III-9, III-10
5	176.026.120	GPRIN1	p.E233_K240del	–	0	rs142779818	yes	III-6, III-9, III-10, III-11, III-12
10	95.111.015	MYOF	p.A1274V	4/ 126264 (0.0032)	24.1	rs553662967	yes	III-9
12	7.510.156	CD163L1	p.E1460Q	–	0.3	–	no	III-6
12	51.740.409	CELA1	p.Y5S	0/90746 (0.00)	9.7	rs117443541	N/A	III-6
12	51.740.410	CELA1	p.Y5H	0/90066 (0.00)	4.7	rs116944010	N/A	III-6
12	93.205.175	**EEA1**	p.V693A	1/106372 (0.00094)	18.2	–	yes	
12	113.617.052	DDX54	p.M154V	1/126470 (0.00097)	20.8	rs752571479	yes	III-7
17	54.990.846	**TRIM25**	p.C168*	–	36	–	yes	
17	74.075.385	ZACN	p.G14W	3/111514 (0.0026)	23.2	rs201264257	N/A	III-6

gDNA numbering according to the human reference sequence (Genome Reference Consortium Human Build 3/human genome 19, GRCh37/hg19).
Abbreviations: CADD, Combined Annotation Dependent Depletion (http://cadd.gs.washington.edu) (Kircher et al.[14]); Chr., chromosome; GTEx, Genotype-Tissue Expression, brain expression according to the Human Protein Atlas (https://www.proteinatlas.org); dbSNP147, the Single Nucleotide Polymorphism Database version 147; GnomAD_NFE, Genome Aggregation Consortium_Non-Finnish European (http://exac.broadinstitute.org/); MAF, minor allele frequency (%); NA, not available; WES, whole exome sequencing.

as a double band on the western blot, providing evidence that TRIM25 p.C168* does not interfere with WT TRIM25 auto-ubiquitination and activity (Figure 3A).

The TRIM25 p.C168* mutant is truncated before the coiled-coil domain, thus it is likely unable to homodimerize or heterodimerize with the WT TRIM25. To test this, we performed co-immunoprecipitation experiments with T7-TRIM25 p.C168* and eGFP-TRIM25 fusion protein on the background of TRIM25 knock-out cells. We found that the TRIM25 C168* mutant protein was not able to form a dimer with WT TRIM25 (Figure 3B). Thus, the mutant does not participate in or interfere with the formation of a functional heterodimer.

4 | DISCUSSION

This family-based exome sequencing study in an EOD family with autosomal dominant inheritance identified a LOF, co-segregating, novel variant in the *TRIM25* gene, which can be considered the cause of the disease.

For several years there have been doubts as to the underlying disease in this family, as the siblings had mixed phenotypes (as frequently occurs in EOD), and a neuropathological study has not been available to date. Studied biomarkers in a first sibling indicated underlying amyloidosis (positive amyloid PET and decrease Aβ42 in CSF) and very likely a full AD pathological phenotype (as consistent with elevated p-tau181 in CSF). More recently, biomarkers analyzed in plasma samples of affected siblings, including P5 with very incipient disease, further supported AD. Although plasma biomarkers have not yet been widely implemented in clinical practice for the diagnosis of AD, the Aβ42/40 ratio seems to have a high accuracy to detect underlying amyloidosis.[22] Given the predisposition of the siblings to develop parkinsonism in early stages of the disease, we speculate that the AD could be associated with another proteinopathy, likely Lewy body disease, which is the most common copathology in AD and particularly frequent in genetic forms.[23,24] The duration of the disease, ≈14 years, is more consistent

TABLE 3 Screening of TRIM25 and EEA1 variants in the Spanish–Belgian cohorts. Data shown are variants with minor allele frequency (MAF) < 0.01 and pathogenic prediction with CADD score > 20

			TRIM25		*EEA1*	
	Group	N	N variant	Carrier freq (%)	N variant	Carrier freq (%)
Spanish	EOD	34	0	0	-	-
	FTD	583	0	0	5	1
	CON	496	1	0,2	2	0,4
	Total	1113	1	0,09	7	0,74
CSVS	Patients	181	1	0,5	3*	3,3
	Controls	1169	6	0,7	19*	5,9
	Total^	2094	7	0,4	33**	5,2
Belgian	AD	236	0	0	6	3,8
	FTD	269	2*	0,7	6	3,7
	Total	505	2	0,3	11	3,7

Notes: Patients refer to the group of mental/behavioral disorders and neurologic diseases. Controls are specific for these groups. ^Total includes the entire database (additional diseases and controls).
All variants are missense except three stopcodon variants labelled with an asterisk.
Abbreviations: AD, Alzheimer's disease; CADD, Combined Annotation Dependent Depletion; CON, control; CSVS, Collaborative Spanish Variant Server; EOD, early-onset dementia; FTD, frontotemporal dementia.

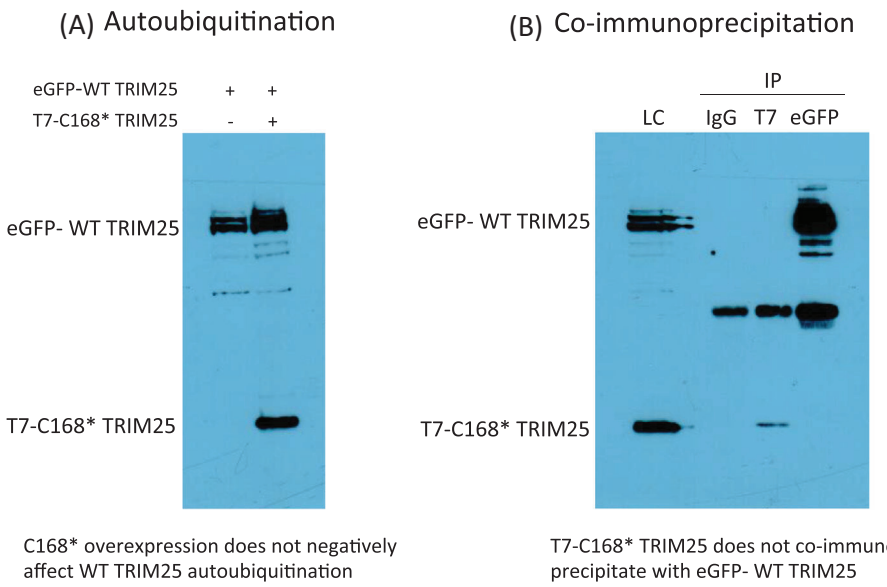

FIGURE 3 C168* TRIM25 does not interact with wild-type (WT) TRIM25 and does not affect its ubiquitination activity. A, Co-expression of enhanced green fluorescent protein (eGFP)-tagged WT and T7-tagged C168* mutant forms of TRIM25 shows that WT TRIM25 is still able to auto-ubiquitinate itself, indicating that WT TRIM25 can still form functional heterodimers, as assayed by western blot analysis. B, Co-immunoprecipitation of eGFP-tagged WT and T7-tagged C168* mutant forms of TRIM25 show that they do not form a dimer (mutant is not able to bind as it is truncated before the coiled-coil domain)

with AD than with a pure presenile Lewy body dementia, expected to be more aggressive.

Four out of five affected siblings were *APOE* ε4/ε4, and it is possible that this genotype could have influenced both the risk for underlying AD and the early age at onset. However, the kindred better fitted a Mendelian autosomal dominant pattern than a semidominant *APOE* ε4-related inheritance, because 5/8 siblings were affected, age at onset of all siblings was very homogeneous ≈60 years of age, and unaffected siblings were also *APOE* ε4 homo- or heterozygous carriers.

The genetic study finally pointed to only two variants with full co-segregation, a missense mutation in *EEA1* (p.V693A), and a stop mutation in *TRIM25* (p.C168*). We considered the implication of the

EEA1 variant as unlikely because it is reported at a frequency of 1/106372 (0.00094) in GnomAD; its CADD score of pathogenicity was under 20; and because the o/e ratio of the gene is 0.95, indicating a high tolerance for gene variations. This o/e score is the ratio of the observed/expected number of LOF/missense variants in that gene, and it is a continuous measure of how tolerant a gene is to the certain class of variation. When a gene has a low o/e value (suggested threshold is < 0.35) it is under stronger selection for that class of variation than a gene with a higher value. In addition, the cohort study revealed a similar presence of *EEA1* missense variants in patients and controls and one stopcodon variant in each.

Therefore, most data strongly support the *TRIM25* nonsense variant as the cause of the disease. The p.C168* variant has not been described previously. It fulfills all American College of Medical Genetics and Genomics criteria for consideration as "likely pathogenic,"[25] as it changes protein length and is a LOF mutation, is only found in cases, segregates with disease, and is predicted to affect protein by two in silico programs. Furthermore, unlike *EEA1*, *TRIM25* is a gene highly intolerant of stop/gain changes as shown by an o/e ratio of 0.34. There is almost no genetic variability around the C168 codon, with only a few missense variants found in < 1/100,000 individuals and no homozygous cases reported. In agreement with this, potentially pathogenic rare variants were found at very low frequencies in the studied cohorts (0% to 0.7%) and there were no LOF variants in controls.

To address which is the link between TRIM25 and neurodegeneration/AD opens a new line of research. TRIM25 is a cytoplasmic protein composed of 630 amino acids that functions as an E3 ubiquitin ligase, enzymes that are involved in the selective recognition and ubiquitination of proteins to be degraded through the ubiquitin–proteasome system (UPS). E3 ubiquitin ligases are therefore involved in the degradation of misfolded proteins that, when aggregated, are associated with neurodegenerative diseases.[7,26,27] Examples of this are mutations in parkin and malin (both E3 ubiquitin ligases), which are associated with development of juvenile recessive Parkinson's disease[9] and Lafora disease,[10,11] respectively.

It is not known whether TRIM25 particularly interacts with misfolded Aβ42 or p-tau on its way to degradation through UPS. However, several studies show that defects in the UPS play a role in AD pathophysiology and may hold the molecular link between Aβ and tau.[28] A significant decrease in UPS activities has been evidenced in the hippocampus, middle temporal gyri, and inferior temporal lobe of AD patients.[29,30] Disruption of UPS correlates with Aβ accumulation, tau hyper-phosphorylation, and autophagy impairment.[31–33] Both Aβ and tau accumulation were reported in transgenic mice after direct inhibition of proteasome activity.[34] In addition, many genetic variants of key regulators in the UPS have been found to be associated with AD.[29,35,36] There is also the possibility that TRIM25 dysfunction could be associated with Lewy body formation or other mechanisms of striatonigral degeneration.

TRIM25 is currently well known for its role in triggering the ubiquitin-dependent antiviral innate immune response through two pathways, RIG-I/INF (retinoid acid inducible gene/interferon) and ZAP (zinc finger antiviral protein).[5,37–39] With emerging evidence suggesting that viral infections could contribute to neurodegenerative diseases,[40–43] a mutation in one of the key factors in the innate immunity against viruses is highly relevant.

The effect of p.C168* on TRIM25 dysfunction was speculated to occur through haploinsufficiency and loss of 50% of the functional protein, or the truncated protein could exert a dominant–negative effect interfering with the remaining WT protein and inducing complete depletion of functional protein. To function properly, TRIM25 requires dimerization and higher-order assembly.[5] Our data showed that the mutant transcript escapes NMD and that the truncated mutated form of TRIM25 is not able to dimerize with WT TRIM25 and does not interfere with its capacity for ubiquitination. Thus, the WT form of TRIM25 is still able to form functional heterodimers, while the p.C168* truncated protein does not bind and is not functional. This provides support that the effect of p.C168* on TRIM25 dysfunction is due to loss of function.

In summary, this study shows that WES of well-characterized families can result in the identification of new variants with significant roles in the pathogenesis of neurodegenerative diseases. TRIM25 adds a new gene to the list of rare genetic causes of presenile dementia and, more importantly, opens the study of a new physiopathological mechanism to the development of amyloidosis and of potentially novel therapeutic interventions.

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CONFLICTS OF INTEREST

Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

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