

Distinctive pattern of temporal atrophy in patients with frontotemporal dementia and the I383V variant in *TARDBP*

INTRODUCTION

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are closely related disorders, linked pathologically and genetically by the TAR DNA-binding protein-43 (TDP-43). Pathogenic variants in *TARDBP* encoding for TDP-43 have been described less frequently in FTD than in ALS, and clinicopathological studies are scarce.¹ We previously observed a high frequency of the I383V variant in *TARDBP* in a Dutch cohort of FTD patients.² Here, we provide further evidence for the pathogenicity of this variant and present its clinicopathological characteristics.

METHODS

We ascertained all FTD (n=13) and ALS patients (n=4) with the I383V variant (NM_007375.3: c.1147A>G, p.Ile383Val) in *TARDBP* from three university medical centres in the Netherlands (Amsterdam, Rotterdam and Utrecht), as identified by whole-exome or whole-genome sequencing in either clinical or research setting. Concurrent pathogenic variants in 20 other genes associated with ALS, FTD or other forms of dementia were excluded in all patients.

Brain imaging (CT or MRI) was available for all FTD patients. Quantitative assessment of volume loss across lobar brain regions was performed in those patients with T1-weighted MRI images of sufficient quality (n=5), and compared with a gender-matched/age-matched reference population.

Family histories were classified into adjusted Goldman categories, which were described previously.² Additionally, we performed extensive genealogical research to investigate possible relatedness between the index patients.

Brain autopsy and routine immunohistochemistry was performed for two FTD patients by the Netherlands Brain Bank. One patient (4M) was reported previously as M008015-001.¹ Detailed information on the genetic, neuroimaging, genealogical and pathological analyses can be found in the 1.

RESULTS AND DISCUSSION

The variable clinical phenotype and reduced penetrance of the I383V variant

All 13 FTD patients with the I383V variant in *TARDBP* presented with a combination of behavioural changes and semantic deficits. The diagnoses of semantic variant of primary progressive aphasia (svPPA) are intriguing since this is usually considered a sporadic disorder. One patient (4M) showed additional motor symptoms, but not fulfilling ALS criteria. Of the 4 ALS patients with the I383V variant, 3 had a relatively slow progression with the longest disease duration of 9 years. None of the ALS patients exhibited cognitive or behavioural symptoms. Clinical details are presented in online supplemental tables 1,2.

Six FTD patients and one ALS patient were found to be related (family 1). Additionally, two FTD patients and two ALS patients (families 2 and 3) could be linked to family 1 through a distant common ancestor (figure 1). The variable phenotype of the I383V variant is exemplified by family 1, in which different family members were affected by svPPA, behavioural variant of FTD, unspecified dementia, ALS or progressive spinal muscular atrophy, with a wide range in age at onset (44–69 years) and disease duration (7–23 years). Interestingly, several obligate carriers were unaffected, suggesting incomplete penetrance even at an advanced age (>80 years). Larger prospective studies are required to estimate age-related penetrance.

Four remaining families (online supplemental figure 1) did not show a clear pattern of autosomal dominant inheritance (Goldman 2–5). In one of these families, an affected relative with the I383V variant was clinically diagnosed with Alzheimer's disease (AD), but AD biomarker changes were not evaluated in cerebrospinal fluid. A possible explanation is that the dementia in this patient is coincidental and unrelated to the I383V variant. Alternatively, increased susceptibility for AD caused by the I383V variant may be considered. Another interesting hypothesis is that *TARDBP* variants might be associated with limbic-predominant age-related TDP-43 encephalopathy, a common age-related disorder with TDP-43 proteinopathy that clinically mimics AD.³

Several other relatives, including obligate carriers, were affected by psychiatric disorders such as psychosis and schizophrenia with onset around 40–50 years. Unfortunately, detailed clinical information or DNA were not available for these

subjects. Whether psychiatric disorders are part of the I383V–*TARDBP* spectrum remains to be investigated in future studies. Altogether, our observations illustrate large phenotypic variability of the I383V variant and incomplete penetrance.

Isolated bitemporal atrophy in FTD patients with the I383V variant

The most discriminating feature of the I383V variant is the predominant and severe atrophy of the temporal lobes in all FTD patients, with relative sparing of the other lobes (figure 1 and online supplemental figure 2). This is in line with previous observations in I383V FTD patients and the frequent occurrence of semantic deficits and prosopagnosia in our patients (online supplemental table 1). Other pathogenic *TARDBP* variants (eg, K263E) are associated with a more variable pattern of lobar atrophy.¹ However, predominant temporal involvement has also been reported for other *TARDBP* variants located nearby the I383V variant (eg, A382T),⁴ suggesting a specific effect of missense variants in this part of the C-terminal domain of TDP-43. Further functional studies are needed to elucidate these possible genotype–phenotype correlations.

Heterogeneous pathological features in *TARDBP* patients

A remarkable observation is the scarcity of TDP-43 reactivity in the cortical areas of two FTD patients (patient 1F and the previously reported patient 4M¹), despite the underlying pathogenic *TARDBP* variant. Only several TDP-43 cytoplasmic inclusions of various morphologies were found in the frontal cortex, dentate gyrus and caudate nucleus (figure 1). A possible explanation for the scarce temporal pathology might be the severe neurodegeneration, especially considering the long disease duration of patient 1F (23 years). Interestingly, we also detected tau positive inclusions in the hippocampus and tufted astrocytes in the putamen and caudate nucleus (figure 1). A single other neuropathological study of a I383V carrier reported similar low amounts of TDP-43 inclusions, and the presence of α -synuclein deposits and tauopathy, including tufted astrocytes in the amygdala.⁵ It appears that the neuropathological changes in FTD caused by variants in *TARDBP* are not readily classifiable. Whether the detected co-pathologies occurred by chance needs to be determined in additional cases with TDP-43 variants.

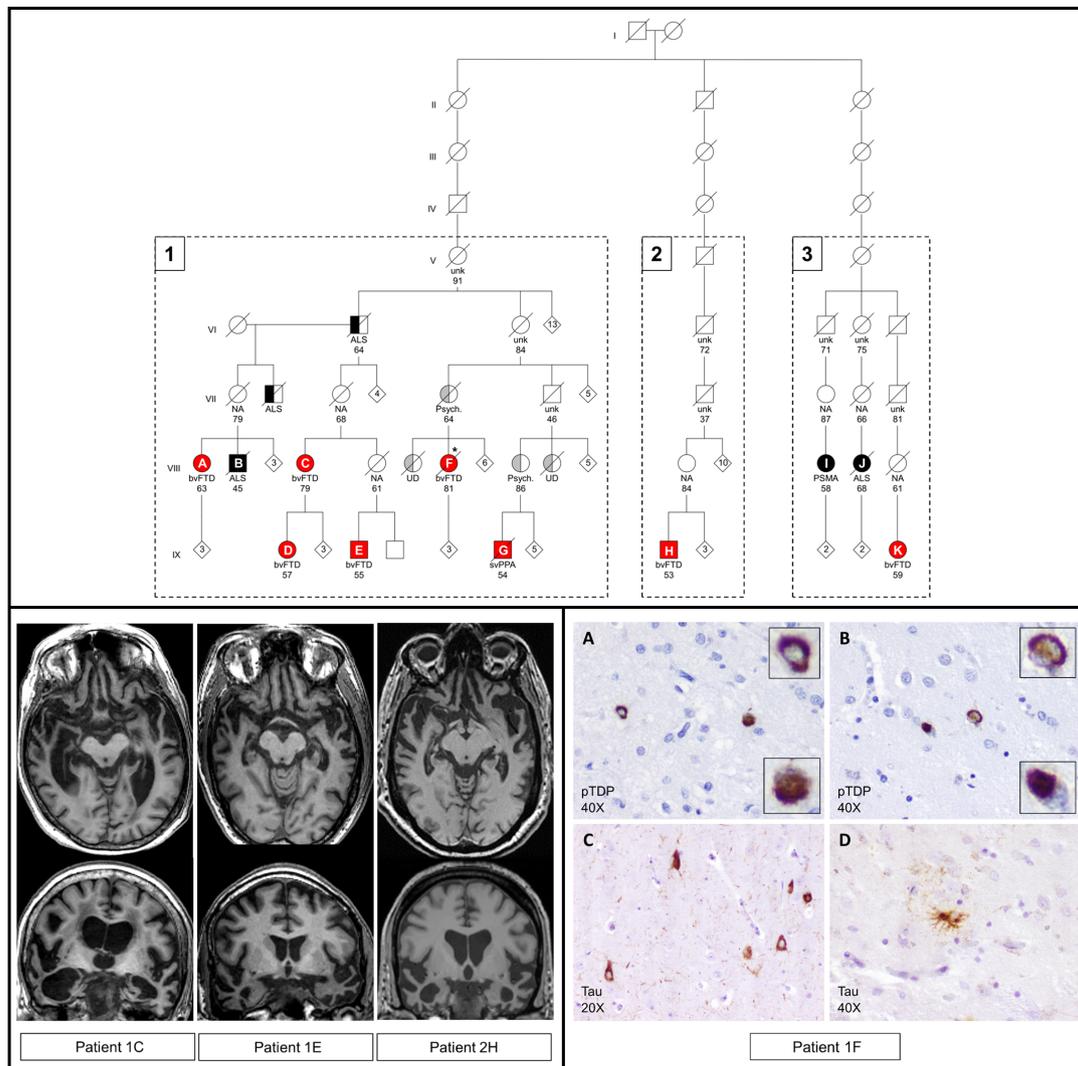


Figure 1 Pedigree of families 1–3 with radiological and pathological features.

Upper panel: three families were found to have a common ancestor following genealogical research. These families include 8 FTD patients and 3 ALS patients with a confirmed I383V variant in *TARDBP* (numbered A–K; fully coloured). Half coloured symbols represent patients with a clinical diagnosis without genetic testing. Red: clinical diagnosis of FTD or PPA. Black: clinical diagnosis of ALS or PSMA. Grey: relatives of index patients affected by other forms of dementia or psychiatric disorders. Numbers inside symbols represent additional family members without further clinical information. Numbers below the symbols indicate age at death or current age. Clinical diagnoses: bvFTD, behavioural variant of frontotemporal dementia; svPPA, semantic variant of primary progressive aphasia; ALS, amyotrophic lateral sclerosis; PSMA, progressive spinal muscular atrophy; UD, unspecified dementia; Psych, psychiatric disorder; NA, not affected based on family history; unk, disease status unknown. *Neuropathological examination (patient 1F).

Lower left panel: neuroimaging of three FTD patients with the I383V variant showing predominant bitemporal atrophy. MRI scans were obtained 7 years (1C), 6 years (1E) and 9 years (2H) after symptom onset. Quantitative analysis of volumetric loss per brain region is shown in online supplemental figure 2. Lower right panel: immunohistochemistry of patient 1F revealed several pTDP-43 positive neuronal cytoplasmic inclusions (NCI) of various morphologies in the frontal cortex (A) and nucleus caudate (B). Compared to other FTD-TDP cases, the amount of inclusions is low and intranuclear inclusions were not found. Therefore, this patient could not be readily classified into one of the FTLD-TDP subtypes. Staining with AT8 antibody revealed NCI in the hippocampus (C) and tufted astrocytes in nucleus caudate (D). Although this patient was 81 years at death, the observed tau pathology is not compatible with normal aging.

Classification of the I383V variant as likely pathogenic

Our findings indicate a pathogenic effect of the I383V variant, which was previously debated due to the more conservative amino acid substitution and the benign in silico predictions by SIFT and PolyPhen. The current families, especially family 1, clearly show segregation of the variant with the disease, although penetrance

appears incomplete. In addition to the patients described here, the I383V variant has been previously reported in 16 FTD and 8 ALS patients (online supplemental table 3), with frequencies ranging from 0% to 0.9% in ALS cohorts and from 0% to 2.5% in clinical FTD cohorts, while the variant is consistently absent in large groups of healthy controls from different populations. These data additionally

support its pathogenicity. This conclusion has clinical implications for genetic counselling of patients and unaffected family members, to whom presymptomatic testing and counselling can now be offered.

CONCLUSION

Our study provides sufficient evidence for the pathogenicity of the I383V variant

and contributes to the characterisation of *TARDBP*-related FTD. We demonstrate the large phenotypic variability and incomplete penetrance of the I383V variant. Marked isolated bitemporal volume loss in all FTD patients should prompt clinicians to genetically test for causal variants in *TARDBP*.

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Contributors MOM and SWRN designed and conceptualised the study, analysed and interpreted all data, and drafted and submitted the manuscript. YALP, SJL, LDK, MRJM, WR, MAE and JHV played a major role in the acquisition of data and revised the manuscript. JGJR, RM and RMLS analysed and interpreted the genetic data, and revised the manuscript. MV analysed and interpreted the neuroimaging data, and revised the manuscript. AJMR analysed and interpreted the pathological data. EAMH conducted genealogical research. JCVS, PCH, HS and EGPD designed and conceptualised the study, revised the manuscript and are responsible for the overall content as guarantors.

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SUPPLEMENTARY METHODS

Patient selection

We ascertained FTD patients ($n=13$) with the variant I383V (NM_007375.3: c.1147A>G, p.Ile383Val) in the *TARDBP* gene, from a large combined cohort of dementia patients who underwent clinical and genetic evaluation in two medical centers in the Netherlands (Amsterdam UMC, Vrije Universiteit Amsterdam, and Erasmus Medical Center, Rotterdam). All but one patient were included in either the Amsterdam Dementia Cohort¹ or an ongoing genetic-epidemiologic study of frontotemporal dementia.²

Subsequently, ALS patients ($n=4$) with the I383V variant in *TARDBP* were selected from the largest ALS cohort in the Netherlands (ALS Center, University Medical Center Utrecht), comprising over 4000 ALS patients included in Project MinE.³ Cognitive status was evaluated in ALS patients on indication only.

Neurological examination and neuroimaging

All FTD patients included in this study underwent neurological and cognitive assessment, and routine neuroimaging (MRI or CT) as part of standard clinical practice. Clinical diagnoses were made according to international consensus criteria.⁴⁻⁶ No neurophysiological assessment was performed. All imaging data were evaluated by experienced neuroradiologists. Additionally, volume loss across all lobar brain regions was quantitatively assessed in patients when 3D-acquired T1-weighted MRI scans with sufficient quality were available ($n=5$). Quantib® ND 1.6 software (Quantib, Rotterdam, The Netherlands), was used to generate automated segmentation and quantification of brain tissue. Volumes were compared to a gender-/age-matched reference population.

Genetic analyses – FTD patients

In all FTD patients, whole-exome sequencing (WES) or whole-genome sequencing (WGS) was performed in either clinical or research setting. Besides the I383V variant, concurrent pathogenic variants in 20 other genes associated with ALS, FTD or other forms of dementia were excluded (Table 1). The presence of a *C9orf72* repeat expansion was tested either using repeat-primed PCR (research setting) or a commercial kit (Asuragen® AmpliDx PCR/CE; diagnostic setting) with repeat length ≥ 30 considered pathogenic.⁷ The variant I383V in *TARDBP* was confirmed by Sanger sequencing.

In 11 out of 13 FTD patients, whole-exome sequencing was performed. DNA was enriched using Agilent SureSelect Clinical Research Exome V2 capture, fragmented to 150 to 200 base pairs, end paired, adenylated, and ligated to adapters. The SeqCap capturing kit for Illumina Paired-End Sequencing library (version 2.0.1; NimbleGen) was used. The captured fragments were purified, and sequenced on either an Illumina HiSeq2000 (Erasmus Medical Center) or HiSeq4000 platform (Amsterdam University Medical Center) using 100 bp paired-end reads. The aim was to obtain 8.1 Giga base pairs per exome with a mapped fraction of 0.99. The average coverage of the exome is $\sim 50\times$ with a minimum depth of >30 reads. Duplicate reads were excluded. Data were demultiplexed with bcl2fastq Conversion Software from Illumina. All sequence reads were mapped to GRCh37/hg19 reference genome using Burrows-Wheeler Aligner (BWA) Tool.⁸ GATK was used for variant calling and quality control according to best practice (McKenna, et al., 2010). Population database frequencies (gnomAD v2.1.1), functional and impact-score annotations were assigned to variants using ANNOVAR.⁹

In 2 out of 13 FTD patients, whole-genome sequencing was performed as part of another study¹⁰, at the Mayo Clinic Genome Analysis Core. Paired end libraries were prepared using 500ng of genomic DNA according to the manufacturer's instructions for the Nextera DNA Flex Library Prep

Kit (Illumina). Libraries were sequenced at an average coverage of ~30X (24 samples/S4 Flow cell) following Illumina's standard protocol using the Illumina NovaSeq™ 6000 and S4 flow cell. The flow cells were sequenced as 150X 2 paired end reads using NovaSeq S4 sequencing kit and NovaSeq Control Software v1.6.0. Base-calling is performed using Illumina's RTA version 3.4.4. Fastq files were processed through the Mayo Genome GPS v4.0 pipeline in a single batch of 48 samples. Briefly, reads were mapped to the human reference sequence (GRCh38 build) using the Burrows–Wheeler Aligner⁸, and local realignment around indels was performed using the Genome Analysis Toolkit (GATK).¹¹ Variant calling was performed using GATK HaplotypeCaller followed by variant recalibration (VQSR) according to the GATK best practice recommendations.^{12, 13} Joint genotyping including all samples was performed using GATK GenotypeGVCF. Quality control (QC) analysis of the data was conducted using a Mayo Clinic in house developed next generation sequencing (NGS) QC pipeline.

Genetic analyses – ALS patients

The four ALS patients described in this study were included in project MinE, a large-scale whole-genome sequencing study in ALS. For methodological details, we refer to previously published papers on the project MinE ALS sequencing consortium.^{3, 14} A *C9orf72* repeat expansion was excluded in these patients by repeat-primed PCR¹⁵ or using the WGS data and the software tool ExpansionHunter.¹⁶

Genealogical analysis

Family histories for FTL spectrum disorders (bvFTD, PPA, ALS, CBS or PSP) were classified into one of the following Goldman categories, which were adjusted and described in more detail previously¹⁰:

1) Autosomal dominant pattern; 2) Familial aggregation; 3) Possible familial with onset <65 years; 4) Possible familial with onset >65 years; 5) Negative family history for a FTL spectrum disorder, any other type of dementia, or Parkinson's disease (PD).

Psychiatric family history was assessed separately.

We performed genealogical research to trace a common link between the FTD patients and the ALS patients. The used sources included Dutch civil registries of births, marriages and deaths (1811-2020) and church archives with baptism, marriage, and death registers (before 1811).

Pathological examination

Brain autopsy was performed in two FTD patients by the Netherlands Brain Bank (NBB) within four hours after death. Routine immunohistochemistry was also carried out by the NBB and FTL diagnosis was confirmed by a neuropathologist based on the criteria by Cairns et al.¹⁷ We performed additional immunohistochemistry on multiple brain regions including all cortical areas, hippocampus and caudate/putamen as previously described.² One patient (4M) was reported previously as M008015-001.¹⁸

Symbol	Name	Reference
<i>ANG</i>	Angiogenin	Greenway et al., 2006
<i>APP</i>	Amyloid beta precursor protein	Goate et al., 1991
<i>CHCHD10</i>	Coiled-coil-helix-coiled-coil-helix domain containing 10	Claussenot et al., 2014
<i>CHMP2B</i>	Charged multivesicular body protein 2B	Skibinski et al., 2005
<i>FUS</i>	FUS RNA binding protein	Vance et al., 2009, Huey et al., 2012
<i>GRN</i>	Granulin Precursor	Cruts et al., 2006
<i>HNRNPA1</i>	Heterogeneous Nuclear Ribonucleoprotein A1	Kim et al., 2013
<i>HNRNPA2B1</i>	Heterogeneous nuclear ribonucleoprotein A2/B1	Kim et al., 2013
<i>MAPT</i>	Microtubule associated protein tau	Hutton et al., 1998
<i>OPTN</i>	Optineurin	Belzil et al., 2011; Pottier et al., 2018
<i>PRKAR1B</i>	Protein kinase cAMP-dependent type I regulatory subunit beta	Wong et al., 2014
<i>PSEN1</i>	Presenilin 1	Sherrington et al., 1995
<i>PSEN2</i>	Presenilin 2	Levy-Lahad et al., 1995
<i>SIGMAR1</i>	Sigma Non-Opioid Intracellular Receptor 1	Luty et al., 2010, Belzil et al., 2013
<i>SOD1</i>	Superoxide Dismutase 1	Rosen et al., 1993
<i>SQSTM1</i>	Sequestosome 1	Le Ber et al., 2013, Thelen et al., 2014
<i>TARDBP</i>	TAR DNA Binding Protein	Caroppo et al., 2016
<i>TBK1</i>	TANK binding kinase 1	Van der Zee et al., 2017
<i>TREM2</i>	Triggering Receptor Expressed On Myeloid Cells 2	Borroni et al., 2014
<i>UBQLN2</i>	Ubiquilin 2	Dillen et al., 2013
<i>VCP</i>	Valosin Containing Protein	Watts et al., 2004; Wong et al., 2018

Table 1. A total of 21 genes associated with FTD, ALS, FTD-ALS, and Alzheimer's disease were screened for variants using whole-exome or whole-genome sequencing, which was performed in all patients.

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	1A	1C	1D	1E	1F ^a	1G	2H	3K	4L	4M	5O	6P	7Q
Gender	F	F	F	M	F	M	M	F	M	M	M	M	M
Age at onset	55	69	55	44	58	46	42	47	54	65	49	60	49
Disease duration (years)	8	10	2	10	23*	8*	11	11	5	7*	17*	8	12
Clinical diagnosis	bvFTD	bvFTD	bvFTD	bvFTD	bvFTD	svPPA	bvFTD	bvFTD	bvFTD	bvFTD	svPPA	bvFTD	bvFTD
Clinical symptoms													
<i>Behavioral disinhibition</i>	++	++	+	+	+	-	++	-	+	++	+	-	+
<i>Apathy/loss of sympathy</i>	+	+	-	+	±	+	++	+	±	±	++	+	-
<i>Compulsivity/stereotypes</i>	-	-	-	+	+	+	+	+	+	-	+	+	-
<i>Hyperorality/dietary changes</i>	+	-	-	-	+	-	-	-	++	-	++	-	-
<i>Executive deficits</i>	+	+	+	-	+	-	+	-	-	+	±	-	+
<i>Episodic memory deficits</i>	-	+	-	+	-	-	-	-	+	-	+	+	-
<i>Impaired confrontation naming</i>	+	++	±	+	+	++	+	++	+	-	+	+	+
<i>Impaired language comprehension</i>	+	+	+	-	±	++	+	-	-	-	+	±	-
<i>Prosopagnosia</i>	+	+	-	-	+	+	+	-	+	-	-	+	+
Family history^b													
<i>Goldman score</i>	1	3	1	3	3	2	4	5	5	4	4	2	3
<i>Psychiatric Family history</i>	-	-	-	-	+	+	-	-	+	-	-	-	+
Neuroimaging													
<i>Temporal atrophy at first scan</i>	R>L (3)	R>L (5)	L>R (2)	L>R (5)	S (6) ^c	S (6)	S (8)	R>L (8)	R>L (4)	S (6)	R>L(5)	S (5) ^c	S (10)
<i>Temporal atrophy at follow-up</i>		S (7)		S (6)				R>L (11)					

Supplementary Table 1. Clinical characteristics of 13 FTD patients carrying I383V TARDBP variant.

Patients are numbered according to their ID in the pedigrees (Figure 1 / Supplementary Figure 1). Four out of 13 patients have died (disease duration marked with asterisk), whereas current disease duration is presented for the nine living patients. *Clinical diagnoses*: bvFTD = behavioral variant of FTD; svPPA = semantic variant of primary progressive aphasia. *Clinical symptoms*: - = absent; ± = mild; + = moderate; ++ = severe. *Neuroimaging*: R = right; L = left; S = symmetric. The numbers in brackets indicate the number of years after symptom onset when neuroimaging was performed.

^a Neuropathologic examination.

^b Goldman score based on family history (up till 2nd degree).

^c Computed tomography (CT) was performed instead of Magnetic Resonance Imaging (MRI).

	1B	3I	3J	5N
Gender	M	F	F	M
Age at onset	39	53	59	62
Disease duration (years)	6*	6	9*	1*
Clinical diagnosis	ALS	PSMA	ALS	ALS
Clinical symptoms				
<i>Onset</i>	Spinal	Spinal	Spinal	Spinal
<i>EMG affected regions (N)^a</i>	1	0 ^d	2	3
<i>Diagnostic delay (months)^b</i>	12	64	24	8
<i>Cognitive impairment</i>	-	-	-	-
Family history^c				
<i>Goldman score</i>	1	5	5	5
<i>Psychiatric Family history</i>	-	-	-	-

Supplementary Table 2. Clinical characteristics of four ALS patients carrying I383V *TARDBP* variant.

Patients are numbered according to their ID in the pedigrees (Figure 1 / Supplementary Figure 1). Three patients have died (disease duration marked with asterisk), whereas current disease duration is presented for patient 3I. Neuroimaging was not performed in these patients. *Clinical diagnoses*: ALS = amyotrophic lateral sclerosis; PSMA = progressive spinal muscular atrophy.

^a Number of regions with features consistent with ALS according to the revised El Escorial criteria.¹

^b The diagnostic delay is presented to indicate the relative slow progression in patients 3I and 3J.

^c Goldman score based on family history (up till 2nd degree).

^d Denervation and reinnervation changes were present, though not fulfilling El Escorial criteria.

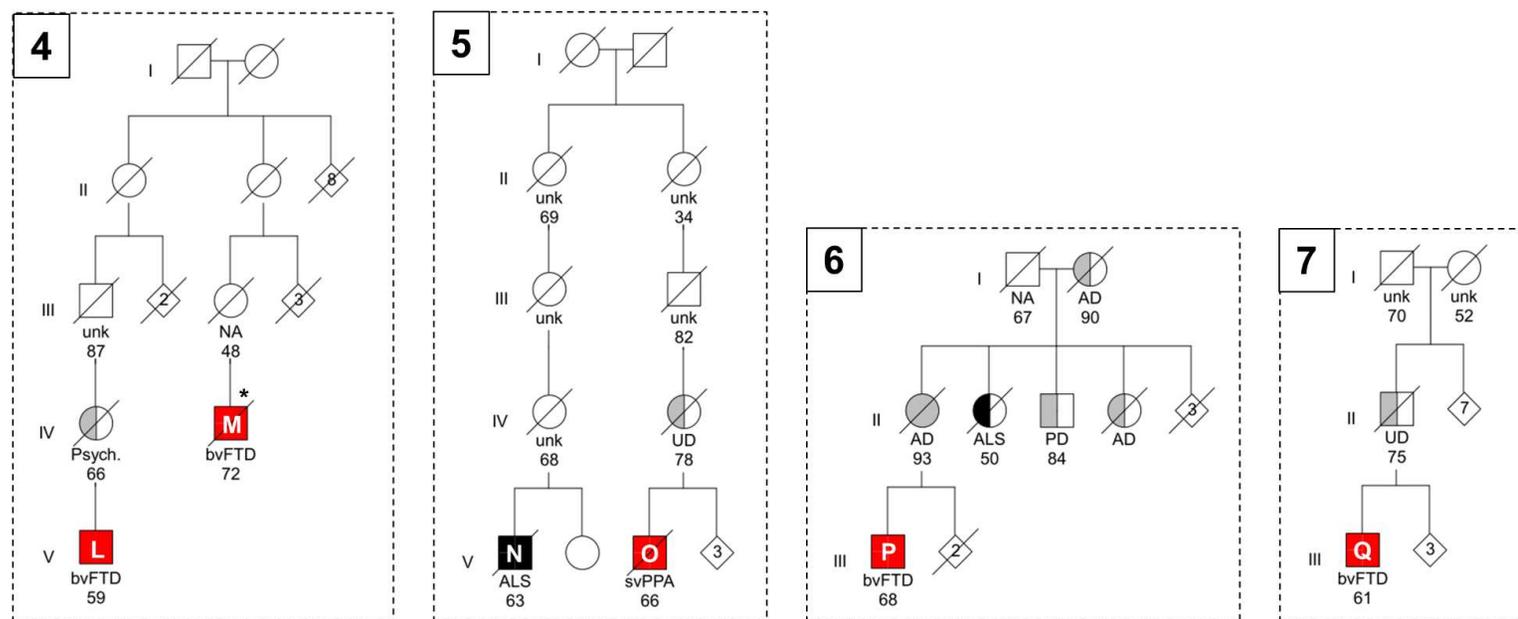
Authors	Number of patients (families)	Age at onset	Clinical Diagnosis	Predominant atrophy distribution	Family history
Ramos et al., 2020 ²	4 (2)	ND	FTD	ND	Positive
		60	bvFTD	Temporal (left>right)	Negative
Ramos et al., 2019 ³	3 (3)	58	svPPA	Temporal (bilateral) + Frontal	Positive
		66	svPPA	Temporal (left>right) + Frontal	Positive
Gelpi et al., 2014 ⁴	1 (1)	60	svPPA	Temporal (left, bilateral at autopsy)	Dementia
		65	bvFTD-ALS	Temporal (bilateral) + Parietal (mild)	ALS
Caroppo et al., 2016 ⁵	4 (3)	63	bvFTD	Temporal (bilateral) + Frontal	bvFTD
		51	bvFTD	Temporal (right>left) + Frontal + Hippocampal	bvFTD
		51	svPPA	Temporal (left>right)	Negative
		38	ALS	No atrophy	FTD, ALS
Cheng et al., 2016 ⁶	3 (1)	64	svPPA	Temporal (left>right)	FTD, ALS
		62	bvFTD-ALS	Temporal (bilateral) + Frontal	FTD, ALS
Coyle-Gilchrist et al., 2016 ⁷	1 (1)	49	svPPA	Temporal (bilateral)	Unknown
Gonzalez-Sanchez et al., 2018 ⁸	2 (1)	51	svPPA	Temporal (left>right)	dementia, ALS
		52	ALS	ND	dementia, svPPA
Rutherford et al., 2008 ⁹	1 (1)	59	ALS	ND	ALS
		66	ALS	ND	ALS
Ticozzi et al., 2011 ¹⁰	3 (1)	25	ALS	ND	ALS
		57	ALS	ND	ALS
Ozoguz et al., 2015 ¹¹	2 (1)	42	ALS	ND	ALS
		47	ALS	ND	ALS

Supplementary Table 3. Clinical characteristics of previously reported patients carrying the I383V *TARDBP* variant.

A total of 24 patients with the I383V variant from 17 different families have been reported in the literature. Eight of these patients were diagnosed with isolated ALS, whereas the majority was diagnosed with FTD or FTD-ALS. Neuroimaging of most FTD patients described the presence of temporal atrophy, in some cases combined with volume loss in other areas. *Clinical diagnoses*: ALS = amyotrophic lateral sclerosis; svPPA = semantic variant of primary progressive aphasia; bvFTD = behavioral variant of FTD. ND = not described.

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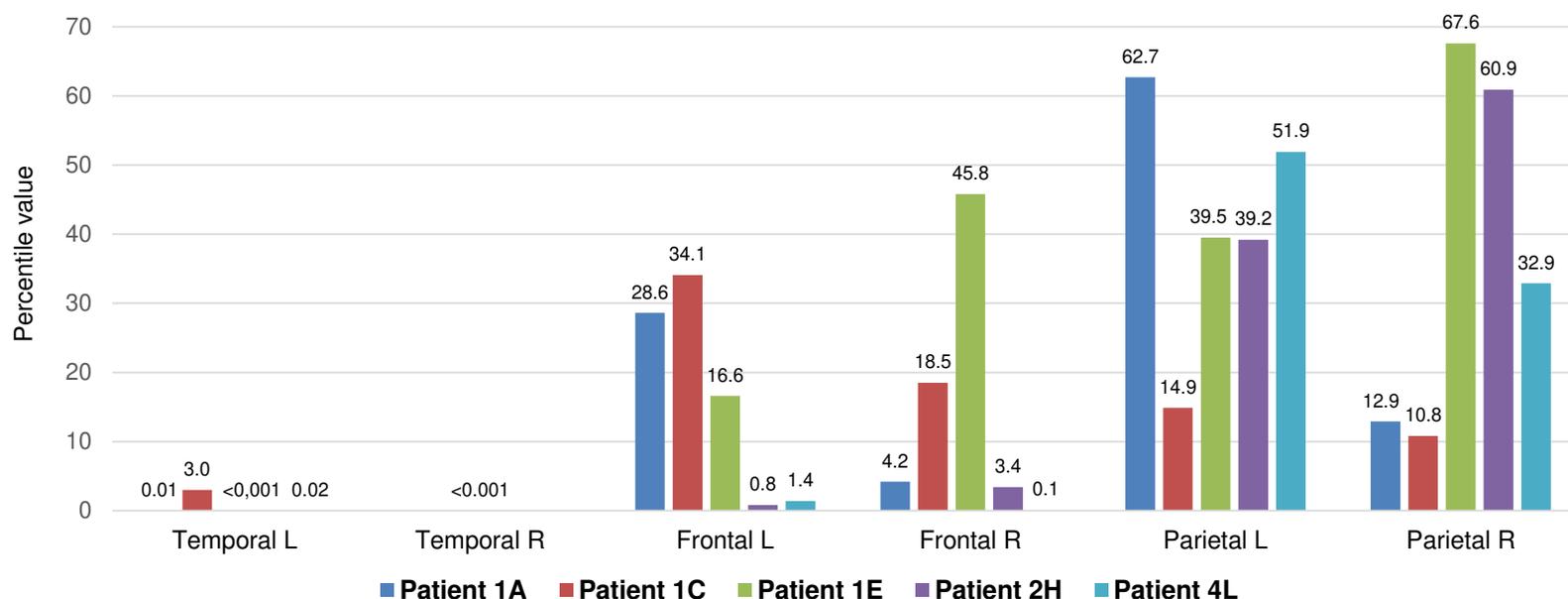


Supplementary Figure 1. Pedigrees of the families 4-7.

Four out of seven pedigrees are presented here, including five FTD patients and one ALS patient with a confirmed I383V variant in *TARDBP* (numbered L-Q). Fully colored symbols represent confirmed carriers of the I383V variant in *TARDBP* ($n=7$), including one relative clinically diagnosed with late-onset AD (family 6). Half colored symbols represent patients with a clinical diagnosis without genetic testing. *Red* = clinical diagnosis of FTD or PPA. *Black* = clinical diagnosis of ALS or PSMA. *Grey* = relatives of index patients affected by other forms of dementia or psychiatric disorder. Numbers inside symbols represent additional family members without further clinical information. Numbers below the symbols indicate age at death or current age.

Clinical diagnoses: bvFTD = behavioral variant of frontotemporal dementia; svPPA = semantic variant of primary progressive aphasia; ALS = amyotrophic lateral sclerosis; AD = Alzheimer's disease; PD = Parkinson's disease; UD = unspecified dementia; Psych. = psychiatric disorder; NA = not affected based on family history; unk = disease status unknown.

* Neuropathologic examination (patient 4M).



Supplementary Figure 2. Brain volumetric quantification of 5 FTD patients carrying the I383V *TARDBP* variant.

Patients are numbered according to their ID in the pedigrees (Figure 1 / Supplementary Figure 1). Volume loss across different brain regions was assessed in five patients using Quantib® ND 1.6 software and compared to a gender and age matched reference population, using so-called reference percentile curves. On the y-axis, age-specific and gender-specific percentile values are shown for left and right temporal, frontal, and parietal lobes. In general, percentile values <5% are considered abnormal.

Clearly visible is the marked bitemporal volume loss in all five patients. More subtle atrophy of the frontal lobes is present in three patients (1A, 2H, 4L). Asymmetry between the left and right temporal lobe, as visually observed, was not found with this method likely due to a floor effect. MRI scans were obtained 3 years (1A), 7 years (1C), 6 years (1E), 9 years (2H) and 4 years (4L) after symptom onset.