Underlying genetic variation in familial frontotemporal dementia: sequencing of 198 patients

Merel O. Mol,⁎, Jeroen G.J. van Rooij,⁎, Tsz H. Wong, Shamiram Melhem,⁠ a, Annemieke J.M.H. Verkerk,⁎ b, Anneke J.A. Kievit, c Rick van Minkelen, d Rosa Rademakers, e Cyril Pottier, d Laura Donker Kaat, e Harro Seelaar, e John C. van Swieten, e Elise G.P. Dopper e

⁎ Department of Neurology & Alzheimer Center, Erasmus Medical Center, Rotterdam, the Netherlands
① Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands
② Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands
③ Neurodegenerative Brain Diseases Group, VIB Center for Molecular Neurology, University of Antwerp, Antwerp, Belgium

1. Introduction

Frontotemporal dementia (FTD) is one of the main causes of presenile dementia (Coyle-Gilchrist et al., 2016). FTD constitutes a heterogeneous spectrum with large variability in clinical and pathological features (Mackenzie and Rademakers, 2007; Mann and Snowden, 2017). It has a strong genetic component, and autosomal dominant inheritance is observed in 10%–25% of patients (Convery et al., 2019; Seelaar et al., 2008). Mutations in C9orf72, GRN, and MAPT account for ~30% of familial cases, with substantial geographical variability in mutation frequencies (Fostinelli et al., 2018; Kim et al., 2018; Moore et al., 2020; Oijerstedt et al., 2019; Seelaar et al., 2008; Tang et al., 2016; Wood et al., 2013). In the past decade, whole-exome sequencing (WES) has emerged as a method to identify novel pathogenic variants not only in these genes, but also likely pathogenic variants or variants of uncertain significance (VUS) in an increasing number of other dementia-associated genes such as TARDBP, VCP, TBK1, and SQSTM1 (Blauwendraat et al., 2018; Dols-Icardo et al., 2018; Ramos et al., 2019, 2020). Nonetheless, around two-thirds of familial cases remain without a known genetic cause, implying yet undiscovered variants (Pottier et al., 2019).

In this study, we systematically assessed a broad set of dementia-related genes in our large cohort of patients with FTD and a positive family history using WES, C9orf72 repeat-primed PCR, and copy number variation analysis. Our objectives were to investigate the frequencies of pathogenic variants in the Netherlands and to identify potential novel variants, which might ultimately provide new pathophysiological insights.

2. Materials and methods

2.1. Clinical data collection

Patients were selected from our large FTD cohort in the Netherlands (Erasmus Medical Center, Rotterdam) (Seelaar et al., 2016). In the past decade, whole-exome sequencing (WES) has emerged as a method to identify novel pathogenic variants not only in these genes, but also likely pathogenic variants or variants of uncertain significance (VUS) in an increasing number of other dementia-associated genes such as TARDBP, VCP, and SQSTM1 (Blauwendraat et al., 2018; Dols-Icardo et al., 2018; Ramos et al., 2019, 2020). Nonetheless, around two-thirds of familial cases remain without a known genetic cause, implying yet undiscovered variants (Pottier et al., 2019).

In this study, we systematically assessed a broad set of dementia-related genes in our large cohort of patients with FTD and a positive family history using WES, C9orf72 repeat-primed PCR, and copy number variation analysis. Our objectives were to investigate the frequencies of pathogenic variants in the Netherlands and to identify potential novel variants, which might ultimately provide new pathophysiological insights.
2008), which currently includes 656 patients with a clinical diagnosis of either the behavioral variant of FTD (bvFTD) or primary progressive aphasia (PPA), classified into 3 different forms (semantic variant [svPPA], nonfluent variant [nfvPPA], and logopenic variant [lvPPA]). We excluded patients and relatives with a pathological diagnosis other than frontotemporal lobar degeneration (FTLD). The family history was considered positive with the presence of at least one first- or second-degree relative affected by an FTLD spectrum disorder (besides bvFTD and PPA, this includes FTLD with motor neuron disease, amyotrophic lateral sclerosis [ALS], progressive supranuclear palsy, and corticobasal syndrome [CBS]) or another type of dementia or Parkinson’s disease [PD]). Family history was further classified into one of the following adjusted Goldman categories (Goldman et al., 2005). Psychiatric disorders were not considered in this classification as these were not known for all patients.

(1) Autosomal dominant: > 2 relatives with either an FTLD spectrum disorder at any age or another type of dementia or PD < 65 years, occurring in at least 2 generations with one person being a first-degree relative of both other 2;
(2) Familial aggregation: ≥ 3 relatives (first, second, or third degree) with an FTLD spectrum disorder; another type of dementia or PD at any age, not meeting criteria for autosomal dominant inheritance;
(3) Possible familial: > 1 first- or second-degree relative with an FTLD spectrum disorder at any age or another type of dementia or PD < 65 years;
4) Possible familial late-onset: > 1 first-degree relative with any type of dementia or PD > 65 years;
(5) Negative family history: none of the above.

From the total cohort (n = 656), we selected 198 unrelated patients with a positive family history (Goldman 1–4) and DNA availability (Supplementary Fig. A.1). For 41 familial patients, DNA was not available.

2.2. Sequencing and variant filtering

In 38 patients, targeted Sanger sequencing of MAPT or GRN, or C9orf72 repeat-primed PCR had previously revealed a pathogenic variant. WES was performed in 151 patients, and 9 were whole-genome sequenced at the Mayo Clinic Genome Analysis Core as part of another study. As the data were collected from various sources, different capture kits were used (see Appendix A for bioinformatics details). The presence of a C9orf72 repeat expansion was tested using either repeat-primed PCR or a commercial kit (AmpliDEX PCR/CE, Asuragen), with a repeat length ≥30 considered pathogenic variants (Renton et al., 2011).

We analyzed 26 prespecified genes, based on an extensive literature search of genes associated with FTD, FTD-ALS, and Alzheimer’s disease (AD), as AD may clinically resemble FTD (Supplementary Table A.1). Variants were selected based on the following criteria: (1) affecting coding (missense, nonsense, frameshift) or splicing regions; (2) with a minor allele frequency of <0.1% in the Genome Aggregation Database (gnomAD); and (3) with a quality by depth score ≥5. The untranslated regions (UTRs) of the genes GRN, MAPT, and TARDBP were investigated for the presence of known pathogenic regulatory variants. Variants reported as pathogenic in the AD&FTD Mutation Database (http://www.molgen.ua.ac.be/ADMutations) were classified accordingly. We classified novel variants as pathogenic, likely pathogenic, or as VUS in a conservative and systematic approach according to the recently refined guidelines by The American College of Medical Genetics and Genomics (ACMG) (Nykamp et al., 2017; Richards et al., 2015). The following criteria were jointly considered to obtain evidence of pathogenicity: (1) bioinformatic in silico prediction scores: SIFT, PolyPhen2, MutationTaster, FATHMM, combined annotation dependent depletion; score ≥10; (2) Human Splicing Finder, and MaxEnt; (2) presence in other online genetic databases [OMIM, HGMD, ClinVar, AlzGene, Healthy Exomes (HEX) (Guerreiro et al., 2018)]; (3) existing literature on the variant or a different variant in the same position; (4) segregation analysis if available; (5) functional biomarker if available (blood progranulin levels for GRN); and (6) pathological confirmation of disease if available. Variants reported in the previously mentioned genetic databases as likely benign were only discarded if these reports were consistent and in concordance with in silico prediction tools. Pathogenic and likely pathogenic variants were confirmed by Sanger sequencing.

2.3. SNP array and CNV detection

We performed copy number variant (CNV) analysis of the same 26 genes using single nucleotide polymorphism (SNP) array data to identify deletions or duplications in subjects without a pathogenic variant (including those with a VUS). The SNP array platform used was Illumina GSA BeadChip GSA MD, v2 (Illumina GSA Arrays “Infinium iSelect 24x1 HTS Custom BeadChip Kit”). Samples were processed using the Illumina manufacturer’s recommended protocol. CNV calling was performed using Nexus Copy Number software (v4.1, BioDiscovery, Inc, El Segundo, CA, USA) with default parameters.

2.4. Neuropathology

Neuropathological examination was available in 76 subjects (46 probands and 30 affected relatives). Immunohistochemistry was performed as previously described (Seelaar et al., 2008), and FTLD diagnosis was based on the criteria by Cairns et al. (2007). The pattern of FTLD with TDP-43 or FET pathology was classified into different subtypes according to the morphology and distribution of neuronal inclusions as proposed by Neumann and Mackenzie (2019).

3. Results

3.1. Frequencies of known pathogenic variants

We detected a pathogenic or likely pathogenic genetic variant in 74 of 198 (37%) patients (Table 1). The most common cause was the C9orf72 repeat expansion identified in 21% (42/198), followed by pathogenic variants in MAPT in 6% (11/198; 6 unique variants), GRN in 4.5% (9/198; 8 unique variants, 3 of which were not reported previously), and TARDBP in 3.5% (7/198, 2 unique variants). Clinical and pathological characteristics of patients carrying genetic variants in these 4 genes are shown in Fig. 1 and Supplementary Table A.2. Furthermore, we identified 2 different pathogenic missense variants in VCP (1%), one nonsense variant in TBK1 (0.5%), one missense variant in PSEN1 (0.5%), and one novel homozygous variant in OPTN (0.5%). Subsequent CNV analysis performed in all remaining cases (n = 124) did not reveal any deletions or duplications. No cases were identified with a double pathogenic variant, although this could not be excluded in 38 cases tested for single genes.

3.2. Novel pathogenic and likely pathogenic variants

The novel OPTN variant is a homozygous splice-site variant (c.1242+1G>A) in a patient with lvPPA, decreased frontotemporal...
FDG uptake on positron emission tomography-computed tomography, and a normal profile in cerebrospinal fluid of ptau and amyloid-β, which is incompatible with AD. Family history revealed a sibling diagnosed with nvfPPA and consanguinity between parents (Goldman 3). No other relatives were known to have dementia, PD, or ALS. We considered the variant likely pathogenic

| Gene     | Nucleotide change | Amino acid change | gnomAD MAF | CADD | #Probands | #Relatives |
|----------|-------------------|-------------------|------------|------|-----------|------------|
| C9orf72  | NA                | NA                | NA         | NA   | 42        | 16         |
| GRN (NM_002087) | c.243delC     | SK22YfsX174       | 0          | NA   | 1         | 28         |
| GRN     | c.373C>T          | Q125X             | 0          | 35.0 | 1         | 5          |
| GRN     | c.1231_1232delGT  | V411Sfs*2         | 0          | NA   | 1         | 0          |
| GRN     | c.945_946delTG    | C315X             | 0          | NA   | 1         | 0          |
| GRN     | c.1160dupG        | C388LinsX26       | 0          | NA   | 1         | 0          |
| GRN     | c.19T>G           | W7G               | 0          | 26.0 | 1         | 0          |
| GRN     | c.19T>C           | W7R               | 0          | 25.9 | 2         | 0          |
| GRN     | c.1A>C            | M17 (p.0)         | 0          | 23.9 | 1         | 0          |
| MAPT (NM_005910) | c.902C>T       | P301L             | 0          | 32.0 | 3         | 34         |
| MAPT    | c.815G>T          | G272V             | 0          | 29.8 | 2         | 6          |
| MAPT    | c.944T>G          | L315R             | 0          | 33.0 | 1         | 6          |
| MAPT    | c.1216C>T         | R406W             | 1.6e-05    | 29.8 | 3         | 3          |
| MAPT    | c.959C>T          | S320F             | 0          | 32.0 | 1         | 0          |
| OPTN (NM_001008211) | c.841_843delAAG | L281del         | 2.6e-05    | NA   | 1         | 0          |
| MAPT    | c.1242+1G>A       | NA                | 4.0e-06    | 28.6 | 1         | 0          |
| ISEN     | c.791C>T         | P264L             | 4.0e-06    | 32.0 | 1         | 0          |
| TARDBP (NM_007375) | c.1147A>G       | I383V             | 1.9e-05    | 18.6 | 6         | 1          |
| TARDBP  | c.787A>G          | K263G             | 0          | 28.9 | 1         | 0          |
| TBK1 (NM_013254) | c.1335G>A       | W445X             | 0          | 39.0 | 1         | 1          |
| TBK1     | c.785G>C          | T262S             | 0          | 23.2 | 1         | 0          |
| TBK1     | c.472A>G          | M158V             | 0          | 23.8 | 1         | 0          |
| TOTAL    |                    |                   |            | 74   | 100       |            |

Key: AD, Alzheimer’s disease; ALS, amyotrophic lateral sclerosis; CADD, combined annotation dependent depletion; FTD, frontotemporal dementia; NA, not available/applicable.

a Minor allele frequency of the total population (141,456 exome/genome sequences).
b Version CADD score: GRch37-v1.4.
c Relatives are all confirmed carriers of the variant.

d FG uptake on positron emission tomography-computed tomography, and a normal profile in cerebrospinal fluid of ptau and amyloid-β, which is incompatible with AD. Family history

Fig. 1. Circos plots showing correlations between the major genetic subtypes and (A) clinical diagnosis (n = 292) and (B) pathological diagnosis (n = 76), whereas large heterogeneity is revealed in cases without identified genetic cause. Patients carrying variants in the genes OPTN, ISEN1, TBK1, and VCP were not included in these figures because of small numbers. The group ‘unknown’ includes patients with a VUS. Other – other clinical diagnosis (nvPPA, mixed PPA, or benign FTD). TDP-other – type D, type E, or unclassified. Details of all patients can be found in the Supplementary Tables A.2-3. Abbreviations: VUS, variants of uncertain significance; nvPPA, logopenic variant of PPA; PPA, primary progressive aphasia; FTD, frontotemporal dementia.
for the following reasons: (1) it is extremely rare in gnomAD (minor allele frequency, 8.8e-06) and has not been reported in the homozygous state; (2) it is predicted to change the canonical splice donor site resulting in skipping of exon 12 (MaxEnt, NNSplice, HSF), leading to a shift of the open reading frame; (3) the variant segregates with the disease as the sibling with nfVPPA carried the same homozygous variant.

Three variants in GRN have not been reported previously, including 2 truncating (p.C388LfsX26 and p.C315X) and 1 missense variant (p.W7G). The truncating variants were found in 2 patients with bvFTD leading to death within 5 years. Family history revealed an autosomal dominant pattern in the patient with the C388LfsX26 variant (Goldman 1), whereas the patient with the C315X variant only had 2 relatives with dementia at old age (Goldman 4). Segregation analysis could not be performed because of lack of DNA from family members and serum was not available to measure progranulin levels. However, all truncating variants in GRN are currently considered as likely pathogenic.

The GRN missense variant was identified in a patient who presented with apathy, severe visual hallucinations, fluctuations in cognitive functioning, and a mild asymmetrical hypokinetic rigid syndrome, leading to a differential diagnosis of dementia with Lewy bodies, bvFTD, and CBS. Neuroimaging showed severe left frontal atrophy, suggestive of underlying FTLD. The patient’s brother was clinically diagnosed with CBS and also suffered from prominent visual hallucinations. Their father had died at the age of 59 years with severe behavioral and memory disturbances. DNA of these unaffected relatives was not available for testing. Its pathogenicity is further supported by absence in gnomAD, reduced serum progranulin levels (13.4 ng/mL) in the carrier, and the previous report of a variant (p.W7R) (Saracino et al., 2019).

We found 15 different VUS (Table 2 and Supplementary Table A3). The variant we identified in TUBA4A (p.R105C) seems most relevant, as it was found in a proband with an autosomal dominant inheritance pattern, and segregation analysis revealed the same variant in 4 additional affected relatives (2 with bvFTD and 2 with unspecified dementia), whereas it was absent in an unaffected relative (aged >70 years). Its pathogenicity is further supported by its absence in gnomAD, and in silico tools predict a deleterious effect. FTLD-TDP pathology was confirmed in the proband, with features fitting subtype A. Based on the ACMG guidelines, without supporting functional data thus far, we interpreted the variant as VUS.

Three other variants (K389Rfs*23 in VCP, p.W541C in GRN, and p.P1084S in DCTN1) in patients with familial aggregation (Goldman 2) are potential candidates, but DNA of family members was not available for segregation analysis. The frameshift variant in VCP, due to an insertion resulting in a truncated protein, was found in a patient with bvFTD. Family history was positive for dementia and PD. Its pathogenicity is unknown as frameshift or nonsense variants have not been previously reported in VCP. Therefore, this variant was classified as VUS. The missense variant in GRN (p.W541C), predicted to be damaging, was found in a patient with nfVPPA, but plasma progranulin levels were not available. The p.P1084S variant in DCTN1 was found in a patient with bvFTD and additional semantic deficits, without parkinsonism or motor neuron disease.

For the remaining 11 variants, pathogenicity remains questionable either because of benign or contradictory in silico predictions or because DNA from other family members was not available for segregation analyses. Of note, the VUS in SQSTM1 (p.A33V) was detected in 2 unrelated patients. This variant was also found in the Healthy Exomes database (minor allele frequency, 0.004).

### 3.4. Patients with unknown genetic cause

We did not identify any pathogenic variant, likely pathogenic variant, or VUS in the 26 screened genes in the remaining 108 (55%) patients. Although >75% had Goldman scores 3–4, this group also included 6 (6%) patients with Goldman 1 and 18 (17%) with Goldman 2. The majority (65%) was diagnosed with bvFTD; a relatively large proportion (21%) in this group had svPPA. Other diagnoses included nfVPPA (13%) and ivPPA (1%). Concomitant parkinsonism was present in 14 patients and 6 suffered from ALS. Seventeen patients underwent pathological examination and showed a variety of FTLD pathologies (Supplementary Tables A.3 and A.4).

### 4. Discussion

In the present study of a large cohort of familial FTD, we revealed pathogenic variants in 8 FTD-related genes, with the following mutations:

### Table 2

| Gene   | Transcript | Nucleotide change | Amino acid change | gnomAD MAF<sup>a</sup> | Pat. Tools<sup>b</sup> | CADD score<sup>c</sup> | Goldman score |
|--------|------------|-------------------|------------------|-------------------------|-------------------------|-------------------------|---------------|
| TUBA4A | NM_006000  | exon3:c.313C>T    | R105C            | 0                       | D/D/D/T                 | 32                      | 1             |
| GRN    | NM_000287  | exon12:c.1623G>C  | W541C            | 0                       | D/D/D/T                 | 34                      | 2             |
| DCTN1  | NM_004082  | exon28:c.2350C>T  | P1084S           | 3.6e-05                 | T/D/T                   | 22.7                    | 2             |
| UNC13A | NM_001080421| exon10:c.1005G>T  | E335D            | 7.6e-05                 | T/T/T                   | 15.7                    | 2             |
| TREM2  | NM_018965  | exon4:c.514C>T    | P172S            | 2.4e-05                 | T/T/T                   | 14.8                    | 2             |
| VCP    | NM_007126  | exon10:c.1064_1065insA | K389Rfs*23 | 0                       | NA                      | NA                      | 2             |
| NEK1   | NM_00119937| exon32:c.3728A>G  | D1243G           | 0                       | D/D/D/T                 | 32                      | 3             |
| PRKAR1B| NM_000273 | exon3:c.259C>G    | P87A             | 3.9e-05                 | T/T/T/D                 | 16.3                    | 3             |
| DPP6   | NM_130797  | exon8:c.805G>A    | G269R            | 3.2e-05                 | T/T/T                   | 24.1                    | 4             |
| SIGMAR1| NM_001282205| exon4:c.463G>C    | A155P            | 1.2e-04                 | NA                      | 18.3                    | 4             |
| UBQ7N2 | NM_013444 | exon1:c.401C>T    | T134I            | 2.5e-05                 | T/T/D                   | 17.5                    | 4             |
| DPP9   | NM_130797  | exon17:c.1673G>A  | G558D            | 0                       | T/T/T                   | 16.3                    | 4             |
| NEK1   | NM_00119937| exon24:c.2023G>A  | V673I            | 1.27e-05                | T/T/T                   | 15.9                    | 4             |
| TBR1   | NM_013254 | exon9:c.1008A>G   | I334V            | 3.2e-05                 | T/T/T                   | 14.3                    | 4             |
| SQSTM1 | NM_003900 | exon1:c.58C>T     | A33V             | 7.7e-04                 | T/T/T/D                 | 13.2                    | 4             |

The variant in SQSTM1 was detected in 2 patients. Variants are ordered to Goldman score and subsequently to CADD score. Variants with a CADD score <10 were discarded. Key: NA, not available.

<sup>a</sup> Minor allele frequency of the total population (141,456 exome/genome sequences).

<sup>b</sup> Prediction tools: SIFT/PolyPhen2/MutationTaster/FATHMM, with T = tolerated and D = damaging.

<sup>c</sup> Version CADD score: gRch37-v1.4.

<sup>d</sup> Large insertion of 124 nucleotides leading to frameshift with stopgain.
C9orf72 repeat expansion as most common, followed by variants in MAPT and GRN. Furthermore, we identified an unexpected high frequency of the p.I383V variant in TARDBP; a novel homozygous OPTN variant, and 3 novel GRN variants. Finally, we found 15 VUS, including a promising variant in TUBA4A that cosegregated with the disease. The overall frequency of pathogenic variants sums up to 37%. Confining the analysis to patients with a strong family history (Goldman 1–2; n=70) raises this to 57%. Nonetheless, it indicates that still a substantial proportion of familial cases remains genetically unresolved.

4.1. Frequencies of known pathogenic variants

We found relatively high frequencies of variants in MAPT (6%) and TARDBP (3.5%) compared with other cohorts (Supplementary Table A.5). Variants in TARDBP have been reported in around 4% of familial ALS (Zou et al., 2017) but much less often in FTD (Blauwendraat et al., 2018; Ramos et al., 2019, 2020). Surprisingly, 5 unrelated TARDBP carriers harbored the same variant (p.I383V), suggestive of a possible founder effect. The same variant was found in other FTD cohort screens across the world (Caroppo et al., 2016; Ramos et al., 2019, 2020). Family history of our patients was not consistent with autosomal dominant transmission (i.e., high Goldmann scores), possibly indicating reduced penetrance of this variant, as also suggested by others (Caroppo et al., 2016).

The repeat expansion in C9orf72 is the most common genetic cause of familial FTD in our cohort, accounting for 21% of cases. This is in line with previous studies revealing it as the major genetic cause of familial and sporadic FTD and ALS (Majounie et al., 2012). However, there is substantial geographical variation with frequencies up to 40% in Scandinavian countries (Fostinelli et al., 2018; Oijerstedt et al., 2019; Ramos et al., 2019), contrasting with its absence in Asian cohorts (Kim et al., 2018; Tang et al., 2016). We found a GRN variant in 4.5%, which is less than in other cohorts, especially compared with an Italian study that reported a remarkably high frequency (Fostinelli et al., 2018) (Supplementary Table A.5). The pathogenic variant in TBK1 (p.W445X) identified in a proband and an affected sibling is the first FTD kindred caused by a variant in TBK1 in the Netherlands. In contrast, other studies have reported variants in TBK1 as the fourth most common genetic cause in FTD (Greaves and Rohrer, 2019). Studies of French and Belgian cohorts found frequencies between 1% and 2% in bvFTD and even higher frequencies in FTD-ALS (Gijselinck et al., 2015; Le Ber et al., 2015; van der Zee et al., 2017).

4.2. Novel likely pathogenic variant in OPTN

The presence of a novel homozygous splice-site variant in OPTN (c.1242+1G>A) in a patient with bvPPA extends the clinical spectrum of OPTN variants, as it has never been associated with this phenotype. OPTN variants are extremely rare in FTD; only a few cases have been described with variants in compound heterozygous state or in combination with a TBK1 variant, which are functionally related genes (Pottier et al., 2015, 2018). Homozygous nonsense/mis sense OPTN variants were first described to cause autosomal recessive ALS (Maruyama et al., 2010). Subsequently, numerous heterozygous variants were reported in ALS as either disease causing or as risk factor (Markovinovic et al., 2017). Thus far, the proband and sibling with nfvPPA are the first FTD cases without motor neuron disease caused by a homozygous OPTN variant. The parents of our patient were unaffected. A heterozygous variant in the same position was reported in a patient with familial ALS (c.1242+1delGinsAA) (Belzil et al., 2011). In this case, a second defect—possibly intrinsic or a copy number variation—in either OPTN or TBK1 cannot be ruled out because the authors performed targeted sequencing of OPTN only. Others have also suggested a complex mode of inheritance regarding OPTN with an oligogenic basis (Pottier et al., 2015). A recent study on patients with dementia identified heterozygous missense variants in OPTN, but functional or segregation analyses were not available (Bartoletti-Stella et al., 2018).

4.3. Variants of uncertain significance

The segregation of a TUBA4A variant (p.R105C)—a gene mostly associated with ALS—in several affected family members seems promising. Neuropathologic findings in the proband resembled FILD-TDP pathology type A. Other groups have also reported likely pathogenic TUBA4A variants in clinical ALS and FTD cases, yet without neuropathologic confirmation, suggesting a plausible role for this gene (Perrone et al., 2017; Smith et al., 2014). Functional studies investigating the pathogenicity of the p.R105C variant are currently ongoing.

A novel frameshift variant in VCP (p.K389RFs*23) is also a plausible candidate. This variant was found in a patient with bvFTD and familial aggregation, without any symptoms of motor neuron disease or myopathy. Variants in VCP are associated with the classical phenotype of inclusion body myopathy with Paget’s disease of bone and frontotemporal dementia (Watts et al., 2004), but cases with pure FTD or ALS have also been described, including 2 other patients in our cohort (Johnson et al., 2016; Wong et al., 2018). Some of the previously reported variants are located in the same D1 domain as this frameshift variant (Abrahaoo et al., 2016; Watts et al., 2004), which is predicted to lead to a truncated protein. A loss of function mechanism has not been described for VCP. Therefore, segregation and/or neuropathological findings consistent with previous VCP cases are needed to confirm its pathogenicity.

For the other identified VUS in our cohort (Table 2), genetic screens in additional cohorts, segregation analyses, and functional studies should provide further insight. Of note, the p.A33V variant in SQSTM1 has been considered as pathogenic despite the lack of functional evidence (Dols-Icardo et al., 2018; Fecto et al., 2011; Le Ber et al., 2013), and it was detected in controls in another study (van der Zee et al., 2014).

4.4. Patients with unknown genetic cause

The wide variety of clinical syndromes and pathologies in the patients with FTD and without an identified genetic cause likely fit various underlying molecular mechanisms. The tau pathology in 4 patients may suggest the presence of unknown causal variants in genes related to MAPT, which may have an impact in its transcription or on the physiology of the tau protein. The strong family history in 2 svPPA cases with confirmed TDP type C was remarkable, as svPPA is nearly always sporadic (Convery et al., 2019). In addition, the presence of FUS pathology in a patient with a family history of dementia, PD, and psychiatric disorders contrasts with the sporadic occurrence of FUS cases in the literature (Neumann and Mackenzie, 2019). As FUS is part of the FET protein family, an undefined variant in 1 of the other FET genes, TAF15 or EWSR1, could be considered. Variants in these genes have been reported in a small number of patients, although these were not confirmed to have FUS pathology (Ramos et al., 2019). In our patient, we did not identify any potential causal variants in these genes.

We have not identified variants in patients with bvFTD and concomitant parkinsonism or motor neuron disease, but could not exclude variants in all genes related to these disorders. It might be worthwhile to extend generic screening to a larger set of genes, as a recent study on sporadic FTD has shown potential variants in genes associated with a variety of disorders (Ciani et al., 2019). Such
neurodegeneration continuously grows, and the phenotypical generation sequencing studies: the list of genes associated with that this work re

Oijerstedt et al., 2019; Ramos et al., 2019). Nonetheless, we believe family history, we may have missed the presence of several GRN or C9orf72 carriers (Lanata and Miller, 2016). Finally, as a substantial family history being rather unconstrained, we might have missed de novo variants in other genes (e.g., in C9orf72 carriers) (Giannoccaro et al., 2017; van Blitterswijk et al., 2013).

5. Conclusions

We present the genetic screen of a large cohort of familial FTD in which we identified a genetic cause in 37% of the patients, including novel pathogenic variants in OPTN and GRN. A large proportion of carriers of the p.1383V variant in TARDBP was found, suggestive of a common founder. We found several VUS, of which the novel variants in TUBA4A and VCP seem most promising. Future studies are needed to confirm their potential pathogenicity. As a whole, our study contributes to the disentanglement of the wide genetic landscape of FTD.

Disclosure statement

The authors declare no conflict of interest. Several authors of this publication are members of the European Reference Network for Rare Neurological Diseases—Project ID No 739510.

CRediT authorship contribution statement

Merel O. Mol: Data curation, Investigation, Formal analysis, Writing - original draft. Jeroen G.J. van Rooij: Conceptualization, Methodology, Writing - review & editing. Tsz H. Wong: Investigation, Writing - review & editing. Shamiram Melhem: Investigation. Annemieke J.M.H. Verkerk: Resources, Writing - review & editing. Anneke J.A. Kievit: Writing - review & editing. Rick van Minakken: Resources, Writing - review & editing. Laura Donker Kaat: Conceptualization, Writing - review & editing. Elise G.P. Dopper: Conceptualization, Supervision, Writing - review & editing.

Acknowledgements

The authors are indebted to all the patients who made this study possible. The authors also thank Prof. A.J.M. Rozemuller from the Netherlands Brain Bank for the neuropathologic examination of the cases. This research was funded by Alzheimer Nederland and by The Dutch Research Council (NWO).

Ethical assurances: Approval of the study was provided by the Medical Ethics Review Board of the Erasmus Medical Center of Rotterdam (MEC-2009-170). Written informed consent was obtained from all participants or their legal representatives. Brain autopsy was performed in accordance with the Legal and Ethical Code of Conduct of the Netherlands Brain Bank.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2020.07.014.

References

Abrahao, A., Abath Neto, O., Kok, F., Zanotelli, E., Santos, B., Pinto, W.B., Barsottini, O.G., Oliveira, A.S., Pedroso, J.L., 2016. One family, one gene and three phenotypes: a novel VCP (valosin-containing protein) mutation associated with myopathy with rimmed vacuoles, amyotrophic lateral sclerosis and fronto-temporal dementia. J. Neurol. Sci. 362, 352–358.

Bartorelli-Stella, A., Biaardi, S., Stanzani-Maserati, M., Piras, S., Caffarra, P., Raggi, A., Pantieri, R., Baldassari, S., Caporali, L., Abu-Rumeileh, S., Linarelli, S., Ligori, R., Parchi, P., Capellari, S., 2018. Identification of rare genetic variants in Italian patients with dementia by targeted gene sequencing. Neurobiol. Aging 66, 180.e23–180.e31.

Belzil, V.V., Daud, H., Desjarlais, A., Bouchard, J.P., Dupre, N., Camu, W., Dixon, P.A., Rouleau, G.A., 2011. Analysis of OPTN as a causative gene for amyotrophic lateral sclerosis. Neurobiol. Aging 32, 555.e13–555.e14.

Blauwendraad, C., Wilke, C., Simon-Sanchez, J., Jansen, I.E., Reisfischer, A., Capell, A., Haasis, C., Castillo-Lizardo, M., Biskup, S., Maetzler, W., Rizzu, P., Heutink, P., Synofzik, M., 2018. The wide genetic landscape of clinical fronto-temporal dementia: systematic combined sequencing of 121 consecutive subjects. Genet. Med. 20, 240–249.

Cairns, N.J., Bigio, E.H., Mackenzie, I.R., Neumann, M., Lee, V.M., Hatapaa, K.J., White T.S., CL. Schneider, J.A., Grinberg, L.T., Halliday, G., Duqueaerts, C., Lowe, J.S., Holm, I.E., Tolnay, M., Okamoto, K., Yokoo, H., Murayama, S., Woulfe, J., Munoz, D.G., Dickson, D.W., Ince, P.G., Trojanowski, J.Q., Mann, D.M., Consortium for Frontotemporal Lobar Degeneration, 2007. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol. 114, 5–22.

Caroppo, P., Camuzat, A., Guillot-Noel, L., Thomas-Anterion, C., Couriatier, P., Wong, T.S., Teschmann, M., Golfer, V., Auriacombe, S., Bellard, S., Lawton, R., Lattante, S., Millecamps, S., Clot, F., Dubois, B., van Swieten, J.C., Brice, A., Le Ber, I., 2016. Defining the spectrum of frontotemporal dementias associated with TARDBP mutations. Neurol. Genet. 2, e80.

Ciani, M., Bonvicini, C., Scassellati, C., Carrara, M., Maj, C., Fostinelli, S., Binetti, G., Ghidoni, R., Benussi, L., 2019. The missing heritability of sporadic fronto-temporal dementia: new insights from rare variants in neurodegenerating candidate genes. Int. J. Mol. Sci. 20.

Convery, R., Mead, S., Rohrer, J.D., 2015. Review: clinical, genetic and neuroimaging features of frontotemporal dementia. Neuropathol. Appl. Neurobiol. 41, 6–18.

Coyle-Gilchrist, L.T., Dick, K.M., Patterson, K., Vazquez Rodriquez, P., Wehmann, E., Velcic, A., Lansdall, C.J., Dawson, K.E., Wiggins, J., Mead, S., Brayne, C., Rowe, J.B., 2016. Prevalence, characteristics, and survival of frontotemporal lobar degeneration syndromes. Neurology 86, 1736–1743.

Dols-Icardo, O., Garcia-Redondo, A., Rojas-Garcia, R., Borrego-Hernandez, D., Illan-Grau, I., Munoz, Blanco-Blanco, J.L., Rabano, A., Cervera-Carles, L., Juarrez-Rufian, A., Spataro, N., De Luna, N., Galan, L., Cortes-Vicente, E., Fortea, J., Blesa, R., Graaij, Riveria, O., Lleo, A., Esteban-Perez, J., Gelpi, E., Clarimon, J., 2018. Analysis of known amyotrophic lateral sclerosis and frontotemporal dementia genes reveal a substantial genetic burden in patients manifesting both diseases not carrying the C9orf72 expansion mutation. J. Neurol. Neurosurg. Psychiatry 89, 162–168.

Fecto, F., Yan, J., Vemula, S.P., Liu, E., Yang, Y., Chen, W., Zheng, J.G., Shi, Y., Siddique, N., Arrat, H., Donkervoort, S., AJroud-Driss, S., Suff, R.L., Heller, S.L., Davies, H.X., Siddique, T., 2013. SOTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch. Neurol. 68, 1440–1446.

Fostinelli, S., Ciani, M., Zanardini, R., Zanetti, O., Nettan, O., Binetti, G., Ghidoni, R., Benussi, L., 2018. The heritability of frontotemporal lobar degeneration: validation of...
