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Frontotemporal dementia spectrum: first genetic screen in a Greek cohort

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ABSTRACT

Frontotemporal dementia (FTD) is a heterogeneous group of neurodegenerative syndromes associated with several causative and susceptibility genes. Herein, we aimed to determine the incidence of the most common causative dementia genes in a cohort of 118 unrelated Greek FTD spectrum patients. We also screened for novel possible disease-associated variants in additional 21 genes associated with FTD or amyotrophic lateral sclerosis. Pathogenic or likely pathogenic variants were identified in 16 cases (13.6%). These included repeat expansions in *C9orf72* and loss-of-function *GRN* variants, and likely pathogenic variants in *TARDBP*, *MAPT*, and *PSEN1*. We also identified 14 variants of unknown significance in other rarer FTD or amyotrophic lateral sclerosis genes that require further segregation and functional analysis. Our genetic screen revealed a high genetic burden in familial Greek FTD cases (30.4%), whereas only two of the sporadic cases (3.5%) carried a likely pathogenic variant. A substantial number of familial cases still remain without an obvious causal variant, suggesting the existence of other FTD genetic causes besides those currently screened in clinical routine.

1. Introduction

Frontotemporal dementia (FTD) encompasses a spectrum of clinically, pathologically, and genetically heterogeneous neurodegenerative syndromes. Three main clinical syndromes are defined based on distinct patterns of behavioral, language, and motor symptoms: the behavioral variant of frontotemporal dementia (bvFTD) affecting social skills, emotions, personal conduct, and self-awareness; and the FTD language variants, progressive nonfluent variant (nfvPPA) and semantic variant (svPPA) primary progressive aphasia (Rascovsky and Grossman, 2013). Some patients with FTD also develop motor symptoms, such as weakness or muscle wasting, characteristic of amyotrophic lateral sclerosis (ALS). There is also a significant clinical overlap with atypical parkinsonian syndromes, mainly progressive supranuclear palsy (PSP) and

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corticobasal syndrome (CBS). Neuropathologically, FTD is characterized by selective degeneration of the frontal and temporal lobes, and loss of motor neurons for FTD-ALS, with abnormal protein aggregates. These inclusions are either composed of mostly fibrillar hyperphosphorylated tau (FTLD-tau) or are immunoreactive to TDP-43 (FTLD-TDP), whereas a small subset are immunoreactive to components of the ubiquitin-proteasome system (FTLD-UPS) or the fused in sarcoma protein (FTLD-FUS) but negative for both tau and TDP (Mackenzie and Neumann, 2016).

FTD has a strong genetic component, with up to 40% of cases reporting a family history of dementia, psychiatric or motor symptoms, and at least 10% showing an autosomal dominant transmission (Rohrer et al., 2009). Pathogenic variants in the granulin (*GRN*) (Snowden et al., 2006) and microtubule-associated protein tau (*MAPT*) (Clark et al., 1998) genes are estimated to be associated with 5%–20% of familial FTD cases each, whereas the repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) (DeJesus-Hernandez et al., 2011; Renton et al., 2011) gene is a major cause of both familial FTD and ALS. Other rare pathogenic variants have been identified in genes encoding for TAR DNA-binding protein 43 (*TARDBP*) (Benajiba et al., 2009), RNA-binding protein fused in sarcoma (*FUS*) (Van Langenhove et al., 2010), charged multivesicular body protein 2B (*CHMP2B*) (Skibinski et al., 2005), valosin containing protein (*VCP*) (Watts et al., 2004) and sequestosome 1 (*SQSTM1*) (Rubino et al., 2012). Recently, two new genes have been associated with FTD: TANK-binding kinase 1 (*TBK1*) (Freischmidt et al., 2015) and RNA-binding protein T cell-restricted intracellular antigen-1 (*TIA1*) (Mackenzie et al., 2017). In addition, while presenilin 1 (*PSEN1*) is one of the main genetic causes of Alzheimer's disease (AD), there are a few reports of *PSEN1* variants associated with FTD phenotypes (Bernardi et al., 2009; Mahoney et al., 2013; Riudavets et al., 2013; Robles et al., 2009).

The goal of this study was to determine the overall genetic contribution of the most common known FTD and AD genes in the first series of Greek patients with FTD, and to evaluate 21 rare FTD- and ALS-associated genes for the presence of rare, predicted deleterious variants.

2. Materials and methods

2.1. Cohort description

We screened 118 unrelated Greek patients with FTD spectrum consecutively recruited at the Attikon University General Hospital in Greece, from November 2011 to December 2016, after obtaining their informed consent and approval from the hospital Bioethics Committee. After clinical review by a neurologist, these patients were categorized into 68 bvFTD, 10 nfvPPA, 14 svPPA, 5 FTD-ALS, 12 PSP, and 9 CBS (Table 1). This cohort consisted of 61 female and 57 male patients, with a mean age at onset of 60.8 ± 10.2 years (ages ranging from 36 to 79 years, data not available for 28 of the cases). Positive family history was reported in 46 cases, whereas 57 had no reports of family members known to suffer from dementia or psychiatric problems (family history was not available for the remaining 15 cases).

We also screened 51 unrelated Greek individuals with no evidence of neurodegenerative dementia. This control group included 31 female and 20 male individuals, with an average age of 62.4 ± 9.1 years (range: 48–81 years).

2.2. Targeted sequencing

DNA was isolated from peripheral EDTA blood with the High Pure PCR Template Preparation Kit. Samples were screened using targeted sequencing of a panel of genes previously implicated in neurodegenerative disorders, including the most common causative genes for Mendelian forms of FTD and AD. Exonic regions were captured using a custom-designed library (SeqCap EZ Choice Library, NimbleGen) and sequenced on an Illumina HiSeq4000 at the UCLA Neuroscience Genomics Core (http://www.semel.ucla.edu/ungc). Sequence reads were mapped to the GRCh37/hg19 reference genome and variants were joint-called with GATK according to GATK Best Practices recommendations (McKenna et al., 2010). The joint variant calling file was annotated using ANNOVAR and the Ensembl Variant Effect Predictor tool (McLaren et al., 2016; Wang et al., 2010).

Table 1
Characteristics of the Greek FTD cohort

Total number of cases	118
Clinical syndrome	
bvFTD	68 (57.6%)
nfvPPA	10 (8.5%)
svPPA	14 (11.9%)
FTD-ALS	5 (4.2%)
CBS	9 (7.6%)
PSP	12 (10.2%)
Family history	
Familial	46 (39.0%)
Sporadic	57 (48.3%)
Unknown	15 (12.7%)
Average age at onset[a]	
All subjects	60.81 ± 10.22 (36–79)
Familial subjects	58.88 ± 10.42 (36–78)
Sporadic subjects	63.26 ± 8.99 (48–79)

Key: bvFTD, behavioral frontotemporal dementia; CBS, corticobasal syndrome; FTD-ALS, frontotemporal dementia-amyotrophic lateral sclerosis; nfvPPA, nonfluent progressive aphasia; PSP, progressive supranuclear palsy; svPPA, semantic progressive aphasia.
[a] Age at onset was available for 90 of the cases, including 40 familial and 46 sporadic cases (family history not available for 4 cases).

2.3. Dementia genes screening

The coding and exon-intron boundary regions of the seven most common FTD and AD genes (*APP, TARDBP, FUS, GRN, MAPT, PSEN1,* and *PSEN2*) were screened for known (listed in the AD&FTD Mutation Database: http://www.molgen.ua.ac.be/ADMutations) or novel (likely) pathogenic variants (according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology published guidelines) (Richards et al., 2015). Transcripts NM_001136129 (*APP*), NM_001170634 (*FUS*), NM_002087 (*GRN*), NM_001123066 (*MAPT*), NM_000021 (*PSEN1*), NM_000447 (*PSEN2*), and NM_007375 (*TARDBP*) were used as reference. Potentially pathogenic variants were confirmed by Sanger sequencing.

2.4. C9orf72 repeat screening

The presence of a pathological hexanucleotide repeat expansion in *C9orf72* was detected using both fluorescent and repeat-primed PCR, as previously described (DeJesus-Hernandez et al., 2011). Fragment length analysis was performed on an ABI 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA), and data were analyzed using the Peak Scanner Software, including a positive control sample for reference.

2.5. Other rare FTD and ALS genes screening

Coding and exon-intron boundaries of 21 additional genes previously reported as associated with FTD or ALS (Supplementary Table 1) were examined. Variants were filtered for 1) protein-

truncating (nonsense, frameshift, canonical splice sites) and missense variants that were 2) novel or rare (minor allele frequency [MAF] < 0.0001 in the non–Finnish European [NFE] population from the Genome Aggregation Database [gnomAD, http://gnomad.broadinstitute.org/], as it corresponds to the highest MAF of known [likely] pathogenic variants in the *GRN* and *MAPT* genes) and 3) predicted to be damaging by at least one of the following *in silico* software algorithms: SIFT, Polyphen-2, and CADD (score ≥20, corresponding to the 1% most deleterious variants in the genome). For genes associated with recessive FTD-ALS we also filtered for homozygous variants. The filtered candidate variants were then classified according to American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines (Richards et al., 2015).

3. Results

3.1. Pathogenic and likely pathogenic variants in common FTD and AD genes

Eleven of the 118 Greek patients with FTD harbored a pathogenic variant: six carried an expanded *C9orf72* repeat expansion and five a *GRN* loss-of-function pathogenic variant. We also identified five additional cases harboring likely pathogenic variants (one in *MAPT*, one in *PSEN1*, and three in *TARDBP*); none of these were found in our control group. Overall, this corresponded to a total frequency of 13.6% (16 of 118) carriers of pathogenic or likely pathogenic variants in the Greek FTD series (Fig. 1). Family history in these variant carriers was positive in 87.5% (14 of 16) cases, whereas two cases were sporadic. Fourteen of 46 (30.4%) familial cases carried a pathogenic or likely pathogenic variant, whereas only two of 57 (3.5%) sporadic cases carried a likely pathogenic variant. Age at onset was lower in the variant carriers (55.9 ± 7.9 years), than in the noncarriers (61.9 ± 10.4 years, Mann *U* test, two-tailed *p*-value = 0.01931).

C9orf72 repeat expansion carriers presented with either bvFTD (n=4) or FTD-ALS (n = 2) (Table 2, cases 1–6). Ages at onset ranged from 49 to 72 years (58.5 ± 8.9 years), and all had a family history of neurodegenerative disease. Four of the five *GRN* cases carried splicing variants. A patient with svPPA (case 7) carried a splice-donor site variant (c.349+1G>C), which was previously reported in a patient with bvFTD (Feneberg et al., 2016). Two patients (cases 8 and 9), diagnosed with bvFTD and nfvPPA, carried a novel splice-acceptor site variant (c.350−2A>G), whereas a novel splice-donor c.264+1delG variant was found in a patient diagnosed with bvFTD (case 11). The other patient (case 10) carried a large 64-bp deletion preceded by a single nucleotide change in the same allele that together are predicted to result in p.Gln401LeufsTer69. This novel protein-truncating variant was also found in his affected brother, not included in this series, who has a clinical diagnosis of probable Parkinson's disease. All of these *GRN* pathogenic variants were absent from the gnomAD database. Altogether, there was sufficient evidence to classify these variants as pathogenic. Ages at onset for the five carriers ranged from 48 to 61 years (52.4 ± 5.0 years), and all had a positive family history.

In addition to these pathogenic variants, we also identified one *MAPT* missense variant (p.Val698Ile) in a CBS case (Table 2, case 12). While this variant has been previously reported in an nfvPPA case (Munoz et al., 2007), and it is within a mutational hot-spot where other missense variants have been reported as pathogenic, it is also predicted to be tolerated by both SIFT and Polyphen, and observed in 3 of 63,361 NFE individuals in gnomAD (MAF = 2.367e-5). Therefore, it can only be classified as likely pathogenic.

Aside from variants in the three most common FTD genes, we also identified three unrelated patients with bvFTD and svPPA (Table 2, cases 14–16) carrying the same missense variant (p.Ile383Val) in *TARDBP*. This variant is predicted to be tolerated by both PolyPhen and SIFT; it was observed in 3 of 59,352 NFE individuals in the gnomAD database (MAF = 2.527e-5), and in four familial ALS cases reported in the literature (Rutherford et al., 2008; Ticozzi et al., 2011). Biochemical analysis of an ALS patient cell line carrying this variant revealed a substantial increase in TDP-43 caspase-cleaved fragments (Gendron et al., 2013; Rutherford et al., 2008). Based on these data, this variant was classified as likely pathogenic.

Although pathogenic variants in *PSEN1* are the main genetic causes of AD, there are a few reports of *PSEN1* variants linked to FTD phenotypes (Bernardi et al., 2009; Mahoney et al., 2013; Riudavets et al., 2013; Robles et al., 2009). We identified one bvFTD case with a missense variant (p.Tyr115Cys) in the *PSEN1* gene (Table 2, case 13). This variant is absent in gnomAD, is predicted to be damaging, and has been associated with multiple cases of AD (Cruts et al., 1998; Janssen et al., 2003; Rogaeva et al., 2001; Wallon et al., 2012), warranting classification as likely pathogenic. Notably, this patient

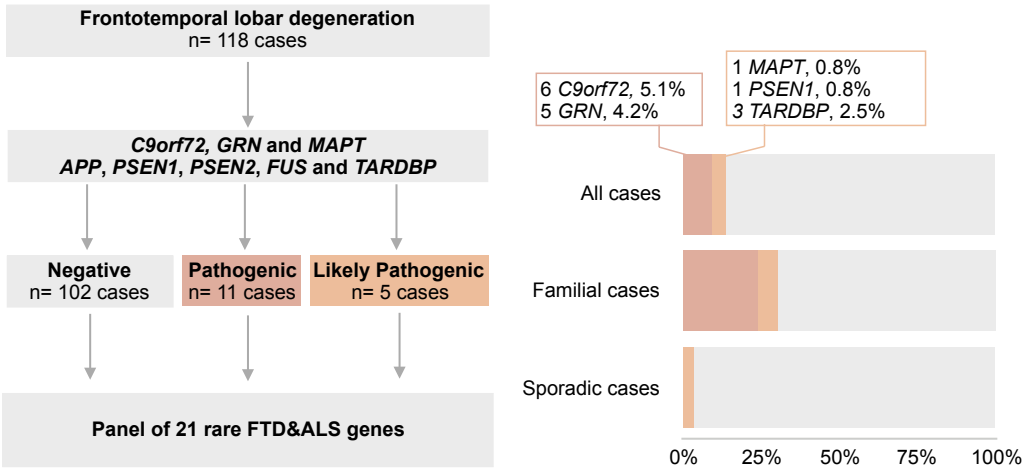

Fig. 1. Relative frequency of pathogenic and likely pathogenic variants in a series of 118 unrelated FTD Greek cases. Eleven cases carried pathogenic variants (*C9orf72* repeat expansion and loss-of-function *GRN* variants) while five cases carried likely pathogenic variants (*MAPT*, *PSEN1*, and *TARDBP* rare missense variants). Pathogenic and likely pathogenic variants were found in 30.4% of familial cases, whereas 3.5% of sporadic cases carried likely pathogenic variants. Abbreviations: ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia.

Table 2
Demographic and clinical characteristics of cases carrying pathogenic or likely pathogenic variants

Case	Variant	gnomAD NFE_MAF	Gender	FH	AO	FTD syndrome	Symptoms	MRI
C9orf72, 6 carriers (5.1%)								
1	Repeat expansion	-	M	+	62	FTD-ALS	Depression, disinhibition, risky behavior, aggressiveness, motor deficits	Diffuse lobar atrophy
2	Repeat expansion	-	M	+	49	FTD-ALS	Dysarthria, dysphagia, disinhibition, behavioral alterations	Bilateral frontal, temporal and parietal lobe atrophy
3	Repeat expansion	-	F	+	50	bvFTD	Logopenia, disinhibition, irritability, concentration deficits, binge eating	No atrophy
4	Repeat expansion	-	F	+	55	bvFTD	Mild memory impairment, apathy	Bilateral frontal lobe atrophy
5	Repeat expansion	-	F	+	63	bvFTD	Executive deficits, apathy, stubbornness, spending money on useless things, memory deficits, anxiety	Generalized moderate atrophy, left hippocampal atrophy more prominent
6	Repeat expansion	-	F	+	72	bvFTD	Apathy, stubbornness, executive deficits, phobias, disinhibition, poor speech, memory problems, oral behavior	Frontal and temporal lobe atrophy
GRN, 5 carriers (4.2%)								
7	c.349+1G>C	-	F	+	52	svPPA	Apathy, memory problems	Temporal lobe atrophy (L>>R)
8	c.350−2A>G	-	F	+	48	bvFTD	Apathy, social withdrawal, emotional blunting, logopenia, syntax errors	Frontal and temporal lobe atrophy (L>R)
9	c.350−2A>G	-	F	+	51	nfvPPA	Depression, reduced speech production with preserved comprehension, obsessive behavior, sweet tooth, mild memory problems	Left frontal and temporal lobe atrophy
10	p.Gln401LeufsTer69	-	M	+	50	bvFTD	Memory problems (AD phenotype), stubbornness, obsessions, bulimia	Temporal and parietal lobe atrophy
11	c.264+1delG	-	F	+	61	bvFTD	Behavioral changes, inadequate in her professional duties, loss of initiative, apathy	Frontal lobe atrophy and to a lesser extent parietal atrophy, mild hippocampal atrophy
MAPT, 1 carrier (0.8%)								
12	p.Val698Ile	2.367e-5	F	-	56	CBS	Left upper limb rigidity and bradykinesia, apraxia, apathy, logopenic speech, concentration deficits, mild memory problems, euphoria	Mild atrophy of the right frontal and temporal lobe
PSEN1, 1 carrier (0.8%)								
13	p.Tyr115Cys	-	F	+	41	bvFTD	Apathy, depression, executive deficits, language disorders (expression)	Left temporal lobe atrophy, mild frontoparietal atrophy
TARDBP, 3 carriers (2.5%)								
14	p.Ile383Val	2.527e-5	M	-	60	bvFTD	Memory deficits, difficulty in naming, apathy, obsessive behavior, disinhibition	Temporal lobe atrophy (L>R)
15	p.Ile383Val	2.527e-5	M	+	58	svPPA	Language disorders (comprehension, expression, reduction of speech), memory, visuospatial and executive deficits, irritability, dietary changes	Bilateral frontal and temporal lobe atrophy
16	p.Ile383Val	2.527e-5	M	+	66	svPPA	Difficulties in the comprehension of language, reduction of speech, memory deficits, collection of useless objects, sweets craving, swearing, apathy	Bilateral frontal and temporal lobe atrophy (L>R)

Key: AO, age at onset; bvFTD, behavioral frontotemporal dementia; CBS, corticobasal syndrome; FH, family history; FTD-ALS, frontotemporal dementia-amyotrophic lateral sclerosis; FTD, frontotemporal dementia; MAF, minor allele frequency; MRI, magnetic resonance imaging; NFE, non-Finnish European; nfvPPA, nonfluent progressive aphasia; svPPA, semantic progressive aphasia; R, right side; L, left side.

had a positive family history for dementia (affected father at the age of 50) and normal cerebrospinal fluid biomarkers (amyloid A-beta 42, tau protein and phospho-tau).

3.2. Variants of unknown significance in rare FTD and ALS genes

In addition to variants in the presumed common FTD and AD genes, we also identified 14 rare, predicted deleterious variants in genes less commonly associated with FTD or ALS (Table 3). These were found in 11 cases (three cases carried two variants), two of which were also carriers of a pathogenic *C9orf72* repeat, and one case carried a likely pathogenic variant in *TARDBP*. None of these variants were found in our control group.

One of these variants, a heterozygous frameshift variant in *OPTN*, p.Lys360ValfsTer18, was predicted to result in a truncated optineurin protein that lacks its ubiquitin-binding domain (Table 3, case 5). While there are reports for both dominant and recessive *OPTN* pathogenic variants in the literature, this deletion has been found only in two Turkish consanguineous families with cognitive impairment andALS (Ozoguz et al., 2015). This, in addition to the fact that it was found in a bvFTD case that also carried a pathogenic *C9orf72* repeat expansion, led to uncertainty on the pathogenicity of this variant. Another example is the *TREM2* missense p.Thr66Met variant found in a patient with nfvPPA (Table 3, case 23). This variant in homozygosity was originally associated with polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, also known as Nasu-Hakola disease, and more recently with an FTD-like syndrome without bone pathology in consanguineous families (Guerreiro et al., 2013a; Le Ber et al., 2014). Functional studies have shown that mutant Thr66Met severely reduces maturation of TREM2 resulting in a significant loss of function, including impaired phagocytosis (Kleinberger et al., 2014). While there are reports of FTD and ALS cases carrying this variant in heterozygous state (Borroni et al., 2014; Guerreiro et al., 2013b), it is still unclear whether one mutant *TREM2* allele is sufficient to cause pathogenicity or if only increases the risk for dementia.

The remaining variants are, by definition, either novel or extremely rare (NFE MAF$<$ 0.0001), predicted deleterious, missense variants in nine different genes (one variant in *CHCHD10*, *EWSR1*, *NEK1*, *TAF15*, *TIA1* and *VCP*, and 2 variants in *DCTN1*, *SQSTM1*, *TBK1*). However, the current evidence is not sufficient to classify these as potentially disease-causing. Indeed, a few of these were identified in cases that also carried known (likely) pathogenic FTD variants (case 6, who carries a *C9orf72* pathogenic repeat expansion and two rare variants in *NEK1* and *TIA1*; and case 15, who carries a likely pathogenic *TARDBP* variant and a rare *DCTN1* variant).

4. Discussion

Our study presents the first genetic screen of a clinical series of patients with FTD from Greece. We identified 16 (likely) pathogenic variant carriers, including two seemingly sporadic cases, corresponding to a total frequency of 13.6% carriers in this Greek FTD series. The frequency of genetic forms increased to 30.4% when considering only familial cases. When ascertained by clinical syndrome, pathogenic variants accounted for up to 13.2% of bvFTD, where we found four *C9orf72* expansions, three pathogenic *GRN* variants, and one likely pathogenic in *PSEN1* and one in *TARDBP*. While the total number of cases for the other clinical syndromes was quite smaller, we also found that two of the five (40.0%) FTD-ALS cases carried a *C9orf72* expansion, whereas three of 14 (21.4%) svPPA cases carried (likely) pathogenic variants in *GRN* or *TARDBP*. We only found one likely pathogenic *MAPT* variant in a CBS case (11.1%) and one pathogenic *GRN* variant in one (10.0%) nfvPPA case, whereas no causative variant was found in any of the 12 PSP cases in our series.

Several studies have demonstrated that *C9orf72* is the major cause of familial (~25%) and sporadic (~5%) FTD, with higher frequencies in northern Europe, especially in isolated populations such as Finland (Majounie et al., 2012). Comparable with other FTD genetic screenings, our series showed that *C9orf72* repeat expansions accounted for 13.0% of familial FTD Greek cases, whereas we did not detect any repeat expansions among the 57 apparently sporadic cases. In terms of clinical presentation, these carriers were diagnosed with bvFTD or FTD-ALS with first symptoms manifesting around 58 years of age (onset ranging from 49 to 72 years). This notable difference in age at onset among *C9orf72* may be a result of different expanded repeat sizes, with longer repeats being associated with earlier onset, as observed in other repeat-associated diseases, such as Huntington's disease. However, it cannot be excluded the possibility that other rare and common variants may contribute to this variability, as we have identified rare, predicted deleterious variants in other FTD and ALS genes in two *C9orf72* expansion carriers.

GRN mutations are estimated to be responsible for another 5%–20% of familial and 1%–5% of sporadic FTD cases (Rademakers et al., 2012), and in our series, they accounted for 10.9% of cases with positive family history. All variants found in *GRN* were loss-of-function variants predicted to lead to nonsense-mediated decay

Table 3
Variants of unknown significance in rare FTD and ALS genes

Case	Gene	Variant	gnomAD NFE_MAF	SIFT	Polyphen	CADD	Gender	AO	FH	FTD syndrome
5[a]	*OPTN*	p.Lys360ValfsTer18	-	-	-	35.0	F	63	+	bvFTD
6[a]	*NEK1*	p.Pro835Leu	0.000018	D	D	30.0	F	72	+	bvFTD
	TIA1	p.Gly336Ser	0.000072	D	P	24.1				
15[a]	*DCTN1*	p.Leu1094Pro	-	T	B	25.1	M	58	+	svPPA
17	*CHCHD10*	p.Tyr104His	-	D	D	24.1	M	53	-	bvFTD
18	*EWSR1*	p.Thr108Ala	0.000090	T	P	11.5	F	66	+	nfvPPA
19	*DCTN1*	p.Arg274Gln	0.000009	D	B	22.9	F	66	-	bvFTD
	SQSTM1	p.Ser328Leu	-	T	B	23.5				
20	*SQSTM1*	p.Pro438Ser	-	D	D	22.6	M	60	-	svPPA
	TAF15	p.Phe212Val	-	T	P	17.4				
21	*TBK1*	p.Ser268Gly	0.000018	D	B	21.1	M	47	N/A	bvFTD
22	*TBK1*	p.Asn725Ser	-	T	P	16.1	F	55	+	PSP
23	*TREM2*	p.Thr66Met	0.000027	D	D	29.4	M	54	+	nfvPPA
24	*VCP*	p.Asp395Gly	-	D	P	23.8	F	36	+	bvFTD

SIFT predictions—T, Tolerated; D, Deleterious. Polyphen predictions—D, Probably Damaging; P, Possibly Damaging; B, Benign.
Key: AO, age at onset; bvFTD, behavioral frontotemporal dementia; FH, family history; FTD, frontotemporal dementia; MAF, minor allele frequency; N/A, not available; NFE, non-Finnish European; nfvPPA, nonfluent progressive aphasia; svPPA, semantic progressive aphasia; PSP, progressive supranuclear palsy.
[a] Carrier of a known pathogenic or [b]likely pathogenic variant in one of the eight most common FTD and AD genes.

of mutant *GRN* mRNA and reduced expression of progranulin. The main clinical diagnosis associated with Greek *GRN* carriers was bvFTD (three cases) followed by aphasia (one svPPA and one nfvPPA), with significant apathy and language dysfunction, which is consistent with previous reports (Benussi et al., 2015). Interestingly, the patient with bvFTD who carried a large *GRN* deletion predicted to result in a truncated progranulin presented with memory impairment at onset that led to an initial clinical diagnosis of AD, whereas his brother had parkinsonism. Although rarely observed at onset, parkinsonian features often manifesting as CBS have been reported in about 40% of patients with *GRN* mutations (Le Ber et al., 2008). The third most common FTD gene is *MAPT,* with a frequency ranging between 5% and 20% of familial FTD cases. Surprisingly, we did not find any (likely) pathogenic *MAPT* variants among our 46 familial FTD cases, as the only carrier we identified was a CBS case with apparent no family history. As with *GRN* mutations, the most common presentation of *MAPT* mutations is bvFTD; however, patients with a primary parkinsonian syndrome have also been reported.

Mutations in *TARDBP* have been associated with both ALS and FTLD-TDP, but while 5% of familial ALS cases carry a *TARDBP* mutation, they are rarely observed in FTD. Among the few cases reported so far, the most common presentation is bvFTD followed by PPA, in particular svPPA (Benussi et al., 2015), which is consistent with our findings. Interestingly, the three cases identified herein carried the same likely pathogenic variant in *TARDBP.* As these cases were not related, it is also possible that this mutation originated from a common founder in the Greek population. Indeed, another missense *TARDBP* variant (p.A382T) has been identified as a major cause of FTD-ALS in patients from Sardinia, a genetic isolate, and haplotype analysis strongly suggested that this mutation originated from a single founder (Chio et al., 2011; Quadri et al., 2011).

Interestingly, we also identified one bvFTD case with a *PSEN1* variant that has been associated with multiple cases of AD (Cruts et al., 1998; Janssen et al., 2003; Rogaeva et al., 2001; Wallon et al., 2012). While it has been shown that *PSEN1* variants can have a clinical presentation of bvFTD (Blauwendraat et al., 2018; Raux et al., 2000; Tang-Wai et al., 2002), it should be noted that a frontal variant of AD, characterized by predominant behavioral or dysexecutive deficits caused by AD pathology, may mimic that of bvFTD in up to 40% of clinically diagnosed bvFTD cases (Ossenkoppele et al., 2015). Therefore, it is important to add common AD genes, especially *PSEN1* and *PSEN2,* to those screened in clinical routine for FTD cases (typically, *MAPT, GRN,* and *C9orf72*).

The substantial number of Greek familial cases in the present series with no obvious causal variant (32 of 46 familial cases) demonstrates that there must be other genetic causes of FTD besides those frequently screened in clinical routine. We therefore examined in our series 21 other genes previously associated with FTD or ALS to uncover possible novel disease-causing variants, and identified rare, predicted deleterious variants in 11 cases, three of which were also carriers of a (likely) pathogenic variant. As these genes are not yet routinely screened in clinical studies, data on frequency of these variants in large clinical cohorts and their functional effects are not available, and therefore it is difficult to assess their relationship to disease susceptibility.

Most clear pathogenic variants identified so far in the *TBK1* gene are loss-of-function variants that have been implicated in ALS and FTD (Cirulli et al., 2015; Freischmidt et al., 2015), although numerous rare missense variants have been identified (Le Ber et al., 2015; van der Zee et al., 2017). In our series, we identified two rare missense variants, p.Ser268Gly, located in the catalytic kinase domain, and p.Asn725Ser, located in the C-terminal in the OPTN binding domain. While these two variants are within functional *TBK1* domains, prediction of its pathogenicity is prevented by the absence of data demonstrating cosegregation and functional studies testing their effect on TBK1 kinase activity and on the interaction with OPTN.

Mutations in *VCP* have been associated with different clinical presentations, including inclusion body myopathy with Paget's disease of bone and FTD, Charcot-Marie-Tooth disease type 2, and ALS. However, mutations in *VCP* are rare and account for less than 1% of cases with familial FTD, with bvFTD and svPPA being the subtypes most frequently reported. In our series, we identified one bvFTD case with a variant located in the ATPase D1 domain, which is involved in oligomerization. However, most pathogenic variants are located within the N-terminal CDC48 domain, which is involved in ubiquitin binding, suggesting that these mutations may affect protein degradation or protein-mediated autophagy in the ubiquitin-proteasome system (Rainero et al., 2017).

Mutations in *SQSTM1*, first implicated in Paget disease of the bone, are predicted to account for up to 3% of FTD cases; however, cosegregation has only been shown in a few families. In our series, we identified two cases with *SQSTM1* rare deleterious variants in the functional LC3 interaction region domain (p.Ser328Leu) and in the C-terminal ubiquitin-associated domain (p.Pro438Ser). While a large resequencing study has shown that *SQSTM1* mutations are clustered in these two domains in patients with FTD compared with controls (van der Zee et al., 2014), cosegregation and functional analysis are necessary to assess their pathogenicity. Furthermore, in our series both carriers of the *SQSTM1* variants also carried another rare deleterious variant (in *DCTN1* and *TAF15*).

Rare mutations impacting the low-complexity sequence domains of *TIA1* have been recently identified in patients with ALS and FTD-ALS (Mackenzie et al., 2017). Herein, we found the rare deleterious missense p.Gly336Ser variant located in the low-complexity sequence domain in one bvFTD case. Interestingly, this case was also a carrier of repeat expansion in the *C9orf72* gene (in addition to a rare variant in the ALS gene *NEK1*). The relevance of this variant is therefore unclear in the absence of further cosegregation and functional studies.

Our study presents some limitations, such as the small number of matching controls. We are aware that comparison against gnomAD data is not optimal, as the gnomAD series (including over 130,000 unrelated individuals) is not population- or age-matched to our Greek FTD cohort. In addition, even if we only considered variants with an MAF up to 0.0001, we cannot exclude the presence of presymptomatic cases within this data set. In summary, we consider gnomAD frequencies as illustrative of the genetic load. Moreover, a segregation analysis, which might have helped in a better assessment of the relevance and impact of the likely pathogenic and unknown significance variants found in our cohort, was not always feasible, as we did not have access to additional family members for most probands included in this study.

5. Conclusions

Here, we present the first in-depth genetic screen of clinical FTD in Greece. These screenings are critical for unraveling the frequencies and distribution of mutations in the common FTD genes, as they can vary substantially across populations, and therefore have important implications for clinical practice, genetic diagnosis, and counseling. Our study also shows that an unbiased sequencing approach, either targeted or whole-exome sequencing, provides important information to assess and determine the role of otherwise nonroutinely screened genes in disease susceptibility.

Disclosure

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2018.10.029.

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