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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 56-63). The phenotype comprised mixed amnestic and behavioral features, and parkinsonism. CSF and plasma biomarkers, and a PET-amyloid study suggested AD. 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.

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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Abbreviations: EOD: early-onset dementia; AD: Alzheimer's disease, FTD: frontotemporal dementia; WES: whole-exome sequencing; NGS: next-generation sequencing. TRIM25: tripartite motif protein 25 (*TRIM25:* gene); SNP: single nucleotide polymorphism; UPS: ubiquitin-proteasome system; WT: wild-type; LOF: loss-of-function variant; NMD: nonsense mediated mRNA decay

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 56-63). The phenotype comprised mixed amnestic and behavioral features, and parkinsonism. CSF and plasma biomarkers, and a PET-amyloid study suggested AD. 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: early-onset autosomal dominant dementia, Alzheimer's disease, TRIM25, E3 ubiquitin ligase, family-based whole-exome sequencing study

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 roughly 0.098% of the population [1,2], and patients with the disease often have a higher genetic burden than late-onset cases, with about 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 EU EOD Consortium collaboration [4]. Biomarkers in CSF and plasma indicated the presence of amyloidosis and likely AD owing to elevated p-tau181, 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 ananalysis 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 ligases 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 56-63 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 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 SNP 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 Genome Combpackage (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 vicinityof indels was performed with the Genome Analysis Toolkit (GATK) [13]. Variants were called at 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 SNP panel (Agilent Technologies Niel, Multiplicom, Belgium) followed by sequencing on a MiSeq® sequencer (Illumina®, San Diego, CA, USA). 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 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 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 previously 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 random primers. 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 eGFP-tagged WT TRIM25 were incubated with protein A agarose with IgG, anti-T7, or anti-eGFP antibody. The bound proteins were separated on a 4–12% SDS 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 orco-expressed with C168* TRIM25. The extracts were analyzed by Western blotting as explained above.

3. RESULTS

3.1 Clinical phenotypes: The main clinical features of the affected siblings are summarized in Table 1.

Initial clinical symptoms included cognitive declinein all affected siblingsconsisting ofmemory loss, anomia, and apraxia. P2 and P3 have developed an Alzheimer'stype 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 hadsubstantial functional declinewithin five years of onset. P5 currently exhibits a behavioral phenotype with depression, apathy, and social withdrawal, together wih 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 (SPECT with HMPAO) in three siblings (P1, P3, and P4). A CT scan of P1 obtained one year before death showed severe atrophy of the temporal lobes (Figure 2A). A brain MRI 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: Aβ42 567 pg/mL (normal >700), Aβ42/40 ratio 0.079 (>0.068), p-tau181 99 pg/mL (<59), and 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, Norway), which was positive (Figure 2C). Plasma AD biomarkers (Aβ42/40 ratio, total 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). APOE testing indicated that four affected patients (P1, P2, P3, and P4) and the unaffected sibling III-6 were homozygous for APOEε4/4. Unaffected sibling III-7 and P5 were APOEε2/4.

3.2 Whole-exome sequencing

WES of four affected (P1 to P4) and one unaffected (III-6) siblings detected a total of 1,315 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 ten 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* (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-segregationto 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 patientsaround the *TRIM25* and *EEA1* genes, with alternative haplotypes in the unaffected siblings (Supplementary Table). Notably, the shared haplotypes around the TRIM25 and EEA1 genes were also absent from the second branch (III-9 to III-12).

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 regards 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 autoubiquitination of WT TRIM25. WT TRIM25 was present as a double band on the Western

blot, providing evidence that TRIM25 p.C168* does not interfere with WT TRIM25 autoubiquitination 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 Aß42 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, around 14 years, is more consistent with AD than with a pure presenile Lewy body dementia, expected to be more aggressive.

Four out of five affected siblings were APOEε4/4, 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 APOEε4-related inheritance, because 5/8 siblings were affected, age at onset of all siblings was very homogeneous around 60 years of age, and unaffected siblings were also APOEε4 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 fullfills all ACMG 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 (0-0.7%) 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 aminoacids 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 Aß42 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 up the study of a new physiopathological mechanism to the development of amyloidosis and of potentially novel therapeutic interventions.

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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. UA: unaffected.

Figure 2. Neuroimaging of affected siblings P1 and P3: A) CT scan of P1 showing severe atrophy at 75 years of age; B) MRI scan of P3 at 68 years of age showing atrophy of left temporal pole and biparietal; C) amyloid PET with 18F-flutemetamol of P3 at 71 years of age.

Figure 3. C168* TRIM25 does not interact with WT TRIM25 and does not affect its ubiquitination activity. (A) Co-expression of eGFP-tagged WT and T7-tagged C168* mutant forms of TRIM25 shows that WT TRIM25 is still able to autoubiquitinate 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).

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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Abbreviations: EOD: early-onset dementia; AD: Alzheimer's disease, FTD: frontotemporal dementia; WES: whole-exome sequencing; NGS: next-generation sequencing. TRIM25: tripartite motif protein 25 (*TRIM25:* gene); SNP: single nucleotide polymorphism; UPS: ubiquitin-proteasome system; WT: wild-type; LOF: loss-of-function variant; NMD: nonsense mediated mRNA decay

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 56-63). The phenotype comprised mixed amnestic and behavioral features, and parkinsonism. CSF and plasma biomarkers, and a PET-amyloid study suggested AD. 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: early-onset autosomal dominant dementia, Alzheimer's disease, TRIM25, E3 ubiquitin ligase, family-based whole-exome sequencing study

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 roughly 0.098% of the population [1,2], and patients with the disease often have a higher genetic burden than late-onset cases, with about 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 EU EOD Consortium collaboration [4]. Biomarkers in CSF and plasma indicated the presence of amyloidosis and likely AD owing to elevated p-tau181, 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 ananalysis 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 ligases 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 56-63 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 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 SNP 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 Genome Combpackage (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 vicinityof indels was performed with the Genome Analysis Toolkit (GATK) [13]. Variants were called at 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 SNP panel (Agilent Technologies Niel, Multiplicom, Belgium) followed by sequencing on a MiSeq® sequencer (Illumina®, San Diego, CA, USA). 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 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 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 previously 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 random primers. 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 eGFP-tagged WT TRIM25 were incubated with protein A agarose with IgG, anti-T7, or anti-eGFP antibody. The bound proteins were separated on a 4–12% SDS 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 orco-expressed with C168* TRIM25. The extracts were analyzed by Western blotting as explained above.

3. RESULTS

3.1 Clinical phenotypes: The main clinical features of the affected siblings are summarized in Table 1.

Initial clinical symptoms included cognitive declinein all affected siblingsconsisting ofmemory loss, anomia, and apraxia. P2 and P3 have developed an Alzheimer'stype 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 hadsubstantial functional declinewithin five years of onset. P5 currently exhibits a behavioral phenotype with depression, apathy, and social withdrawal, together wih 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 (SPECT with HMPAO) in three siblings (P1, P3, and P4). A CT scan of P1 obtained one year before death showed severe atrophy of the temporal lobes (Figure 2A). A brain MRI 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: Aβ42 567 pg/mL (normal >700), Aβ42/40 ratio 0.079 (>0.068), p-tau181 99 pg/mL (<59), and 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, Norway), which was positive (Figure 2C). Plasma AD biomarkers (Aβ42/40 ratio, total 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). APOE testing indicated that four affected patients (P1, P2, P3, and P4) and the unaffected sibling III-6 were homozygous for APOEε4/4. Unaffected sibling III-7 and P5 were APOEε2/4.

3.2 Whole-exome sequencing

WES of four affected (P1 to P4) and one unaffected (III-6) siblings detected a total of 1,315 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 ten 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* (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-segregationto 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 patientsaround the *TRIM25* and *EEA1* genes, with alternative haplotypes in the unaffected siblings (Supplementary Table). Notably, the shared haplotypes around the TRIM25 and EEA1 genes were also absent from the second branch (III-9 to III-12).

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 regards 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 autoubiquitination of WT TRIM25. WT TRIM25 was present as a double band on the Western

blot, providing evidence that TRIM25 p.C168* does not interfere with WT TRIM25 autoubiquitination 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 Aß42 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, around 14 years, is more consistent with AD than with a pure presenile Lewy body dementia, expected to be more aggressive.

Four out of five affected siblings were APOEε4/4, 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 APOEε4-related inheritance, because 5/8 siblings were affected, age at onset of all siblings was very homogeneous around 60 years of age, and unaffected siblings were also APOEε4 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 fullfills all ACMG 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 (0-0.7%) 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 aminoacids 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 Aß42 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 up the study of a new physiopathological mechanism to the development of amyloidosis and of potentially novel therapeutic interventions.

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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. UA: unaffected.

Figure 2. Neuroimaging of affected siblings P1 and P3: A) CT scan of P1 showing severe atrophy at 75 years of age; B) MRI scan of P3 at 68 years of age showing atrophy of left temporal pole and biparietal; C) amyloid PET with 18F-flutemetamol of P3 at 71 years of age.

Figure 3. C168* TRIM25 does not interact with WT TRIM25 and does not affect its ubiquitination activity. (A) Co-expression of eGFP-tagged WT and T7-tagged C168* mutant forms of TRIM25 shows that WT TRIM25 is still able to autoubiquitinate 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).

Figure 1.

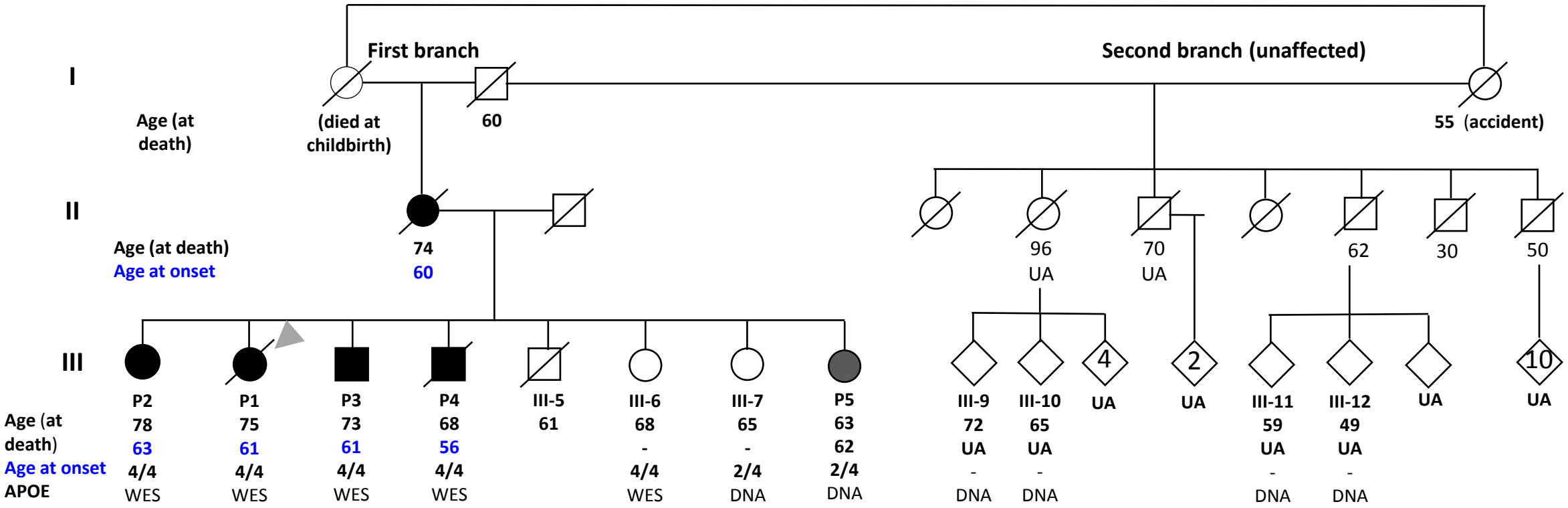

First branch
Second branch (unaffected)
I
II
III
Age (at death)
(died at childbirth)
60
55 (accident)
Age (at death)
Age at onset
74
60
96
UA
70
UA
62
30
50
P2
78
63
4/4
WES
P1
75
61
4/4
WES
P3
73
61
4/4
WES
P4
68
56
4/4
WES
III-5
61
III-6
68
-
4/4
WES
III-7
65
-
2/4
DNA
P5
63
62
2/4
DNA
III-9
72
UA
-
DNA
III-10
65
UA
-
DNA
4
UA
2
UA
III-11
59
UA
-
DNA
III-12
49
UA
-
DNA
UA
10
UA
Age (at death)
Age at onset
APOE

Figure 2.

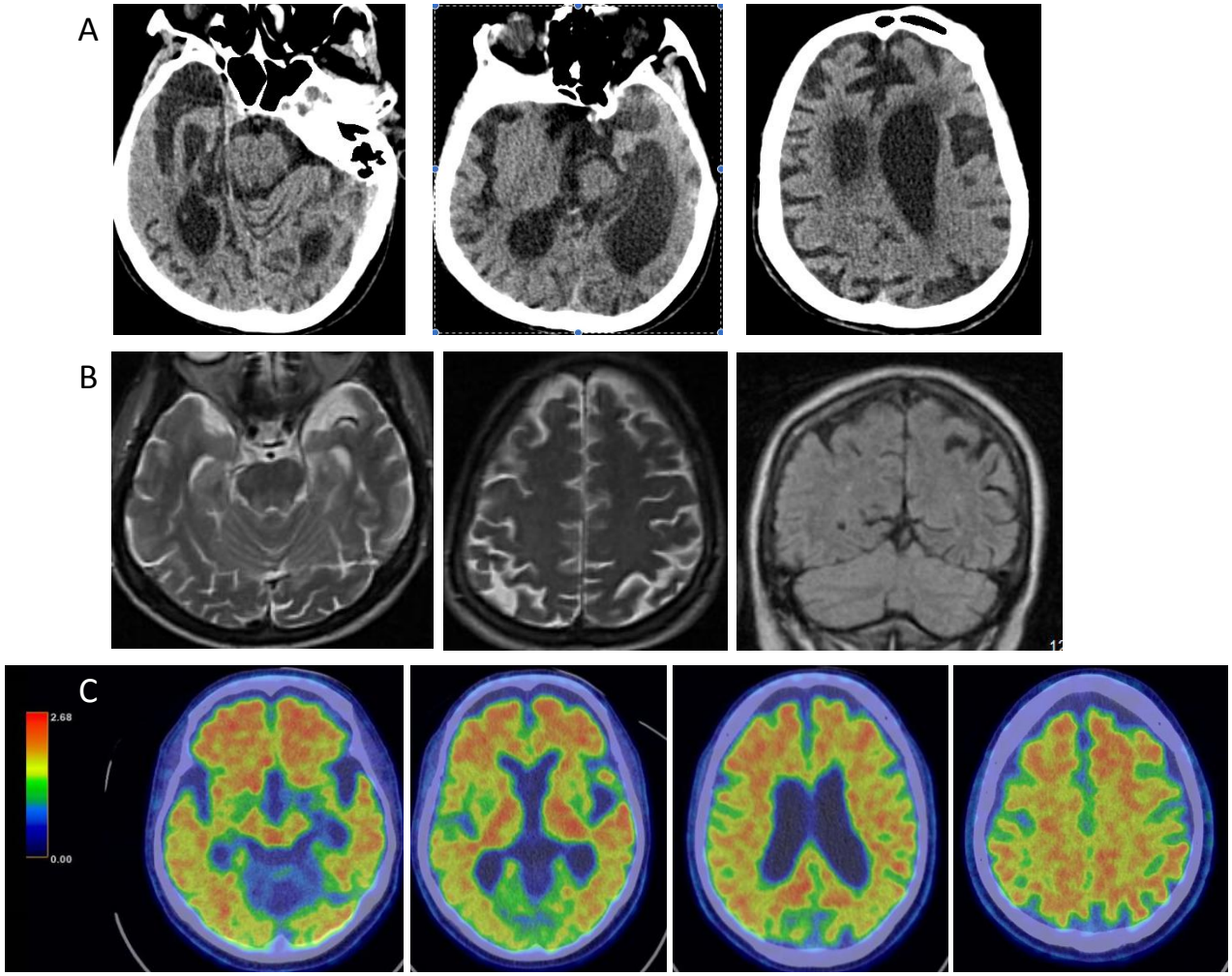
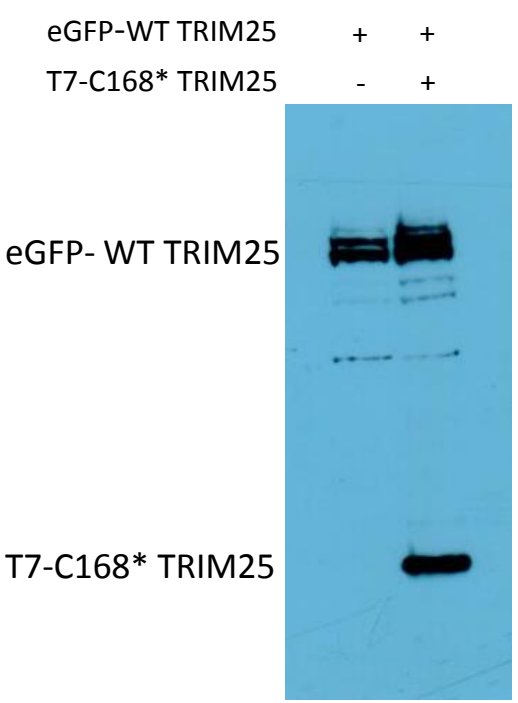


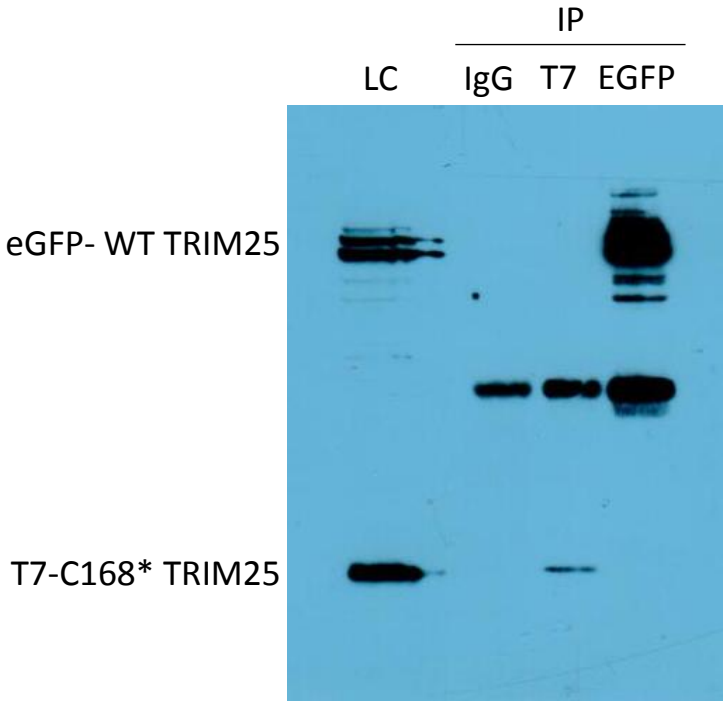
Figure 3.

A) Autoubiquitination



C168* overexpression does not negatively affect WT TRIM25 autoubiquitination

B) Co-immunoprecipitation



T7-C168* TRIM25 does not co-immuno-precipitate with eGFP- WT TRIM25

Table 1. Summary of clinical phenotypes of affected siblings

Case/ gender	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 yrs): 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 yrs):** 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 yrs):** 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 yrs):** 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 0,3-4,5); individuals with advanced dementia mean 3,14 pg/mL (range 0,6-8,0).

Table 2

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 37/human genome 19, GRCh37/hg19);
CADD: Combined Annotation Dependent Depletion (http://cadd.gs.washington.edu) (Kircher et al., 2014); 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.

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.

	Group	N	*TRIM25*		*EEA1*	
			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

CSVS: Collaborative Spanish Variant Server. 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.

Supplementary Table: Haplotype segregation and allele sharing analysis in affected and non-affected members of the family in the two cosegregating candidate genes, *TRIM25* (p.C168*), and *EEA1* (p.V693A). Phased haplotypes using flanking short tandem repeat (STR) markers are shown. Shared alleles are indicated by colors.

		first branch														second branch							
TRIM25	**genomic position**	**P1**		**P2**		**P3**		**P4**		**P5**		**III-6**		**III-7**		**III-9**		**III-10**		**III-11**		**III-12**	
D17S1865	50642148	179	195	179	197	179	195	179	197	179	195	197	197	197	197	183	183	183	183	183	195 or 197?	183	197
D17S790	52798849	254	254	254	256	254	254	254	256	254	254	256	262	256	262	252	258	258	262	252	262	252	258
D17S787	53281885	426	452	426	430	426	452	426	430	426	452	430	430	430	430	426	450	426	426	428	428	426	428
TRIM25* p.C168		**mut**	**wt**	**mut**	**wt**	**mut**	**wt**	**mut**	**wt**	**mut**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**
D17S1853	55584402	240	246	240	236	240	246	240	236	240	246	236	244	236	244	240	248	failed	failed	failed	failed	failed	failed
D17S1604	57977479	185	185	185	191	185	185	185	191	195	193	191	195	193	195	191	195	failed	failed	191	failed	failed	191
D17S1855	59735852	467	465	467	471	467	465	467	471	455	465	471	455	465	455	455	457	455	471	463?	471	455	463

		first branch														second branch							
EEA1	**genomic position**	**P1**		**P2**		**P3**		**P4**		**P5**		**III-6**		**III-7**		**III-9**		**III-10**		**III-11**		**III-12**	
D12S1598	89527906	223	225	223	225	223	225	223	225	225	225	225	227	225	227	225	225	225	231	229	229	225	225
D12S1717	90748764	151	149	151	149	151	149	151	149	151	149	149	149	149	149	153	159	153	153	143	153	153	159
D12S322	92213228	215	213	215	213	215	213	215	213	failed	failed	213	209	213	209	213	217	failed	failed	failed	failed	failed	failed
***EEA1* p.M154V**		**mut**	**wt**	**mut**	**wt**	**mut**	**wt**	**mut**	**wt**	**mut**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**	**wt**
D12S327	94770271	241	233	241	233	241	241	241	241	failed	failed	241	231	241	231	238	239	228	232	228	231	238	239
D12S101	95595693	220	206	220	206	220	206	220	206	220	206	206	210	206	210	206	212	206	210	206	210	206	212
D12S1716	96945072	291	285	291	285	291	285	291	285	291	285	285	285	285	285	285	289	287	289	289	291	285	289
D12S1051	97569768	249	269	249	269	249	249	249	249	249	269	249	249	249	249	249	269	269	269	279	279	249	269

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

- A *TRIM25* nonsense mutation (p.C168*) is associated with autosomal dominant early-onset dementia and parkinsonism with biomarkers suggestive of AD
- TRIM25 protein studies support 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 in association with neurodegeneration
- The role of TRIM25 dysfunction in development of amyloidosis and neurodegeneration merits a new line of research

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

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 up a whole new line of research to address the relationship between TRIM25 and neurodegeneration. As examples, to explore: a) the probable link between amyloidosis/AD 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.