

Clinical variability and onset age modifiers in an extended Belgian GRN founder family



Eline Wauters^{a,b,1}, Sara Van Mossevelde^{a,b,c,1}, Kristel Slegers^{a,b}, Julie van der Zee^{a,b}, Sebastiaan Engelborghs^{b,d}, Anne Sieben^{a,b,e}, Rik Vandenberghe^{f,g}, Stéphanie Philtjens^{a,b}, Marleen Van den Broeck^{a,b}, Karin Peeters^{a,b}, Ivy Cuijt^{a,b}, Wouter De Coster^{a,b}, Tim Van Langenhove^e, Patrick Santens^e, Adrian Ivanoiu^h, Patrick Cras^{b,c}, Jan L. De Bleecker^e, Jan Versijptⁱ, Roeland Crols^d, Nina De Klippel^j, Jean-Jacques Martin^b, Peter P. De Deyn^{a,b,d}, Marc Cruts^{a,b}, Christine Van Broeckhoven^{a,b,*}, on behalf of the Belgian Neurology (BELNEU) Consortium

^a Center for Molecular Neurology, VIB, Antwerp, Belgium

^b Institute Born-Bunge and University of Antwerp, Antwerp, Belgium

^c Department of Neurology, Antwerp University Hospital, Edegem, Belgium

^d Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerp, Belgium

^e Department of Neurology, University of Ghent and University Hospital Ghent, Ghent, Belgium

^f Department of Neurosciences, Faculty of Medicine, KU Leuven, Leuven, Belgium

^g Department of Neurology, University Hospitals Leuven, Leuven, Belgium

^h Department of Neurology, Saint-Luc University Hospital and Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium

ⁱ Department of Neurology, University Brussels (VUB), University Hospital Brussel (UZ Brussel), Brussels, Belgium

^j Neurology Service, Jessa Hospital, Hasselt, Belgium

ARTICLE INFO

Article history:

Received 9 November 2017

Received in revised form 6 February 2018

Accepted 3 March 2018

Available online 10 March 2018

Keywords:

Frontotemporal dementia

Clinical heterogeneity

GRN

Founder pedigree

Modifiers

ABSTRACT

We previously reported a granulin (GRN) null mutation, originating from a common founder, in multiple Belgian families with frontotemporal dementia. Here, we used data of a 10-year follow-up study to describe in detail the clinical heterogeneity observed in this extended founder pedigree. We identified 85 patients and 40 unaffected mutation carriers, belonging to 29 branches of the founder pedigree. Most patients (74.4%) were diagnosed with frontotemporal dementia, while others had a clinical diagnosis of unspecified dementia, Alzheimer's dementia or Parkinson's disease. The observed clinical heterogeneity can guide clinical diagnosis, genetic testing, and counseling of mutation carriers. Onset of initial symptomatology is highly variable, ranging from age 45 to 80 years. Analysis of known modifiers, suggested effects of GRN rs5848, microtubule-associated protein tau H1/H2, and chromosome 9 open reading frame 72 G₄C₂ repeat length on onset age but explained only a minor fraction of the variability. Contrary, the extended GRN founder family is a valuable source for identifying other onset age modifiers based on exome or genome sequences. These modifiers might be interesting targets for developing disease-modifying therapies.

© 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a heterogeneous group of neurodegenerative disorders. Three clinical subtypes have been defined: the behavioral variant of frontotemporal dementia

(bvFTD) and the nonfluent and semantic variant of primary progressive aphasia (PPA) (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). The pathological hallmark of FTLD is neuronal loss in the frontal and temporal lobes of the brain (Neary et al., 2005). FTLD is a proteinopathy and, based on the nature of the inclusion proteins, 5

See Appendix for Members of the Belgian Neurology (BELNEU) Consortium.

* Corresponding author at: Neurodegenerative Brain Diseases Group, VIB Center for Molecular Neurology, University of Antwerp—CDE, Universiteitsplein 1, B-2610 Antwerp, Belgium. Tel.: +32 3 265 1101; fax: +32 3 265 1013.

E-mail address: christine.vanbroeckhoven@molgen.vib-ua.be (C. Van Broeckhoven).

¹ These first authors have contributed equally to this work.

pathological subtypes are recognized. FTLD-tau, with inclusions of microtubule-associated protein tau (*MAPT*), and FTLD-Tat activating regulatory (TAR)DNA-binding protein (FTLD-TDP) are the most frequent pathological diagnoses (Mackenzie and Neumann, 2016). Causal mutations have been identified in 6 genes, namely granulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*), *MAPT*, TANK-binding kinase 1 (*TBK1*), valosin-containing protein (*VCP*), and charged multivesicular body protein 2B (*CHMP2B*) (Baker et al., 2006; Cruts et al., 2006; DeJesus-Hernandez et al., 2011; Freischmidt et al., 2015; Gijssels et al., 2012, 2015; Hutton et al., 1998; Poorkaj et al., 1998; Pottier et al., 2015; Renton et al., 2011; Skibinski et al., 2005; Spillantini et al., 1998; Watts et al., 2004). Variations in these genes have been suggested to modify disease risk, such as the common variation rs5848 in *GRN*, the H1/H2 haplotype of *MAPT*, and intermediate alleles of 7–24 repeat units of the *C9orf72* G₄C₂ repeat (e.g., [Benussi et al., 2014; Borroni et al., 2005; Gijssels et al., 2012; Rademakers et al., 2008; Verpillat et al., 2002]). In addition, a genome-wide association study identified variations in *TMEM106B* as risk factors for FTLD-TDP (Van Deerlin et al., 2010).

GRN mutations explain 10%–25% of all and 3%–26% of familial FTD patients (<http://www.molgen.vib-ua.be/FTDMutations>; Cruts et al., 2012; Sieben et al., 2012). The majority are heterozygous loss-of-function mutations that lead to premature stop codons triggering degradation of the mutant transcript. Consequently, reduced *GRN* levels are measured in blood and cerebrospinal fluid (CSF) (Finch et al., 2009; Ghidoni et al., 2008; Sleegers et al., 2009). In brain, FTLD-TDP pathology type A is typically present (Mackenzie and Neumann, 2016). Clinical phenotypes are diverse, even between patients carrying the same mutation, and include FTD, Alzheimer's dementia (AD), Parkinson's disease (PD), and corticobasal syndrome (e.g., [Brouwers et al., 2007; Kelley et al., 2009]). Mutation carriers show a high interfamilial and intrafamilial variability in onset age ranging from 35 to 89 years (Cruts and Van Broeckhoven, 2008; Cruts et al., 2012; Van Swieten and Heutink, 2008). This suggests that modifiers influence the disease onset and clinical appearance. Variations near sortilin (*SORT1*), a receptor of *GRN*, and prosaposin (*PSAP*) regulate *GRN* levels, but their effect on clinical heterogeneity remains unclear (Carrasquillo et al., 2010; Nicholson et al., 2016).

We identified in an extended Belgian FTD family a *GRN* founder mutation in the splice donor site of intron 1 (mutation alias IVS1+5 G>C, protein p.0). The mutation prevents splicing of intron 1, leading to nuclear retention and degradation of the mutant transcript, which results in haploinsufficiency (Cruts et al., 2006). Since its identification, the pedigree has been extended and additional genealogical and clinical data have been gathered. The Belgian *GRN* founder mutation is one of the most common *GRN* mutations worldwide, in addition to the p.Arg493X mutation (Rademakers et al., 2007) and the p.Thr272SerfsX10 mutation, which is the most frequent *GRN* mutation in Italy (Benussi et al., 2009, 2010; Borroni et al., 2011b). For all 3 mutations, founder effects have been described (Benussi et al., 2013; Borroni et al., 2011a; Cruts et al., 2006; Rademakers et al., 2007; van der Zee et al., 2006). The 10-year follow-up study of the Belgian *GRN* founder pedigree allowed us to describe in detail the clinical heterogeneity of the *GRN* patient carriers. The observed heterogeneity in onset age and the size of the extended pedigree provided an opportunity to investigate the potential onset age-modifying effects of known genetic modifiers of FTD risk or disease presentation.

2. Materials and methods

2.1. Study population

Index patients were ascertained in Belgium through an ongoing multicenter collaboration of neurology departments and

memory clinics partnering in the BELNEU consortium. Additional patients, referred to the diagnostic service facility for genetic testing, were included. Information on family history of neurodegenerative diseases was gathered for the index patients, and relatives were asked to participate in genetic studies. Written informed consent for participation in clinical and genetic studies, and for the brain autopsy when appropriate, was obtained from participants and/or their legal guardians. The clinical study protocol and the informed consent forms for patient ascertainment were approved by the local ethics committees of each of the collaborating neurological centers. The genetic and pathological study protocols and informed consent forms were approved by the ethics committee of the University Hospital of Antwerp and the University of Antwerp, Belgium.

FTD, AD, or PD diagnoses were made in accordance with the international consensus criteria (Gelb et al., 1999; Gorno-Tempini et al., 2011; McKhann et al., 1984, 2011; Postuma et al., 2015; Rascofsky et al., 2011). Patients with a combination of behavioral and language features of FTD at presentation, without clear predominance, were denoted mixed FTD. In addition to index patients, symptomatic relatives with a clinical diagnosis carrying the founder mutation were included in the clinical study. All available clinical records were reviewed by a medical doctor of the research team (Sara Van Mossevelde). A positive familial history was defined as the presence of at least 1 first degree or 2 second degree relatives with neurodegenerative disease. The onset age was defined as the age at which first symptoms were noticed by the patient or his partner/relatives. [Supplementary Fig. S1](#) summarizes the available biomaterials and clinical information.

2.2. Genetic screening

2.2.1. Targeted resequencing of genes involved in neurodegeneration

GRN founder mutation carriers and their family members were screened for mutations using parallel screening of a multi-gene panel of neurodegenerative brain disease-related genes (Agilent, <https://www.agilent.com>). Multiplex polymerase chain reactions (PCR) were performed for target enrichment of the coding regions of 17 causal genes and the apolipoprotein E gene (*APOE*). This was followed by equimolar pooling of the amplicon libraries and purification using Agencourt AMPureXP beads (Beckman Coulter). A universal PCR was performed to incorporate patient-specific barcodes. Samples were pooled, and massive parallel sequencing was performed in-house on an Illumina MiSeq platform. Paired-end reads were generated, and adapters were trimmed using Fastq-mcf. Reads were aligned to the reference genome hg19 with the Burrows-Wheeler Aligner MEMv0.7.5a (Li and Durbin, 2009). Variant calling and annotation was performed using GATKv2.4 UnifiedGenotyper and the GenomeComb package (McKenna et al., 2010; Reumers et al., 2012). We focused on known pathogenic mutations present within the genes amyloid beta precursor protein, presenilin 1, presenilin 2, *GRN*, *MAPT*, *TBK1*, *CHMP2B*, *VCP*, superoxide dismutase 1, TAR DNA-binding protein (*TARDBP*), *FUS* RNA binding protein *FUS*, prion protein, synuclein alpha, leucine rich repeat kinase 2, parkin RBR E3 ubiquitin protein ligase (*PARKN*), parkinsonism associated deglycase (*PARK7*), and PTEN induced putative kinase 1. We have identified 2 carriers of the p.G287S mutation in the *TARDBP* gene in branch DR404 of the founder pedigree ([Table 1](#), [Supplementary Fig. S2](#)). Individual III.3 carried both mutations and was unaffected at 65 years. Sibling III.5 carried only the *TARDBP* mutation and was unaffected at 57 years. According to relatives, the parent II.3 showed a reduction in speech at 80 years, mutism at age 84 years, and loss of interest in the surroundings at 85 years. The spouse of II.3 died unaffected at

80 years. The p.G287S mutation has been identified in patients suffering from amyotrophic lateral sclerosis (Corrado et al., 2009; Kabashi et al., 2008; Kenna et al., 2013; Kirby et al., 2010).

2.2.2. Screening of candidate onset age modifier variations

The gene panel of neurodegenerative brain disease-related genes also targets regions encompassing candidate modifier variations, that is rs429358 and rs7412 for *APOE* genotyping, the *GRN* 3'-untranslated region (UTR) variant rs5848, and *MAPT* rs1052553 to determine the H1/H2 haplotype, with the A-allele tagging H1 and the G-allele tagging H2 (Baker et al., 1999; Cruys et al., 2005; Rademakers et al., 2005). We have also genotyped variant rs1800547, with the A-allele tagging H1 and the G-allele tagging H2 (Baker et al., 1999). With both *MAPT* variants we obtained the same results, as expected since they are in linkage disequilibrium ($R^2 = 1$ and $D' = 1$, HapMap 22). Other candidate modifier variations in *TMEM106B*, *PSAP*, *SORT1*, and *C9orf72* were genotyped using Sanger sequencing on a 3730 DNA Analyzer (Applied Biosystems) followed by sequence analysis using SeqMan software (DNASTAR). PCR amplification followed by size separation of amplicons on the 3730 DNA Analyzer was used to determine the size of the $(G_4C_2)_n$ repeat in *C9orf72*. Genotypes were assigned using the in-house developed TracI genotyping software. Individuals who appeared homozygous for the short (≤ 6 units) or

intermediate (7–24 units) G_4C_2 repeat allele were screened with a reverse repeat-primed PCR to detect a possible repeat expansion (Gijssels et al., 2012).

2.3. Estimation of the effect of risk variations on onset age

Effects of candidate modifier variations on onset age were estimated using a reversible jump Markov chain Monte Carlo algorithm for censored quantitative traits in Loki (Heath, 1997). In addition to gender, genotypes or haplotypes of candidate modifier variations were entered in the trait model as a fixed major gene covariate to estimate whether a variation can explain the variance in onset age (Wijsman and Yu, 2004). The founder family was analyzed as one pedigree, linked together by a common founder. Genotypes of 79 mutation carriers and 82 family members without *GRN* mutation were included in the analysis. Ages of individuals with the *TARDBP* p.G287S mutation and of their relatives DR404 II.1, II.2, and II.3 were excluded from analysis, since we could not exclude an effect on onset age (Supplementary Fig. S2). *C9orf72* repeat length and *APOE* genotypes were analyzed as dichotomous variables. The normal lengths of the G_4C_2 repeat were categorized as short (≤ 6 repeat units) and intermediate (7–24 repeat units) alleles (van der Zee et al., 2013). Repeat sizes > 24 units (31 units, unaffected founder mutation carrier of 55 years; 26 units, relative without *GRN* mutation) were included as intermediate alleles. For *APOE*, the $\epsilon 4$ risk allele was analyzed in comparison to the $\epsilon 2$ plus $\epsilon 3$ alleles. A significance threshold of $p < 0.05$ was used.

2.4. GRN serum levels

Serum *GRN* levels were measured in duplicate in 1 experiment by a sandwich enzyme linked-immunosorbent assay (AdipoGen Life Sciences), according to manufacturers' instructions. The average intra-assay coefficient of variability was 4.85%. The individual coefficients of variability were deduced from the calculated concentrations by dividing the standard deviation of the duplicate measures by the duplicate average and multiplying by 100. Duplicate measurements were averaged for further analysis. Nonparametric Kruskal-Wallis or Mann-Whitney *U* test was used to compare levels between mutation carriers and control individuals and between mutation carriers with different genotypes at risk variations. Calculations were performed using SPSS version 22. Correlation between onset age and *GRN* levels was assessed using a Cox mixed effects model where kinship was included in the model to correct for relatedness. Calculations were performed using the R-packages *coxme* and *kinship2* in R (R Core Team, 2014; Sinnwell et al., 2014).

2.5. Haplotype sharing analysis

Polymorphic short tandem repeat (STR) markers, located in and flanking the *GRN* founder haplotype at 17q21, were PCR-amplified, and the products were separated and analyzed on a 3730 DNA Analyzer (Applied Biosystems) (Brouwers et al., 2007; van der Zee et al., 2006). Genotypes were assigned using the in-house developed TracI genotyping software.

2.6. Estimation of shared ancestry

The distance from index patients of the *GRN* founder family to the most recent common ancestor was estimated using ESTIAGE software (Genin et al., 2004). STR marker and haplotype data flanking the *GRN* mutation were included in the analysis for 13 index patients with phase-determined haplotypes. The allele frequencies were

Table 1
Characteristics of index patients carrying the *GRN* founder mutation

Index patient	Sex	Clinical diagnosis	Subtype	OA	AAD
DR2.1	m	FTD ^c	bvFTD	67	71
DR8.1	f	FTD ^{a,b}	bvFTD	62	68
DR25.1	f	FTD ^{a,b,c}	bvFTD	69	75
DR26.1	m	FTD	PPA (PNFA)	65	68
DR27.1	f	FTD ^a	bvFTD	58	63
DR28.1	m	FTD ^{a,b}	PPA (PNFA)	56	62
DR31.1	m	FTD ^{a,b}	PPA (PNFA)	65	70
DR119.1	f	FTD ^a	PPA (PNFA)	45	49
DR142.1	f	AD		66	73
DR205.1	m	PD+D ^{a,b}		55	60
DR404.1 ^Δ	f	AD	PPA (SD)	55	62
DR632.1	f	AD		60	69
DR686.1	f	FTD	unspecified	61	68
DR792.1	f	FTD	PPA (PNFA)	69	/
DR985.1	f	AD	PPA (SD)	57	62
DR1194.1	f	FTD	PPA (PNFA)	62	/
DR1206.1	f	FTD	bvFTD	53	59
DR1207.1	f	FTD ^{a,b}	PPA (PNFA)	62	66
DR1208.1	f	FTD	PPA (PNFA)	63	67
DR1209.1	m	FTD	PPA (PNFA)	60	64
DR1210.1	m	FTD	PPA (SD)	70	/
DR1211.1	m	FTD	bvFTD	56	62
DR1213.1	m	FTD ^{a,b}	bvFTD	58	60
DR1214.1	m	FTD	bvFTD	54	/
DR1241.1	f	FTD	bvFTD	72	76
DR1275.1	m	FTD	bvFTD	54	60
DR1276.1	m	AD		54	61
DR1277.1	f	PD		76	/
DR1278.1	m	PD+D		49	63

Note: For each index patient the sex, clinical diagnosis, and subclass, onset age and age at death are summarized. Families in which a second mutation in a neurodegenerative brain disease-related gene is present, are indicated with Δ . Key: AAD, age at death; AD, Alzheimer's dementia; bvFTD, behavioral variant of frontotemporal dementia; OA, onset age; PD, Parkinson's disease; PD+D, Parkinson's disease with dementia; PNFA, progressive nonfluent aphasia; PPA, primary progressive aphasia; SD, semantic dementia.

^a *GRN* founder (n = 9) patient carriers with autopsy confirmed FTLD-TDP proteinopathy type A (Fig. 3.4), of which.

^b 7 brains underwent a detailed neuropathology examination.

^c In addition, one relative of index patients DR2.1 and DR25.1, had FTLD-TDP proteinopathy type A and a detailed neuropathology examination (R.V., personal communication, 2016; Sieben et al., 2018).

determined in unrelated control individuals ($n = 102$). The recombination fractions were computed using the Kosambi mapping function. We assumed a stepwise mutation model that seems to hold better for STR loci and a mutation rate of 10^{-4} or 10^{-3} (Fan and Chu, 2007; Genin et al., 2004). This corresponds to published mutation rates at STR loci, although these rates can vary greatly (Brinkmann et al., 1998).

3. Results

3.1. Belgian GRN founder family

Since the first description of the GRN founder family (Cruts et al., 2006), the pedigree has been considerably extended. It currently consists of 29 branches of index patients carrying the GRN founder

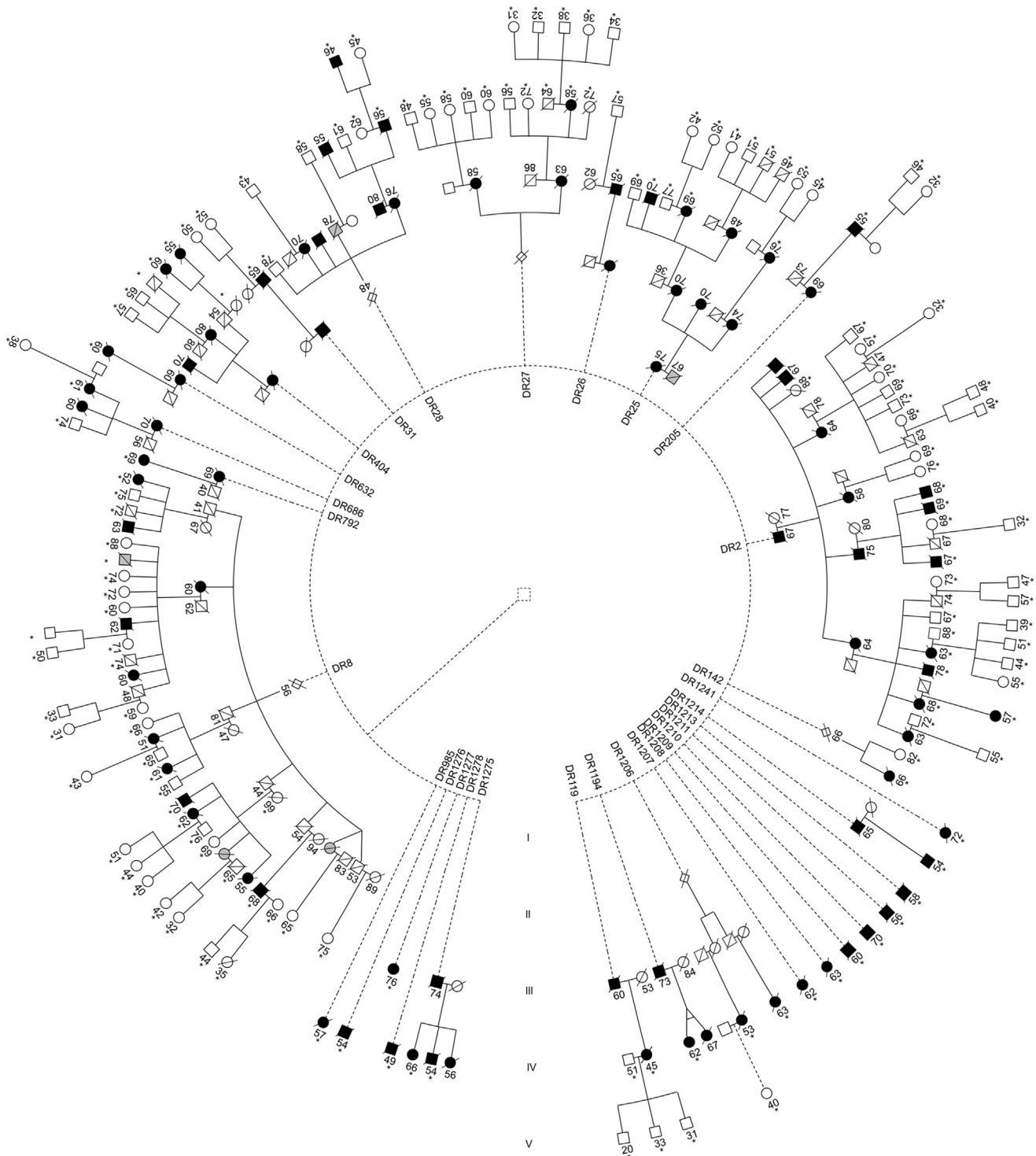


Fig. 1. Merged GRN founder pedigree. The 29 branches are illustrated, aligned to 5 generations based on birth year. The branches are connected to each other and to the common founder via a dotted line, indicating that they are not supported by genealogical evidence and separated by an unknown number of generations. The onset age is indicated for patients, the age at last examination for unaffected individuals and the age at death for deceased individuals. Circles represent females; squares, males; black symbols, patients; gray symbols, individuals with an unclear affection status; slash, deceased individuals; *, DNA material available.

mutation (IVS1+5 G>C; p.0) and spans at least 5 generations (Fig. 1, Table 1). DNA is available of 175 family members, including 79 mutation carriers. The mutation so far, has only been identified in the Flanders-Belgian population. All carriers share a haplotype indicating that they are genetically related and belong to 1 founder pedigree (Supplementary Table S1). The distance to the most recent common ancestor, carrying the founder mutation, was estimated at 40 generations (95% confidence interval [CI] 27–58, mutation rate 10^{-3}) by the Estiage program and increased to 46 generations (95% CI 32–67) when we considered a mutation rate of 10^{-4} .

3.2. Phenotypic heterogeneity in GRN founder mutation carriers

3.2.1. Onset age variability

Considering all patients in the pedigree, independent of the availability of DNA or clinical diagnosis, the onset age ranged from 45 to 80 years with an average of 63.1 ± 7.7 years ($n = 79$) (Fig. 2). The disease duration was 6.1 ± 3.8 years ($n = 66$, range 1–20), and death occurred at 70.0 ± 7.2 years ($n = 72$, range 49–85).

3.2.2. Clinical heterogeneity

For 40 of the 79 patients with onset age data, DNA material was available, and a GRN founder mutation was identified. Three were obligate carriers and had a clinical neurodegenerative disease diagnosis. Of these 43 patients, 32 were diagnosed with FTD (74%, spread over 21 branches of the pedigree), 6 with AD (14%, in 6 branches), 2 with PD (5%, in 2 branches), 2 with PD and dementia (PD+D) (5%, in 2 branches), and 1 with dementia unspecified (2%). Fifteen FTD patients (in 11 branches) were diagnosed with bvFTD, 14 patients (in 13 branches) with PPA, and 2 patients with a mixed FTD phenotype (Table 2, Supplementary Table S4).

Detailed clinical information was available for 38 patients. Thirteen FTD patients were diagnosed with bvFTD, of whom 5 had predominant symptoms of apathy (39%), 5 of disinhibition (39%), 2 of executive dysfunction (15%), and in 1 the predominant feature was unclear. Nine of 14 FTD patients with PPA had a nonfluent aphasia (64%), 2 patients had a semantic dementia (14%), and 3 patients had an unclear language subtype. Overall, behavioral and language symptoms were reported in 27 (71%) and 25 (66%) patients, respectively. Apathy was the most frequent reported behavioral feature (67%), and word-finding difficulties were the most frequent reported language feature (52%). Although only 5 patients were diagnosed with AD, early memory problems were reported in 17 patients (45%), and early orientation problems in 11 patients (29%). Detailed findings of clinical neurological examinations were available for 32 patients. In nearly half of them (47%),

extrapyramidal symptoms were present. Upper motor neuron symptoms were present in 11 patients (34%). An overview of the reported clinical symptoms and signs can be found in Supplementary Tables S2 and S5.

In 30 of 34 patient carriers, cerebral atrophy was reported. The atrophy was in most patients predominantly present in the frontal and/or temporal lobes (80%) and asymmetric in half of the patients (Fig. 3.1). Parietal atrophy was reported in 24% (6/25), and hippocampal atrophy was reported in 20% (5/25). Abnormal findings on functional neuroimaging (fluorodeoxyglucose positron emission tomography or perfusion single-photon emission computed tomography) were reported for 22 of 23 patients of whom 13 had predominant frontal and/or temporal hypometabolism/-perfusion (Fig. 3.2) and 15 (68%) had additional or isolated parietal lobe involvement (Fig. 3.3). In 20 patients (91%), the pattern of the functional abnormalities was reported to be asymmetric (Supplementary Tables S3 and S6). Dopamine transporter imaging was performed in 3 of the 4 PD patients and confirmed a presynaptic dopaminergic deficit.

3.3. GRN as modifier of onset age

We measured serum GRN levels of 22 founder mutation carriers from 17 branches (onset age 62.2 ± 9.3 years, range 45–76). The levels in GRN mutation carriers (median 27.3 ng/mL, range 12.8–39.8 ng/mL) were significantly lower ($p = 0.0001$) in comparison to the levels measured in control individuals (median 74.2 ng/mL, range 56.2–90.3 ng/mL) with no overlap between both groups. There was no evidence of correlation between onset age and GRN levels (Cox mixed effects $p = 0.41$).

In the founder family, the major C-allele of rs5848, situated in the GRN 3'UTR, is located on the mutant haplotype. The onset age of heterozygous rs5848 C/T carriers was on average 3.5 years earlier compared to C/C carriers ($p = 0.042$, minor allele frequency [MAF] = 0.23, Table 3). There were no differences in serum GRN

Table 2
Demographic and clinical characteristics of GRN founder mutation carriers with clinical diagnosis

Patient characteristics	GRN founder mutation
Total, n	43 [38]
Gender, n male (%)	18 (41.9)
Diagnosis, n (%)	
FTD	32 (74.4) [28]
AD	6 (14.0) [5]
D	1 (2.3) [1]
PD	2 (4.7) [2]
PD + D	2 (4.7) [2]
Subtype FTD, n (% within FTD patients with known subtype)	
bvFTD	15 (48.4) [13]
PPA	14 (45.2) [14]
MXD	2 (6.5) [1]
Average OA	($n = 43$) 61.2 ± 7.3 y
Average DD	($n = 33$) 5.5 ± 2.2 y
Average AAD	($n = 33$) 66.4 ± 6.4 y
Familial history, n (%)	
F	33 (76.7)
S	7 (16.3)

Note: Numbers between square brackets indicate the number of individuals with detailed clinical information available.

Key: AAD, age at death; AD, Alzheimer's dementia; bvFTD, behavioral variant of frontotemporal dementia; D, dementia unspecified; DD, disease duration; F, familial; FTD, frontotemporal dementia; LOF, loss-of-function; MXD, mixed frontotemporal dementia (both behavioral and language features without clear predominance); OA, onset age; PD, Parkinson's disease; PD+D, Parkinson's disease with dementia; PPA, primary progressive aphasia; S, sporadic.

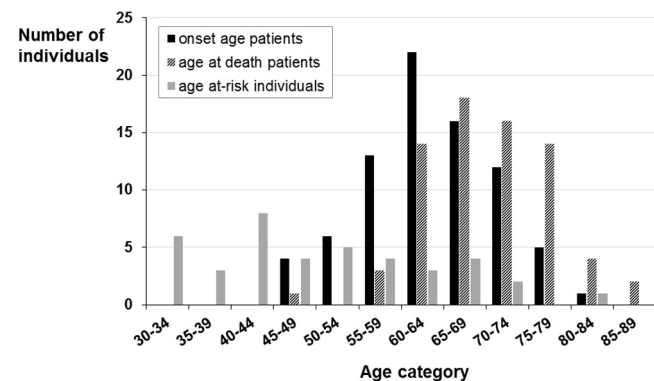


Fig. 2. Age distribution of GRN founder mutation carriers. For patients, the onset age and the age at death are indicated, for at-risk individuals, the age at last examination. (Adapted and extended from [Brouwers et al., 2007]).

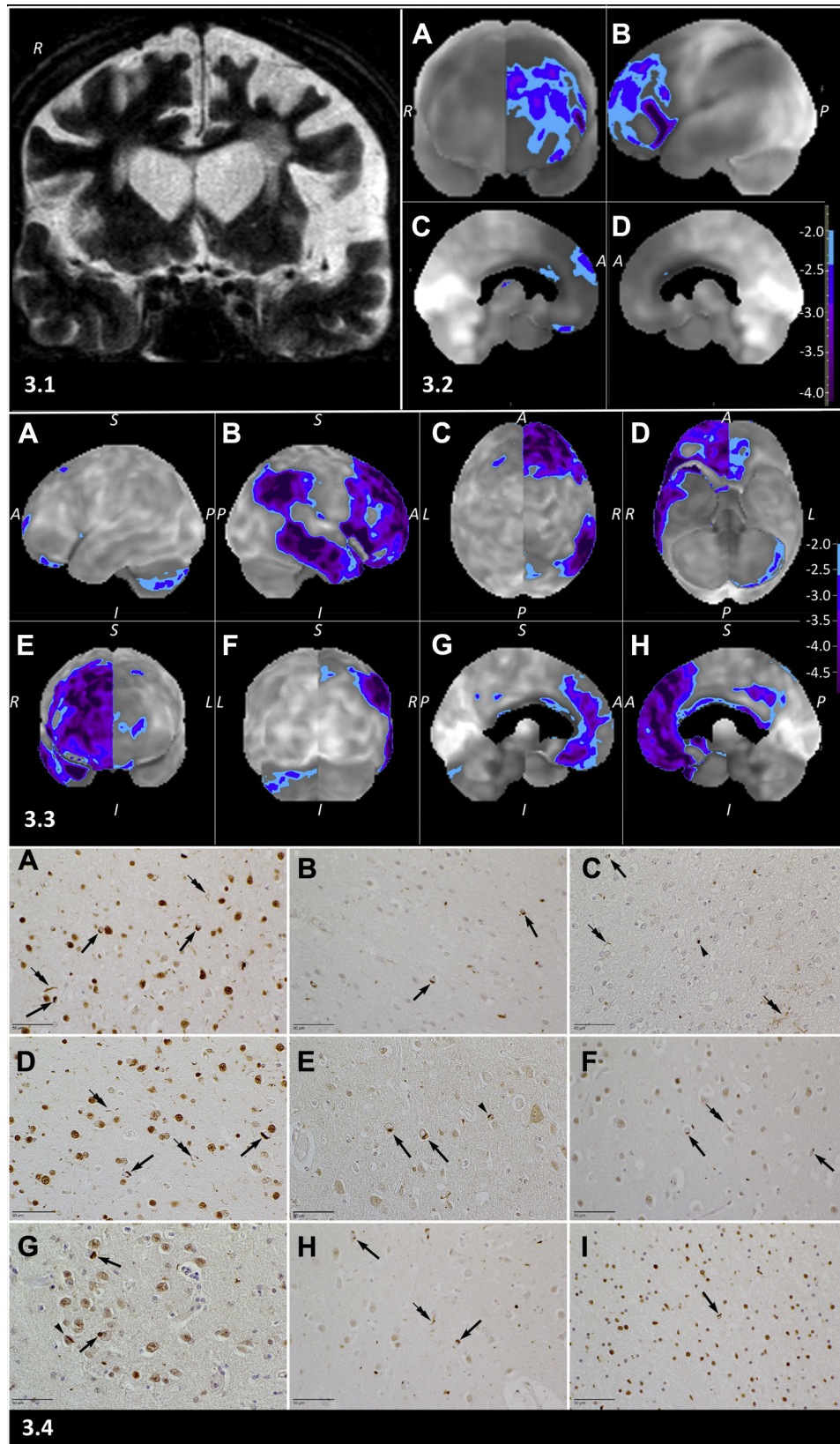


Fig. 3. Distinctive neuroimaging and neuropathological findings in GRN founder mutation carriers. **3.1 Structural neuroimaging DR1208.1:** Coronal MRI T2-weighted image illustrating asymmetric atrophy, which is in this case more pronounced at the left side. **3.2 Functional neuroimaging DR1208.1:** These z-map rendering FDG-PET images, with Z value (scale bar) calculated by comparing the individual glucose metabolic pattern to the normal age-matched control, illustrate asymmetric predominant left frontal hypometabolism. (A) Anterior view. (B) Left lateral view. (C) Left medial view. (D) Right medial view. **3.3 Functional neuroimaging DR28.3:** These z-map rendering FDG-PET images, with Z value (scale bar) calculated by comparing the individual glucose metabolic pattern to the normal age-matched control, illustrate asymmetric hypometabolism in the right frontal, temporal, and parietal lobes. (A) Left lateral view. (B) Right lateral view. (C) Superior view. (D) Inferior view. (E) Anterior view. (F) Posterior view. (G) Left medial view. (H) Right medial view. **3.4 FTLD-TDP type A pathology:** Paraffin-embedded sections stained with anti-hyperphosphorylated TDP-43 antibody. (A) DR31.1 Area 6. Moderate amount of neuronal cytoplasmic

levels between mutation carriers with a T- or C-allele on the normal haplotype (n = 22, p = 0.25).

3.4. Other modifiers of onset age

We did not observe a significant effect of the *SORT1* and *PSAP* variants, *TMEM106B* rs1990622, and *APOE* ε4 on onset age; neither did we detect differences in serum GRN levels between patients carrying different genotypes at *SORT1* and *PSAP* variations. Homozygous carriers of the *MAPT* H2 haplotype developed disease on average 7.8 years later than homozygous H1 carriers (p = 0.032, MAF = 0.36) (Table 3). We also identified a significant effect of the *C9orf72* G4C2 repeat size on onset age. Patients heterozygous for an intermediate repeat developed disease on average 3.8 years earlier compared to patients with 2 short alleles (p=0.035, MAF = 0.27) (Table 3).

4. Discussion

Mutations in *GRN* are an important cause of FTLD and related neurodegenerative disorders. The *GRN* IVS1+5 G>C null mutation was originally described in a Belgian founder family, which was characterized by a wide variability of clinical symptoms and onset ages of disease (Brouwers et al., 2007; Cruts et al., 2006; van der Zee et al., 2006). We have extended the Belgian *GRN* founder pedigree to 29 branches including 85 patients and 40 unaffected carriers. This was achieved by recruiting patients and relatives of known branches and newly identified index patient carriers and relatives. Further, all index patients included in our Belgian neurodegenerative brain diseases patient cohort are routinely screened for causal mutations in genes associated with neurodegenerative brain diseases using parallel sequencing. In this way, we identified since our latest report (van der Zee et al., 2011), an additional 19 index carriers of the founder mutation, bringing the total to 29 branches, and biomaterials of 44 individuals including 29 founder mutation carriers have been added.

In the founder pedigree, three quarters of the patients of whom we have a clinical diagnosis available received a clinical diagnosis of FTD, half bvFTD, and half PPA. Apathy was the most frequent FTD behavioral feature, and nonfluent progressive aphasia was the most frequent PPA variant. Parkinsonism was reported in about half of the patients. On neuroimaging, asymmetric brain damage as well as parietal involvement was frequently reported. These findings are consistent with previous reports (e.g., [Beck et al., 2008; Rohrer et al., 2010; Whitwell et al., 2009]). Other diagnoses were AD, unspecified dementia, PD, and PD+D. Behavioral features were present in 5 and language symptoms in 3 of the 8 patients with a nonFTD dementia diagnosis. In the 5 AD patients, the diagnosis was based on early episodic memory and/or orientation problems with parietal or more global involvement on neuroimaging. In one of them, the CSF biomarker profile was in accordance with AD, while in 3 others, the profile was not typical for AD. The 3 PD patients that had dopamine transporter imaging performed, had a decreased striatal uptake. In 1 patient with clinical PD+D (Table 1, DR205.1),

Table 3
Effect of genetic modifier variations on onset age in the GRN founder family

Variation	Minor allele	MAF	Htz vs WT			Hmz vs WT		
			Δ OA	SD	p-value	Δ OA	SD	p-value
GRN rs5848	T	0.23	-3.5	2.0	0.042			
SORT1 rs611917	C	0.32	-2.5	2.1	0.111	1.3	3.6	0.354
SORT1 rs646776	G	0.27	-0.6	2.0	0.389	2.9	5.4	0.285
PSAP rs1867977	T	0.36	-2.5	2.1	0.113	-2.5	3.0	0.200
PSAP rs7869	T	0.23	-3.3	2.3	0.075	-0.7	4.1	0.431
TMEM106B rs1990622	C	0.26	2.4	2.0	0.110	1.5	7.4	0.430
C9orf72 G4C2 repeat	intermediate	0.27	-3.8	2.0	0.035	5.6	5.9	0.173
C9orf72 rs2814707	T	0.31	-3.6	2.0	0.038	6.1	5.5	0.133
MAPT H1/H2	H2	0.36	5.9	3.9	0.065	7.8	4.2	0.032
APOE	ε4	0.14	-0.8	2.6	0.388	7.7	5.6	0.083

p-values < 0.05 are bold italicized.

Key: Δ OA, difference in onset age in years; Htz, heterozygous; Hmz, homozygous; MAF, minor allele frequency; SD, standard deviation; WT, wild-type.

characterized by severe frontal dysfunction and early episodic memory impairment, postmortem neuropathological examination revealed a combination of FTLT-DTP type A, diffuse Lewy body pathology, and mild AD-related pathology (Brouwers et al., 2007). In total, neuropathological examination was performed in 11 founder mutation carriers, all showing FTLT-DTP type A (Table 1, Fig. 3.4) (R.V., personal communication, 2016; Sieben et al., 2018). In depth examination of 9 patients revealed atherosclerotic changes of the small vessels in cortex and white matter as well as mild AD-related amyloid and/or tau pathology in all patients (Table 1) (Sieben et al., 2018).

Patients carrying the *GRN* founder mutation received their clinical diagnosis in different hospitals and different clinical settings between 1993 and 2014, implicating that the used diagnostic tools and standards were not in all patients equally advanced. This implies that some diagnoses might be misdiagnoses due to a lack of diagnostic tools at the time, such as fluorodeoxyglucose positron emission tomography or amyloid-PET of the brain or lumbar puncture to determine biomarker levels in CSF. However, the frequent combined pathology of FTLT-DTP type A with Alzheimer's and/or synucleinopathy features, suggests that at least a part of the heterogeneous clinical phenotype of *GRN* mutation carriers might be the result of additional pathologies. The question remains why additional neuropathological features occur frequently in *GRN* mutation carriers (Hosokawa et al., 2017; Kelley et al., 2009). Given the function of *GRN* as a neurotrophic factor, which enhances neuronal survival (Van Damme et al., 2008), and the increased cellular aging that was postulated from the cytological changes seen in granulin knockout mice (Ahmed et al., 2010; Wils et al., 2012), it is plausible that *GRN* haploinsufficiency lowers the threshold for other neurodegenerative pathologies or accelerates age-related neurodegeneration. Related to this, it is interesting to note that variations in *TMEM106B* and *GRN* have recently been identified in a genome-wide association study for differential aging (Rhinn and Abeliovich, 2017).

We observed in the founder pedigree a wide variability in onset age, ranging from 45 to 80 years, suggesting the influence of onset age modifiers. We investigated the effect of previously reported

inclusions (NCI) (arrow), mainly in the second cortical layer. The dystrophic neurites (DN) (double arrow) are more evenly spread throughout the entire cortex. (B) DR8.1 Area 11. Mild amount of NCI (arrow) in the second cortical layer. (C) DR8.1 Area 4. Similar findings: mild to moderate NCI (arrow) in the second cortical layer. No involvement of Betz neurons. Note the presence of one neuronal intranuclear inclusion (arrow head) and different DN (double arrow head). (D) DR2.3 Superior temporal gyrus. Mild to moderate TDP-43 proteinopathy type A with NCI (arrow) and DN (double arrow head). (E) DR25.1 Parietal cortex. Mild amount of NCI (arrow) and one neuronal intranuclear inclusion (arrow head) in the second cortical layer. (F) DR31.1 Area striata. Mild amount of NCI (arrow) and DN (double arrow head) in the second cortical layer. (G) DR25.1 Hippocampus. Mild amount of NCI (arrow) and one neuronal intranuclear inclusion (arrow head) in the subiculum. (H) DR31.1 Neostriatum. Large amount of NCI (arrow) and DN (double arrow head) in nucleus caudatus. (I) DR8.1. White matter of the frontal cortex. Presence of glial cytoplasmic inclusions (arrow). Abbreviations: A, anterior; FDG-PET, fluorodeoxyglucose positron emission tomography; FTLT, frontotemporal lobar degeneration; FTLT-DTP FTLT-TAR DNA-binding protein 43; I, inferior; L, left; MRI, magnetic resonance imaging; P, posterior; R, right; S, superior.

modifiers of onset age or disease risk. For the *GRN* rs5848 variation, homozygous carriers of the major C-allele developed symptoms on average 3.5 years later than heterozygous carriers of the risk T-allele. The variation is located in the 3'UTR within a binding site for miR-659. This miRNA binds more efficiently to the T-allele resulting in a translational inhibition of *GRN* (Rademakers et al., 2008). We did not identify a correlation between serum *GRN* levels and onset age, consistent with previous results (e.g., [Meeter et al., 2016; Sleegers et al., 2009]) but in contrast with the finding of an earlier onset in patients with the lowest *GRN* levels (Ghidoni et al., 2012). Analysis of the effect of the H1/H2 haplotypes of *MAPT*, which is located adjacent to *GRN* on chromosome 17, indicated that H2/H2 carriers develop disease on average 7.8 years later than H1/H1 carriers. Though, caution is needed in interpreting this result, because only 3 patients were homozygous H1/H1 carriers (onset ages 49–54 years) due to the location of H2 on the disease haplotype in most branches of the founder family. The effect on onset age is in agreement with the protective effect of H2 in progressive supranuclear palsy (Baker et al., 1999). Of interest, also in Italian patients carrying the *GRN* p.Thr272SerfsX10 mutation, the *MAPT* haplotype is an onset age modifier; H2/H2 carriers developed disease later in comparison to patients with H1/H2 haplotypes (Benussi et al., 2009). In contrast, other studies in clinical FTD patients have reported an earlier disease onset in H2 carriers (Borroni et al., 2005; Laws et al., 2007). Patients of the pedigree heterozygous for an intermediate *C9orf72* G₄C₂ repeat allele, developed disease on average 3.8 years earlier than patients with two short normal alleles. We could not detect a significantly lower onset age in carriers of two intermediate alleles, since there were only very few homozygous carriers of intermediate alleles, i.e. one patient with onset age 70 years and two asymptomatic *GRN* mutation carriers aged 34 and 48 years. No onset age-modifying effects were observed for the genetic variations in the *SORT1* and *PSAP* loci. We could not replicate a significant difference in serum *GRN* levels between patients with different genotypes (Carrasquillo et al., 2010; Nicholson et al., 2016), but we cannot exclude that the low number of samples limited the detection of subtle differences in *GRN* levels. Consistent with our earlier results, no significant onset age-modifying effect of *TMEM106B* rs1990622 or *APOE* ϵ 4 was observed (Cruts et al., 2006; van der Zee et al., 2011). Although we did not see an onset age modifying effect of the rs1990622 variation, we did notice that none of the affected mutation carriers was homozygous carrier of the protective C-allele, supporting a protective effect of *TMEM106B* rs1990622. Variations in *TMEM106B* have been identified as a risk factor for FTLT-TDP, with the greatest effect in *GRN* mutation carriers (Van Deerlin et al., 2010). Furthermore, an effect on onset age has been observed in *GRN* mutation carriers (Cruchaga et al., 2011).

For the loci we investigated, positive as well as negative associations with disease or onset age have been reported indicating that studies in larger cohorts will be needed to obtain solid results (e.g., [Rademakers et al., 2008; Rollinson et al., 2011; Rubino et al., 2013; Van Deerlin et al., 2010; van der Zee et al., 2011]). It is possible that small effects were detected in some families or cohorts, while in other groups of patients, the effect did not reach statistical significance. Also in our study, effect sizes were small and would not reach statistical significance in a genome-wide study of modifier variations. *GRN*, *MAPT*, and *C9orf72* repeat length explained 2.7%, 4.4%, and 5.6% of the variability in onset age in the pedigree, indicating the involvement of other modifying factors. One possibility is that pathogenic mutations in other causal genes associated with neurodegenerative brain diseases could exert a modifying effect. In one branch of the pedigree, we identified the p.G287S mutation in *TARDBP* (Supplementary Fig. S2), a well-established causal gene for amyotrophic lateral sclerosis (Kabashi

et al., 2008; Sreedharan et al., 2008). Rare *TARDBP* mutation carriers have also been identified, who present clinically with FTD (Borroni et al., 2010). Since only 1 nonaffected individual carrying both the *GRN* founder mutation and the *TARDBP* mutation was identified in the founder family, we cannot make a firm statement about the potential of a modifying effect on disease presentation.

The phenotypic heterogeneity in patients carrying the same *GRN* mutation, emphasizes that neurodegenerative brain diseases are rather a continuum of disorders, overlapping in genetics, pathological features, and clinical phenotypes. Appreciating the disease heterogeneity associated with loss-of-function mutations in *GRN* is of major importance for genetic counseling. Within families, clinical heterogeneity could mask the presence of an underlying genetic defect segregating with neurodegenerative disease. Furthermore, the phenotype of a *GRN* mutation carrier cannot be predicted by phenotypical characteristics of relatives. Heterogeneity within families points toward the presence of disease modifiers. Within the founder family, *GRN* rs5848, *MAPT* H1/H2 haplotype, and *C9orf72* G₄C₂ repeat length were identified as putative genetic modifiers of onset age, though their effect size was limited. This implies that other modifying factors still need to be identified in this family, for example, by using exome- or genome-wide approaches. Identifying those novel factors is of great significance since they could be targets for disease-modifying therapies, which are currently not available for FTD patients.

Disclosure statement

The authors declare no conflicts of interest in relation to the research described in this article.

Acknowledgements

The authors thank the personnel of the Neuromics Support Facility at the VIB Center for Molecular Neurology and of the Biobank at the Institute Born-Bunge for their expert support and Dr. Sarah Ceysens for one of the FDG-PET images.

The research was in part funded by the Belgian Science Policy Office Interuniversity Attraction Poles program; the Flemish government initiated Methusalem excellence program; the Flemish government initiated Impulse Program on Networks for Dementia Research; the Queen Elisabeth Medical Foundation; the Alzheimer Research Foundation; the Research Foundation Flanders (FWO), the Agency for Innovation by Science and Technology Flanders (IWT), and the University of Antwerp Research Fund; Belgium. EW and WDC received a PhD fellowship of the IWT and SP of the FWO.

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.2018.03.007>.

References

- Ahmed, Z., Sheng, H., Xu, Y.F., Lin, W.L., Innes, A.E., Gass, J., Yu, X., Wuertzer, C.A., Hou, H., Chiba, S., Yamanouchi, K., Leissring, M., Petrucelli, L., Nishihara, M., Hutton, M.L., McGowan, E., Dickson, D.W., Lewis, J., 2010. Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggest a role for progranulin in successful aging. *Am. J. Pathol.* 177, 311–324.
- Baker, M., Litvan, I., Houlden, H., Adamson, J., Dickson, D., Perez-Tur, J., Hardy, J., Lynch, T., Bigio, E., Hutton, M., 1999. Association of an extended haplotype in the tau gene with progressive supranuclear palsy. *Hum. Mol. Genet.* 8, 711–715.
- Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A.D., Rollinson, S., Cannon, A., Dwosh, E., Neary, D., Melquist, S., Richardson, A., Dickson, D., Berger, Z., Eriksen, J., Robinson, T., Zehr, C., Dickey, C.A., Crook, R., McGowan, E., Mann, D., Boeve, B., Feldman, H., Hutton, M., 2006. Mutations in progranulin cause tau-

- negative frontotemporal dementia linked to chromosome 17. *Nature* 442, 916–919.
- Beck, J., Rohrer, J.D., Campbell, T., Isaacs, A., Morrison, K.E., Goodall, E.F., Warrington, E.K., Stevens, J., Revesz, T., Holton, J., Al-Sarraj, S., King, A., Scallion, R., Warren, J.D., Fox, N.C., Rossor, M.N., Collinge, J., Mead, S., 2008. A distinct clinical, neuropsychological and radiological phenotype is associated with progranulin gene mutations in a large UK series. *Brain* 131 (Pt 3), 706–720.
- Benussi, L., Ghidoni, R., Pegioani, E., Moretti, D.V., Zanetti, O., Binetti, G., 2009. Progranulin Leu271LeufsX10 is one of the most common FTLD and CBS associated mutations worldwide. *Neurobiol. Dis.* 33, 379–385.
- Benussi, L., Ghidoni, R., Binetti, G., 2010. Progranulin mutations are a common cause of FTLD in Northern Italy. *Alzheimer Dis. Assoc. Disord.* 24, 308–309.
- Benussi, L., Rademakers, R., Rutherford, N.J., Wojtas, A., Glionna, M., Paterlini, A., Albertini, V., Bettecken, T., Binetti, G., Ghidoni, R., 2013. Estimating the age of the most common Italian GRN mutation: walking back to Canossa times. *J. Alzheimers Dis.* 33, 69–76.
- Benussi, L., Rossi, G., Glionna, M., Tonoli, E., Piccoli, E., Fostinelli, S., Paterlini, A., Flocco, R., Albani, D., Pantieri, R., Cereda, C., Forloni, G., Tagliavini, F., Binetti, G., Ghidoni, R., 2014. C9ORF72 hexanucleotide repeat number in frontotemporal lobar degeneration: a genotype-phenotype correlation study. *J. Alzheimers Dis.* 38, 799–808.
- Borroni, B., Yancopoulos, D., Tsutsui, M., Padovani, A., Sawcer, S.J., Hodges, J.R., Spillantini, M.G., 2005. Association between tau H2 haplotype and age at onset in frontotemporal dementia. *Arch. Neurol.* 62, 1419–1422.
- Borroni, B., Archetti, S., Del, B.R., Papetti, A., Buratti, E., Bonvicini, C., Agosti, C., Cosseddu, M., Turla, M., Di, L.D., Pietro, C.G., Gennarelli, M., Padovani, A., 2010. TARDBP mutations in frontotemporal lobar degeneration: frequency, clinical features, and disease course. *Rejuvenation Res.* 13, 509–517.
- Borroni, B., Bonvicini, C., Galimberti, D., Tremolizzo, L., Papetti, A., Archetti, S., Turla, M., Alberici, A., Agosti, C., Premi, E., Appollonio, I., Rainero, I., Ferrarese, C., Gennarelli, M., Scarpini, E., Padovani, A., 2011a. Founder effect and estimation of the age of the Progranulin Thr272fs mutation in 14 Italian pedigrees with frontotemporal lobar degeneration. *Neurobiol. Aging* 32, 555–558.
- Borroni, B., Bonvicini, C., Galimberti, D., Tremolizzo, L., Papetti, A., Archetti, S., Turla, M., Alberici, A., Agosti, C., Premi, E., Appollonio, I., Rainero, I., Ferrarese, C., Gennarelli, M., Scarpini, E., Padovani, A., 2011b. Founder effect and estimation of the age of the Progranulin Thr272fs mutation in 14 Italian pedigrees with frontotemporal lobar degeneration. *Neurobiol. Aging* 32, 555–558.
- Brinkmann, B., Klitsch, M., Neuhuber, F., Huhne, J., Rolf, B., 1998. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. *Am. J. Hum. Genet.* 62, 1408–1415.
- Brouwers, N., Nuytemans, K., van der Zee, J., Gijssels, I., Engelborghs, S., Theuns, J., Kumar-Singh, S., Pickut, B.A., Pals, P., Dermaut, B., Bogaerts, V., De, P.T., Serneels, S., Van Den Broeck, M., Cuijt, I., Mattheijssens, M., Peeters, K., Sciot, R., Martin, J.J., Cras, P., Santens, P., Vandenbergh, R., De Deyn, P.P., Cruts, M., Van Broeckhoven, C., Sleegers, K., 2007. Alzheimer and Parkinson diagnoses in progranulin null mutation carriers in an extended founder family. *Arch. Neurol.* 64, 1436–1446.
- Carrasquillo, M.M., Nicholson, A.M., Finch, N., Gibbs, J.R., Baker, M., Rutherford, N.J., Hunter, T.A., DeJesus-Hernandez, M., Bisceglia, G.D., Mackenzie, I.R., Singleton, A., Cookson, M.R., Crook, J.E., Dillman, A., Hernandez, D., Petersen, R.C., Graff-Radford, N.R., Younkin, S.G., Rademakers, R., 2010. Genome-wide screen identifies rs646776 near sortilin as a regulator of progranulin levels in human plasma. *Am. J. Hum. Genet.* 87, 890–897.
- Corrado, L., Ratti, A., Gellera, C., Buratti, E., Castellotti, B., Carlomagno, Y., Ticozzi, N., Mazzini, L., Testa, L., Taroni, F., Baralle, F.E., Silani, V., D'Alfonso, S., 2009. High frequency of TARDBP gene mutations in Italian patients with amyotrophic lateral sclerosis. *Hum. Mutat.* 30, 688–694.
- Cruchaga, C., Graff, C., Chiang, H.H., Wang, J., Hinrichs, A.L., Spiegel, N., Bertelsen, S., Mayo, K., Norton, J.B., Morris, J.C., Goate, A., 2011. Association of TMEM106B gene polymorphism with age at onset in granulin mutation carriers and plasma granulin protein levels. *Arch. Neurol.* 68, 581–586.
- Cruts, M., Van Broeckhoven, C., 2008. Loss of progranulin function in frontotemporal lobar degeneration. *Trends Genet.* 24, 186–194.
- Cruts, M., Rademakers, R., Gijssels, I., van der Zee, J., Dermaut, B., de Pooter, T., de Rijk, P., Del-Favero, J., van Broeckhoven, C., 2005. Genomic architecture of human 17q21 linked to frontotemporal dementia uncovers a highly homologous family of low-copy repeats in the tau region. *Hum. Mol. Genet.* 14, 1753–1762.
- Cruts, M., Gijssels, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenbergh, R., Dermaut, B., Martin, J.J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van Den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P.P., Kumar-Singh, S., Van Broeckhoven, C., 2006. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 442, 920–924.
- Cruts, M., Theuns, J., Van Broeckhoven, C., 2012. Locus-specific mutation databases for neurodegenerative brain diseases. *Hum. Mutat.* 33, 1340–1344.
- DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256.
- Fan, H., Chu, J.Y., 2007. A brief review of short tandem repeat mutation. *Genomics Proteomics Bioinformatics* 5, 7–14.
- Finch, N., Baker, M., Crook, R., Swanson, K., Kuntz, K., Surtees, R., Bisceglia, G., Rovelet-Lecrux, A., Boeve, B., Petersen, R.C., Dickson, D.W., Younkin, S.G., Deramecourt, V., Crook, J., Graff-Radford, N.R., Rademakers, R., 2009. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. *Brain* 132 (Pt 3), 583–591.
- Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Muller, K., Marroquin, N., Nordin, F., Hubers, A., Weydt, P., Pinto, S., Press, R., Millicamps, S., Molko, N., Bernard, E., Desnuelle, C., Soriani, M.H., Dorst, J., Graf, E., Nordstrom, U., Feiler, M.S., Putz, S., Boeckers, T.M., Meyer, T., Winkler, A.S., Winkelman, J., de Carvalho, M., Thal, D.R., Otto, M., Brannstrom, T., Volk, A.E., Kursula, P., Danzer, K.M., Lichtner, P., Dikic, I., Meitinger, T., Ludolph, A.C., Strom, T.M., Andersen, P.M., Weishaupt, J.H., 2015. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat. Neurosci.* 18, 631–636.
- Gelb, D.J., Oliver, E., Gilman, S., 1999. Diagnostic criteria for Parkinson disease. *Arch. Neurol.* 56, 33–39.
- Genin, E., Tullio-Pelet, A., Begeot, F., Lyonnet, S., Abel, L., 2004. Estimating the age of rare disease mutations: the example of Triple-A syndrome. *J. Med. Genet.* 41, 445–449.
- Ghidoni, R., Benussi, L., Glionna, M., Franzoni, M., Binetti, G., 2008. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. *Neurology* 71, 1235–1239.
- Ghidoni, R., Stoppani, E., Rossi, G., Piccoli, E., Albertini, V., Paterlini, A., Glionna, M., Pegioani, E., Agnati, L.F., Fenoglio, C., Scarpini, E., Galimberti, D., Morbin, M., Tagliavini, F., Binetti, G., Benussi, L., 2012. Optimal plasma progranulin cutoff value for predicting null progranulin mutations in neurodegenerative diseases: a multicenter Italian study. *Neurodegener Dis.* 9, 121–127.
- Gijssels, I., Van Langenhove, T., van der Zee, J., Sleegers, K., Philtjens, S., Kleinberger, G., Janssens, J., Bettens, K., Van Cauwenbergh, C., Pereson, S., Engelborghs, S., Sieben, A., De Jonghe, P., Vandenbergh, R., Santens, P., De Bleecker, J., Maes, G., Baumer, V., Dillen, L., Joris, G., Cuijt, I., Corsmit, E., Elinck, E., Van Dongen, J., Vermeulen, S., Van Den Broeck, M., Vaerenberg, C., Mattheijssens, M., Peeters, K., Robberecht, W., Cras, P., Martin, J.J., De Deyn, P.P., Cruts, M., Van Broeckhoven, C., 2012. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet Neurol.* 11, 54–65.
- Gijssels, I., Van Mossevelde, S., van der Zee, J., Sieben, A., Philtjens, S., Heeman, B., Engelborghs, S., Vandenbulcke, M., De, B.G., Baumer, V., Cuijt, I., Van Den Broeck, M., Peeters, K., Mattheijssens, M., Rousseau, F., Vandenbergh, R., De Jonghe, P., Cras, P., De Deyn, P.P., Martin, J.J., Cruts, M., Van Broeckhoven, C., 2015. Loss of TBK1 is a frequent cause of frontotemporal dementia in a Belgian cohort. *Neurology* 85, 2116–2125.
- Gorno-Tempini, M.L., Hillis, A.E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S.F., Ogar, J.M., Rohrer, J.D., Black, S., Boeve, B.F., Manes, F., Dronkers, N.F., Vandenbergh, R., Rascovsky, K., Patterson, K., Miller, B.L., Knopman, D.S., Hodges, J.R., Mesulam, M.M., Grossman, M., 2011. Classification of primary progressive aphasia and its variants. *Neurology* 76, 1006–1014.
- Heath, S.C., 1997. Markov chain Monte Carlo segregation and linkage analysis for oligogenic models. *Am. J. Hum. Genet.* 61, 748–760.
- Hosokawa, M., Kondo, H., Serrano, G.E., Beach, T.G., Robinson, A.C., Mann, D.M., Akiyama, H., Hasegawa, M., Arai, T., 2017. Accumulation of multiple neurodegenerative disease-related proteins in familial frontotemporal lobar degeneration associated with granulin mutation. *Sci. Rep.* 7, 1513.
- Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R.C., Stevens, M., de, G.E., Wauters, E., van, B.J., Hillebrand, M., Joosse, M., Kwon, J.M., Nowotny, P., Che, L.K., Norton, J., Morris, J.C., Reed, L.A., Trojanowski, J., Basun, H., Lannfelt, L., Neystat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P.R., Hayward, N., Kwok, J.B., Schofield, P.R., Andreadis, A., Snowden, J., Craufurd, D., Neary, D., Owen, F., Oostra, B.A., Hardy, J., Goate, A., van, S.J., Mann, D., Lynch, T., Heutink, P., 1998. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393, 702–705.
- Kabashi, E., Valdmanis, P.N., Dion, P., Spiegelman, D., McConkey, B.J., Vande, V.C., Bouchard, J.P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P.F., Camu, W., Meininger, V., Dupre, N., Rouleau, G.A., 2008. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat. Genet.* 40, 572–574.
- Kelley, B.J., Haidar, W., Boeve, B.F., Baker, M., Graff-Radford, N.R., Krefft, T., Frank, A.R., Jack Jr., C.R., Shiung, M., Knopman, D.S., Josephs, K.A., Parashos, S.A., Rademakers, R., Hutton, M., Pickering-Brown, S., Adamson, J., Kuntz, K.M., Dickson, D.W., Parisi, J.E., Smith, G.E., Ivnik, R.J., Petersen, R.C., 2009. Prominent phenotypic variability associated with mutations in Progranulin. *Neurobiol. Aging* 30, 739–751.
- Kenna, K.P., McLaughlin, R.L., Byrne, S., Elamin, M., Heverin, M., Kenny, E.M., Cormican, P., Morris, D.W., Donaghy, C.G., Bradley, D.G., Hardiman, O., 2013. Delineating the genetic heterogeneity of ALS using targeted high-throughput sequencing. *J. Med. Genet.* 50, 776–783.
- Kirby, J., Goodall, E.F., Smith, W., Highley, J.R., Masanzu, R., Hartley, J.A., Hibberd, R., Hollinger, H.C., Wharton, S.B., Morrison, K.E., Ince, P.G., McDermott, C.J., Shaw, P.J., 2010. Broad clinical phenotypes associated with TAR-DNA binding protein (TARDBP) mutations in amyotrophic lateral sclerosis. *Neurogenetics* 11, 217–225.
- Laws, S.M., Perneckzy, R., Drzezga, A., Diehl-Schmid, J., Ibach, B., Baumli, J., Eisele, T., Forstl, H., Kurz, A., Riemenschneider, M., 2007. Association of the tau haplotype

- H2 with age at onset and functional alterations of glucose utilization in frontotemporal dementia. *Am. J. Psychiatry* 164, 1577–1584.
- Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760.
- Mackenzie, I.R., Neumann, M., 2016. Molecular neuropathology of frontotemporal dementia: insights into disease mechanisms from postmortem studies. *J. Neurochem.* 138 (Suppl 1), 54–70.
- McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytzky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., DePristo, M.A., 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20, 1297–1303.
- McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of Health and human Services Task Force on Alzheimer's disease. *Neurology* 34, 939–944.
- McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack Jr., C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rossor, M.N., Scheltens, P., Carrillo, M.C., Thies, B., Weintraub, S., Phelps, C.H., 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7, 263–269.
- Meeter, L.H., Patzke, H., Loewen, G., Dopfer, E.G., Pijnenburg, Y.A., Van, M.R., van Swieten, J.C., 2016. Progranulin levels in plasma and cerebrospinal fluid in granulin mutation carriers. *Dement Geriatr. Cogn. Dis. Extra* 6, 330–340.
- Neary, D., Snowden, J., Mann, D., 2005. Frontotemporal dementia. *Lancet Neurol.* 4, 771–780.
- Nicholson, A.M., Finch, N.A., Almeida, M., Perkerson, R.B., van, B.M., Wojtas, A., Cenik, B., Rotondo, S., Inskeep, V., Almasy, L., Dyer, T., Peralta, J., Jun, G., Wood, A.R., Frayling, T.M., Fuchsberger, C., Fowler, S., Teslovich, T.M., Manning, A.K., Kumar, S., Curran, J., Lehman, D., Abecasis, G., Duggirala, R., Pottier, C., Zahir, H.A., Crook, J.E., Karydas, A., Mitic, L., Sun, Y., Dickson, D.W., Bu, G., Herz, J., Yu, G., Miller, B.L., Ferguson, S., Petersen, R.C., Graff-Radford, N., Blangero, J., Rademakers, R., 2016. Progranulin is a regulator of progranulin levels and oligomerization. *Nat. Commun.* 7, 11992.
- Poorikaj, P., Bird, T.D., Wijsman, E., Nemens, E., Garruto, R.M., Anderson, L., Andreadis, A., Wiederholt, W.C., Raskind, M., Schellenberg, G.D., 1998. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol.* 43, 815–825.
- Postuma, R.B., Berg, D., Stern, M., Poewe, W., Olanow, C.W., Oertel, W., Obeso, J., Marek, K., Litvan, I., Lang, A.E., Halliday, G., Goetz, C.G., Gasser, T., Dubois, B., Chan, P., Bloem, B.R., Adler, C.H., Deuschl, G., 2015. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord.* 30, 1591–1601.
- Pottier, C., Bieniek, K.F., Finch, N., van d, V., Baker, M., Perkerson, R., Brown, P., Ravenscroft, T., van, B.M., Nicholson, A.M., DeTure, M., Knopman, D.S., Josephs, K.A., Parisi, J.E., Petersen, R.C., Boylan, K.B., Boeve, B.F., Graff-Radford, N.R., Veltman, J.A., Gilissen, C., Murray, M.E., Dickson, D.W., Rademakers, R., 2015. Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta Neuropathol.* 130, 77–92.
- R Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rademakers, R., Melquist, S., Cruts, M., Theuns, J., Del-Favero, J., Poorikaj, P., Baker, M., Sleegers, K., Crook, R., De Pooter, T., Bel Kacem, S., Adamson, J., Van den Bosch, D., Van den Broeck, M., Gass, J., Corsmit, E., De Rijk, P., Thomas, N., Engelborghs, S., Heckman, M., Litvan, I., Crook, J., De Deyn, P.P., Dickson, D., Schellenberg, G.D., Van Broeckhoven, C., Hutton, M.L., 2005. High-density SNP haplotyping suggests altered regulation of tau gene expression in progressive supranuclear palsy. *Hum. Mol. Genet.* 14, 3281–3292.
- Rademakers, R., Baker, M., Gass, J., Adamson, J., Huey, E.D., Momeni, P., Spina, S., Coppola, G., Karydas, A.M., Stewart, H., Johnson, N., Hsiung, G.Y., Kelley, B., Kuntz, K., Steinbart, E., Wood, E.M., Yu, C.E., Josephs, K., Sorenson, E., Womack, K.B., Weintraub, S., Pickering-Brown, S.M., Schofield, P.R., Brooks, W.S., Van Deerlin, V.M., Snowden, J., Clark, C.M., Kertesz, A., Boylan, K., Ghetti, B., Neary, D., Schellenberg, G.D., Beach, T.G., Mesulam, M., Mann, D., Grafman, J., Mackenzie, I.R., Feldman, H., Bird, T., Petersen, R., Knopman, D., Boeve, B., Geschwind, D.H., Miller, B., Wszolek, Z., Lipka, C., Bigio, E.H., Dickson, D., Graff-Radford, N., Hutton, M., 2007. Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C>T (Arg493X) mutation: an international initiative. *Lancet Neurol.* 6, 857–868.
- Rademakers, R., Eriksen, J.L., Baker, M., Robinson, T., Ahmed, Z., Lincoln, S.J., Finch, N., Rutherford, N.J., Crook, R.J., Josephs, K.A., Boeve, B.F., Knopman, D.S., Petersen, R.C., Parisi, J.E., Caselli, R.J., Wszolek, Z.K., Uitti, R.J., Feldman, H., Hutton, M.L., Mackenzie, I.R., Graff-Radford, N.R., Dickson, D.W., 2008. Common variation in the miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. *Hum. Mol. Genet.* 17, 3631–3642.
- Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., Van Swieten, J.C., Seelaar, H., Dopfer, E.G., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prileau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 134 (Pt 9), 2456–2477.
- Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., Van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.M., Kaivorinne, A.L., Holtta-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wu, J., Chio, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9orf72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72, 257–268.
- Reumers, J., De Rijk, P., Zhao, H., Liekens, A., Smeets, D., Cleary, J., Van, L.P., Van Den Bosch, M., Catthoor, K., Sabbe, B., Despiere, E., Vergote, I., Hilbush, B., Lambrechts, D., Del-Favero, J., 2012. Optimized filtering reduces the error rate in detecting genomic variants by short-read sequencing. *Nat. Biotechnol.* 30, 61–68.
- Rhinn, H., Abeliovich, A., 2017. Differential aging analysis in human cerebral cortex identifies variants in TMEM106B and GRN that regulate aging phenotypes. *Cell Syst.* 4, 404–415.
- Rohrer, J.D., Ridway, G.R., Modat, M., Ourselin, S., Mead, S., Fox, N.C., Rossor, M.N., Warren, J.D., 2010. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. *Neuroimage* 53, 1070–1076.
- Rollinson, S., Rohrer, J.D., van der Zee, J., Sleegers, K., Mead, S., Engelborghs, S., Collinge, J., De Deyn, P.P., Mann, D.M., Van Broeckhoven, C., Pickering-Brown, S.M., 2011. No association of PGRN 3'UTR rs5848 in frontotemporal lobar degeneration. *Neurobiol. Aging* 32, 754–755.
- Rubino, E., Vacca, A., Govone, F., De, M.P., Pinessi, L., Rainero, I., 2013. Apolipoprotein E polymorphisms in frontotemporal lobar degeneration: a meta-analysis. *Alzheimers Dement.* 9, 706–713.
- Sieben, A., Van Langenhove, T., Engelborghs, S., Martin, J.J., Boon, P., Cras, P., De Deyn, P.P., Santens, P., Van, B.C., Cruts, M., 2012. The genetics and neuropathology of frontotemporal lobar degeneration. *Acta Neuropathol.* 124, 353–372.
- Sieben, A., Van Mossevelde, S., Wauters, E., Engelborghs, S., van der Zee, J., Van Langenhove, T., Santens, P., Praet, M., Boon, P., Miatton, M., Van Hoecke, S., Vandenbulcke, M., Vandenbergh, R., Cras, P., Cruts, M., De Deyn, P.P., Van Broeckhoven, C., Martin, J.J., 2018. Extended FTL pedigree segregating a Belgian GRN-null mutation: neuropathological heterogeneity in one family. *Alzheimers Res. Ther.* 10, 7.
- Sinnwell, J.P., Therneau, T.M., Schaid, D.J., 2014. The kinship2 R package for pedigree data. *Hum. Hered.* 78, 91–93.
- Skibinski, G., Parkinson, N.J., Brown, J.M., Chakrabarti, L., Lloyd, S.L., Hummerich, H., Nielsen, J.E., Hodges, J.R., Spillantini, M.G., Thugaaard, T., Brandner, S., Brun, A., Rossor, M.N., Gade, A., Johannsen, P., Sorensen, S.A., Gydesen, S., Fisher, E.M., Collinge, J., 2005. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat. Genet.* 37, 806–808.
- Sleegers, K., Brouwers, N., Van Damme, P., Engelborghs, S., Gijssels, I., van der Zee, J., Peeters, K., Mattheijssens, M., Cruts, M., Vandenbergh, R., De Deyn, P.P., Robberecht, W., Van Broeckhoven, C., 2009. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. *Ann. Neurol.* 65, 603–609.
- Spillantini, M.G., Murrell, J.R., Goedert, M., Farlow, M.R., Klug, A., Ghetti, B., 1998. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. U S A* 95, 7737–7741.
- Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackersley, S., Durnall, J.C., Williams, K.L., Buratti, E., Baralle, F., De, B.J., Mitchell, J.D., Leigh, P.N., Al-Chalabi, A., Miller, C.C., Nicholson, G., Shaw, C.E., 2008. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319, 1668–1672.
- Van Damme, P., Van Hoecke, A., Lambrechts, D., Vanacker, P., Bogaert, E., van, S.J., Carmeliet, P., Van Den Bosch, L., Robberecht, W., 2008. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. *J. Cell Biol.* 181, 37–41.
- Van Deerlin, V.M., Sleiman, P.M., Martinez-Lage, M., Chen-Plotkin, A., Wang, L.S., Graff-Radford, N.R., Dickson, D.W., Rademakers, R., Boeve, B.F., Grossman, M., Arnold, S.E., Mann, D.M., Pickering-Brown, S.M., Seelaar, H., Heutink, P., Van Swieten, J.C., Murrell, J.R., Ghetti, B., Spina, S., Grafman, J., Hodges, J., Spillantini, M.G., Gilman, S., Lieberman, A.P., Kaye, J.A., Woltjer, R.L., Bigio, E.H., Mesulam, M., Al-Sarraj, S., Troakes, C., Rosenberg, R.N., White III, C.L., Ferrer, I., Llado, A., Neumann, M., Kretzschmar, H.A., Huete, C.M., Welsh-Bohmer, K.A., Miller, B.L., Alzualde, A., Lopez de, M.A., McKee, A.C., Gearing, M., Levey, A.I., Lah, J.J., Hardy, J., Rohrer, J.D., Lashley, T., Mackenzie, I.R., Feldman, H.H., Hamilton, R.L., Dekosky, S.T., van der Zee, J., Kumar-Singh, S., Van, B.C., Mayeux, R., Vonsattel, J.P., Troncoso, J.C., Kiri, J.J., Kwok, J.B., Halliday, G.M., Bird, T.D., Ince, P.G., Shaw, P.J., Cairns, N.J., Morris, J.C., McLean, C.A., DeCarli, C., Ellis, W.G., Freeman, S.H., Frosch, M.P., Growdon, J.H., Perl, D.P., Sano, M., Bennett, D.A., Schneider, J.A., Beach, T.G., Reiman, E.M., Woodruff, E.K., Cummings, J., Vinters, H.V., Miller, C.A., Chui, H.C., Alafuzoff, I., Hartikainen, P., Seilhean, D., Galasko, D., Masliah, E., Cotman, C.W., Tunon, M.T., Martinez, M.C., Munoz, D.G., Carroll, S.L., Marson, D., Riederer, P.F., Bogdanovic, N., Schellenberg, G.D., Hakonarson, H., Trojanowski, J.Q., Lee, V.M., 2010. Common

- variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat. Genet.* 42, 234–239.
- van der Zee, J., Rademakers, R., Engelborghs, S., Gijssels, I., Bogaerts, V., Vandenberghe, R., Santens, P., Caekebeke, J., De Pooter, T., Peeters, K., Lubke, U., Van Den Broeck, M., Martin, J.J., Cruts, M., De Deyn, P.P., Van Broeckhoven, C., Dermaut, B., 2006. A Belgian ancestral haplotype harbours a highly prevalent mutation for 17q21-linked tau-negative FTLD. *Brain* 129 (Pt 4), 841–852.
- van der Zee, J., Van Langenhove, T., Kleinberger, G., Sleegers, K., Engelborghs, S., Vandenberghe, R., Santens, P., Van Den Broeck, M., Joris, G., Brys, J., Mattheijssens, M., Peeters, K., Cras, P., De Deyn, P.P., Cruts, M., Van Broeckhoven, C., 2011. TMEM106B is associated with frontotemporal lobar degeneration in a clinically diagnosed patient cohort. *Brain* 134 (Pt 3), 808–815.
- van der Zee, J., Gijssels, I., Dillen, L., Van Langenhove, T., Theuns, J., Engelborghs, S., Philtjens, S., Vandenbulcke, M., Sleegers, K., Sieben, A., Baumer, V., Maes, G., Corsmit, E., Borroni, B., Padovani, A., Archetti, S., Perneczky, R., Diehl-Schmid, J., de, M.A., Miltenberger-Miltenyi, G., Pereira, S., Pimentel, J., Nacmias, B., Bagnoli, S., Sorbi, S., Graff, C., Chiang, H.H., Westerlund, M., Sanchez-Valle, R., Llado, A., Gelpi, E., Santana, I., Almeida, M.R., Santiago, B., Frisoni, G., Zanetti, O., Bonvicini, C., Synofzik, M., Maetzler, W., vom Hagen, J.M., Schols, L., Heneka, M.T., Jessen, F., Matej, R., Parobkova, E., Kovacs, G.G., Strobel, T., Sarafov, S., Tournev, I., Jordanova, A., Danek, A., Arzberger, T., Fabrizio, G.M., Testi, S., Salmon, E., Santens, P., Martin, J.J., Cras, P., Vandenberghe, R., De Deyn, P.P., Cruts, M., Van Broeckhoven, C. On behalf of the European Early-Onset Dementia (EOD) Consortium, 2013. A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Hum. Mutat.* 34, 363–373.
- Van Swieten, J.C., Heutink, P., 2008. Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. *Lancet Neurol.* 7, 965–974.
- Verpillat, P., Camuzat, A., Hannequin, D., Thomas-Anterion, C., Puel, M., Belliard, S., Dubois, B., Didic, M., Michel, B.F., Lacomblez, L., Moreaud, O., Sellal, F., Golfier, V., Campion, D., Clerget-Darpoux, F., Brice, A., 2002. Association between the extended tau haplotype and frontotemporal dementia. *Arch. Neurol.* 59, 935–939.
- Watts, G.D., Wymer, J., Kovach, M.J., Mehta, S.G., Mumm, S., Darvish, D., Pestronk, A., Whyte, M.P., Kimonis, V.E., 2004. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat. Genet.* 36, 377–381.
- Whitwell, J.L., Jack Jr., C.R., Boeve, B.F., Senjem, M.L., Baker, M., Rademakers, R., Ivnik, R.J., Knopman, D.S., Wszolek, Z.K., Petersen, R.C., Josephs, K.A., 2009. Voxel-based morphometry patterns of atrophy in FTLD with mutations in MAPT or PGRN. *Neurology* 72, 813–820.
- Wijsman, E.M., Yu, D., 2004. Joint oligogenic segregation and linkage analysis using bayesian Markov chain Monte Carlo methods. *Mol. Biotechnol.* 28, 205–226.
- Wils, H., Kleinberger, G., Pereson, S., Janssens, J., Capell, A., Van, D.D., Cuijt, I., Joris, G., De Deyn, P.P., Haass, C., Van, B.C., Kumar-Singh, S., 2012. Cellular ageing, increased mortality and FTLD-TDP-associated neuropathology in progranulin knockout mice. *J. Pathol.* 228, 67–76.