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Rare nonsynonymous variants in *SORT1* are associated with increased risk for frontotemporal dementia

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ABSTRACT

We investigated the genetic role of sortilin (*SORT1*) in frontotemporal dementia (FTD). SORT1 is the neuronal receptor for granulin, encoded by the progranulin gene (*GRN*), a major causal gene for inherited FTD. In Belgian cohorts of 636 FTD patients and 1066 unaffected control individuals, we identified 5 patient-only nonsynonymous rare variants in *SORT1*. Rare variant burden analysis showed a significant increase in rare coding variants in patients compared to control individuals ($p = 0.04$), particularly in the β-propeller domain ($p = 0.04$), with 2 rare variants located in the predicted binding site for GRN ($p = 0.001$). We extended these observations by analyzing 3 independent patient/control cohorts sampled in Spain, Italy, and Portugal by partners of the European Early-Onset Dementia Consortium, together with 1155 FTD patients and 1161 control persons. An additional 7 patient-only nonsynonymous variants were observed in *SORT1* in European patients. Meta-analysis of the rare nonsynonymous variants in the Belgian and European patient/control cohorts revealed a significant enrichment in FTD patients ($p = 0.006$), establishing *SORT1* as a genetic risk factor for FTD.

1. Introduction

Frontotemporal lobar degeneration (FTLD) represents a heterogeneous group of neurodegenerative disorders characterized by neuronal loss in the frontal and temporal lobes of the brain. Clinically, 2 entities are defined: the behavioral variant of frontotemporal dementia (bvFTD) with main changes in personality and behavior (Neary et al., 1998; Rascovsky et al., 2011) and primary progressive aphasia (PPA) with language disturbances (Gorno-Tempini et al., 2011; Neary et al., 1998). PPA is further subdivided into progressive nonfluent aphasia (PNFA), characterized by apraxia of speech and/or agrammatism, semantic dementia (SD), characterized by impaired confrontation naming and single word comprehension, and logopenic variant PPA, characterized by impaired word retrieval and a deficit in repetition of sentences and, in contrast with the other 2 PPA variants, mostly associated with underlying atypical Alzheimer's disease (AD) pathology (Gorno-Tempini et al., 2011; Neary et al., 1998). At neuropathology, FTLD brains show protein inclusions in degenerating neurons, and based on the particular inclusion protein, FTLD can be further subdivided into FTLD-TDP (TAR DNA-binding protein 43 kDa), FTLD-tau (tau), FTLD-FUS (fused in sarcoma), FTLD–ubiquitin proteasome system or FTLD-ni (no inclusions) (Mackenzie et al., 2010).

About 30%–50% of frontotemporal dementia (FTD) patients have a positive family history, indicating a strong genetic contribution. In 10%–23% of FTD families, the segregation is compatible with autosomal dominant inheritance (Goldman et al., 2007). So far, 7 causal FTD genes have been identified: microtubule-associated protein tau (*MAPT*) (Hutton et al., 1998), valosin-containing protein (*VCP*) (Watts et al., 2004), charged multivesicular body protein 2B (*CHMP2B*) (Skibinski et al., 2005), progranulin (*GRN*) (Baker et al., 2006; Cruts et al., 2006), chromosome 9 open reading frame 72 (*C9orf72*) (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011), sequestosome 1 (*SQSTM1*) (Rubino et al., 2012; van der Zee et al., 2014), and TANK-binding kinase 1 (*TBK1*) (Gijselinck et al., 2015; Pottier et al., 2015).

GRN is the second major mutated gene after *C9orf72*, with loss-of-function mutations (Cruts et al., 2006, 2012; Sleegers et al., 2009) leading to a reduction of GRN levels in the CSF and serum or plasma, supporting haploinsufficiency as the underlying disease mechanism (Finch et al., 2009; Ghidoni et al., 2008; Schofield et al., 2010; Sleegers et al., 2009). GRN is widely expressed, but in the brain, intracellular expression is highest in the neurons and activated microglia (Petkau et al., 2010). Sortilin (SORT1) was identified as the main neuronal receptor for GRN and is expressed on the neuronal cell surface (Hu et al., 2010). Under stress conditions, GRN is secreted by activated microglia, binds to the neuronal receptor SORT1, and is rapidly endocytosed, leading to reduced levels of extracellular GRN (Hu et al., 2010). Furthermore, a genome-wide association study of plasma GRN levels in FTD patients and controls identified a significant association with 2 single-nucleotide polymorphisms, rs646776 and rs611917, located 34 kb and 37 kb downstream of *SORT1* (Carrasquillo et al., 2010). In the present study, we investigated a role *SORT1* in the genetic etiology of FTD.

2. Methods

2.1. Patient/control study populations

The Belgian patients and control individuals were ascertained through the Belgian Neurology (BELNEU) Consortium and consisted of 636 index FTD patients (mean onset age 63 ± 10 years) with a clinical diagnosis of probable or possible FTD (n = 593), possible corticobasal syndrome (n = 15), possible progressive supranuclear palsy (PSP) syndrome (n = 27) or a differential diagnosis of progressive supranuclear palsy syndrome or corticobasal syndrome (n = 1). All patients were evaluated by detailed clinical history of patients and family, neurological examination, and neuroimaging. Clinical diagnosis was reached according to established clinical criteria (Alexander et al., 2014; Gorno-Tempini et al., 2011; Litvan et al., 1996; Rascovsky et al., 2011). For 408 FTD patients, information was available on the clinical subtype: 286 received a diagnosis of possible or probable bvFTD (45.0% of the entire cohort) and 123 of PPA (19.3%). From the latter, 50 were diagnosed with SD (7.9%), 42 with PNFA (6.6%), and 4 with logopenic PPA (0.6%). All patients with a clinical diagnosis of FTD combined with motor neuron disease were excluded from this study. For 5.7% of the patients (n = 36), neuropathological examination was available. FTLD-TDP was the most frequent pathology (n = 18; 2.8%), followed by FTLD-tau (n = 11; 1.7%; includes 3 progressive supranuclear palsy and 1 corticobasal degeneration patients) and FTLD–ubiquitin proteasome system (n = 2; 0.3%); few received a pathological diagnosis consistent with FTLD-U not further specified (n = 4; 0.6%), and 1 was reported negative for TDP-43 and tau-positive inclusions (n = 1; 0.2%). A family history of dementia, defined by at least 1 first-degree relative with dementia, was present in 30.3% (n = 193) of patients. We also included 1066 unrelated age-matched and geographically matched control individuals (mean age at inclusion 67 years ±13 years), who were negative for a personal or family history of a neurodegenerative disease and had a mini-mental state examination or Montreal

Cognitive Assessment (MoCA) score above 25 (Folstein et al., 1975; Nasreddine et al., 2005).

Patients and control persons were genetically profiled for common genes associated with the FTD-amyotrophic lateral sclerosis spectrum (*GRN*, *MAPT*, *TBK1*, *VCP*, *TARDBP*, and *FUS*) or AD (*APP*, *PSEN1*, *PSEN2*). Custom gene panels were designed using Amplicon Target Amplification technology (Agilent, https://www.agilent.com) for resequencing on a MiSeq (Illumina) platform. The *C9orf72* repeat was typed by repeat-primed polymerase chain reaction (PCR) and fragment-length analysis, as described (Gijselinck et al., 2012).

Three independently sampled patient/control cohorts were obtained via the European Early-Onset Dementia Consortium, described in detail in previous studies (van der Zee et al., 2013; van der Zee et al., 2017). The European cohorts together consisted of 1155 FTD patients originating from Spain (n = 438), Italy (n = 486), and Portugal (n = 231), and 1161 geographically matched controls from Spain (n = 502), Italy (n = 537), and Portugal (n = 122). Characteristics of the patient and control cohorts are listed in Supplementary Table S1 and have been published in more detail (van der Zee et al., 2017).

2.2. Ethical assurances

For all participants, informed consent for participation in clinical and genetic studies was obtained according to sampling and study protocols that were approved by the local ethics committees of the collaborating medical centers. The protocols for the clinical and genetic studies were approved by the Ethics Committee of the University of Antwerp, Belgium.

2.3. SORT1 sequencing

Genomic DNA was extracted from the peripheral blood according to standard procedures. For the resequencing of all 20 coding exons of *SORT1* (RefSeq NM_002959.5), 2 multiamplicon target panels were designed using the Amplicon Target Amplification assay (Agilent, https://www.agilent.com). Primers for multiplex PCR were designed using the mPCR primer design software (Agilent, https://www.agilent.com; Goossens et al., 2009). Specific target regions were amplified using multiplex PCR, and Individual barcodes (Illumina Nextera XT) were incorporated, in a universal PCR step. Bridge amplification and sequencing of barcoded samples was performed using an Illumina MiSeq platform, with the Illumina V2 reagent kit, generating 250-bp paired-end reads.

After sample demultiplexing, sequence reads were mapped to hg19 using the Burrows-Wheeler Aligner (Li and Durbin, 2010). Sequence variants were called using the Genome Analysis Toolkit (McKenna et al., 2010), and variants were annotated using GenomeComb (Reumers et al., 2012). Identified variants were validated using Sanger sequencing. Flanking intronic primers were designed using Primer 3. Standard PCR amplifications were performed on 20-ng genomic DNA using empirically defined cycling conditions. PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH). Both strands of the purified amplicons were sequenced using PCR primers or internal sequencing primers and the BigDye Terminator Cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA) and analyzed on an automated ABI3730 DNA Analyzer (Applied Biosystems). Sequences were analyzed using both the software packages novoSNP (Weckx et al., 2005) and Lasergene (DNASTAR, Madison WI, USA).

gDNA numbering of *SORT1* variants is relative to nucleotide 1 of the reference sequence NG_028280.1. Coding variants were numbered relative to the translation initiation codon in the largest *SORT1* transcript (RefSeq NM_002959.5). Amino acid numbering is according to the largest SORT1 protein isoform (GenPept accession number NP_002950.3).

2.4. Haplotype sharing analysis

Allele sharing was investigated using 8 polymorphic short tandem repeat (STR) markers at chromosome 1p13.3 and surrounding *SORT1* (D1S239, D1S2688, ATA42G12, D1S248, D1S2778, D1S2792, D1S221, D1S2651). STRs were PCR-amplified using fluorescently labeled primers and sized using GeneScan 500 Liz Size Standard (Applied Biosystems) on an ABI3730xl DNA Analyzer (Applied Biosystems). Alleles were scored with Local Genotype Viewer.

2.5. In silico predictions

The CADD_Phred score (http://cadd.gs.washington.edu/score) was used to predict the impact of nonsynonymous variants (Kircher et al., 2014).

2.6. Statistical analysis

Rare variants were defined as variants with a minor allele frequency $\leq$1% and were included in a rare variant burden analysis for individuals originating from Belgium, Spain, Italy, and Portugal. Rare variant burden analysis was performed by collapsing alleles of all rare variants across the full *SORT1* coding sequence, separately for each functional protein domain, or for rare variants located in the predicted GRN binding site (aa 281–320), using an optimized sequence kernel association test (SKAT-O test), adjusted for sample size <2000. SKAT-O meta-analysis was performed using Meta_SKAT and corrected for ethnicity. Presented SKAT-O *p*-values represent the *p*-values over rho = 1, which represents a burden analysis. Functional protein domains were determined according to UniProt accession number Q99523.

3. Results

3.1. SORT1 variants in the Belgian cohorts

Sequencing of the *SORT1* coding region in the Belgian patient/control cohorts identified 5 rare (minor allele frequency $\leq$1%), nonsynonymous variants in 8 index patients who were absent in the Belgian control cohort. (Table 1). Together, the *SORT1* carrier frequency was 1.3% (8/636 patients) in the Belgian FTD patient cohort. Four variants, p.Lys302Glu, p.Gly310Ala, p.Arg392Gln, and p.Val418Met, were located in the β-propeller domain while one, p.Gln724His, was located in the 10CC module (Fig. 1). Two variants, p.Lys302Glu and p.Gly310Ala, were located in the region predicted to bind ligands including GRN. All 5 variants affected evolutionary highly conserved amino acid codons (Supplementary Fig. S1) and received a pathogenicity CADD_Phred score of above 20. Three variants, p.Lys302Glu, p.Arg392Gln, and p.Val418Met, were present in the ExAc database at low frequencies. Four patients carried the p.Lys302Glu variant, and genotyping of STR markers flanking *SORT1* indicated that all four carriers were sharing alleles in a genomic segment of 1 Mb.

In addition, we identified in *SORT1*, 5 rare variants that were present in both patients and control individuals (Table 2; Supplementary Tables S3 and S4). Three of these variants, p.Ile124Val, p.Asp358Tyr, and p.Glu447Gly, are located in the β-propeller domain of SORT1 but outside the ligand binding region, while the other 2 variants, p.Val650Met and p.Asp656Gly, are located at the 10 CC module (Fig. 1). In the Belgian control cohort, we identified 2 variants that were present only in control persons (Supplementary Table S4), of which p.Glu148Asp is located in the

Table 1
SORT1 patient-only rare variants

Patient identifier	cDNA[a]	Predicted protein[b]	Protein domain	CADD_Phred[c]	ExAc allele frequency	Country of origin	Gender	Family history	AAO (y)	Clinical diagnosis	Clinical subdiagnosis
Belgian cohort											
DR1247	c.904 A>G	p.Lys302Glu	β-Propeller	20.7	0.0021	Belgium	Female	S	79[f,g]	FTD	SD
DR1149	c.904 A>G	p.Lys302Glu	β-Propeller	20.7	0.0021	Belgium	Male	F	77	FTD[e]	bvFTD
DR1193	c.904 A>G	p.Lys302Glu	β-Propeller	20.7	0.0021	Belgium	Female	F	67	FTD	bvFTD
DR1248	c.904 A>G	p.Lys302Glu	β-Propeller	20.7	0.0021	Belgium	Male	F	57[f]	FTD[d]	-
DR1116	c.929 G>C	p.Gly310Ala	β-Propeller	24.4	-	Belgium	Female	S	48	FTD	bvFTD
DR732	c.1175 G>A	p.Arg392Gln	β-Propeller	30.0	0.000033	Belgium	Male	U	64	FTD	bvFTD
DR1252	c.1252 G>A	p.Val418Met	β-Propeller	25.1	0.000058	Belgium	Male	F	56	FTD	bvFTD
DR1345	c.2172 G>C	p.Gln724His	TM	23.0	-	Belgium	Female	S	80[f]	FTD	bvFTD
European cohort											
EOD-P51	c.675 G>T	p.Gln225His	β-Propeller	19.4	0.00014	Spain	Female	S	65	FTD	bvFTD
EOD-P52	c.675 G>T	p.Gln225His	β-Propeller	19.4	0.00014	Spain	Female	F	49	FTD	bvFTD
EOD-P53	c.793 A>C	p.Asn265His	β-Propeller	16.6	0.000025	Spain	Female	U	65	FTD	bvFTD
EOD-P54	c.1243 A>G	p.Ile415Val	β-Propeller	18.6	0.00001648	Spain	Female	S	76	FTD	bvFTD
EOD-P55	c.1261 G>A	p.Glu421Lys	β-Propeller	23.8	-	Portugal	Male	F	62	FTD	bvFTD
EOD-P56	c.1887 T>A	p.Asp629Glu	10 CC	17.2	-	Italy	Female	U	71	FTD	PPA
EOD-P57	c.1948G>A	p.Val650Met	10 CC	25.3	0.00034	Spain	Male	U	73	FTD	PNFA
EOD-P58	c.2267 C>A	p.Ser756Tyr	TM	24.1	0.000017	Italy	Male	F	62	FTD	PNFA
EOD-P59	c.2317 G>T	p.Ala773Ser	TM	23.2	0.0000082	Portugal	Male	F	70	FTD	bvFTD

Key: AAO, age at onset; bvFTD, behavioral variant frontotemporal dementia; F, familial; FTD, frontotemporal dementia; PPA, primary progressive aphasia; PNFA, progressive nonfluent aphasia; S, sporadic; SD, semantic dementia; TM, transmembrane domain; U, unknown.
[a] Relative to the translation initiation codon in NM_002959.5.
[b] According to NP_002950.3.
[c] CADD_Phred score >20 means that the variant is predicted to be among the 1% most deleterious substitutions in the human genome.
[d] Unspecified subtype.
[e] Probably with vascular contributing factor.
[f] Age at referral because age at onset was not available.
[g] Indicates that the patient is deceased.

β-propeller domain and p.Arg716Gln at the 10 CC module (Fig. 1). All rare variants identified in patients and control persons or in control persons only are present in the ExAc database (Supplementary Table S2).

A burden analysis, collapsing all rare *SORT1* variants across the whole protein, showed a significant enrichment in patients (21/1272 alleles = 1.6%) compared to control persons (20/2132 alleles = 0.9%; $p = 0.04$) (Table 3 and Supplementary Table S5). The association appeared to be driven by the rare variants burden in the β-propeller domain in FTD patients (18/1272 alleles = 1.4%) compared to controls (16/2132 = 0.7%; $p = 0.04$) (Supplementary Table S5), and particularly by these variants that are located in the predicted binding region for GRN, 5/1272 alleles = 0.4% in patients and 0/2150 = 0% in controls ($p = 0.001$) (Supplementary Table S5).

3.2. *SORT1* variants in the European cohorts

Aiming at replication of our findings in the Belgian cohorts, we sequenced the entire coding sequence of *SORT1* in 3 independently sampled patient/control cohorts in Spain, Italy, and Portugal by partners of the European Early-Onset Dementia Consortium (Supplementary Table S1), together comprising 1155 FTD patients and 1161 control persons. Eight rare nonsynonymous variants were identified in 9 FTD patients only, 5 Spanish (5/438 = 1.1%), 2 Italian (2/486 = 0.4%), and 2 Portuguese (2/231 = 0.9%) (Table 1) FTD patients. Seven of the 9 patient-only variants were not observed in the Belgian discovery cohort (Fig. 1, Table 1). Four variants, p.Gln225His, p.Asn265His, p.Ile415Val, and p.Glu421Lys, are located in the β-propeller domain and 1 variant, p.Asp629Glu, in the 10CC module and 2 variants, p.Ser756Tyr and p.Ala773Ser, in the transmembrane domain of SORT1 (Fig. 1). While, p.Val650Met was not observed in Spanish control persons, it was present in 1 Belgian control individual (Supplementary Table S4). All patient-only variants, identified in the overall European cohort, affect evolutionary highly conserved amino acid codons (Supplementary Fig. S1), of which 3 received a pathogenicity CADD_Phred score above 20 (Table 1). Two variants, p.Glu421Lys and p.Asp629Glu, were absent in the ExAc database, while the other 5 were observed with varying frequencies (Table 1). Of 4 variants, observed in Belgian patients only (p.Lys302Glu) or in patients and controls (p.Ile124Val, p.Asp358Tyr, and p.Glu447Gly), 3 were present in European patients and control persons and 1 in controls only (Fig. 1). In the European cohorts, 6 newly observed variants were all observed only in control individuals (Fig. 1 and Supplementary Table S3 and S4).

A SKAT-O meta-analysis was performed, including the patient/control cohorts of Belgium, Spain, Italy, and Portugal, together with 1791 FTD patients and 2227 control individuals. The data confirmed the enrichment of rare variants in FTD patients compared to control persons (SKAT-O *p*-value 0.006, rare allele frequency in patients [RAF] 1.7% [63/3582], RAF in controls 1.2% [53/4454]) (Table 3 and Supplementary Table S5). The most significant enrichment of variants was found in the β-propeller domain of SORT1 (SKAT-O *p* value 0.008, RAF in patients 1.6% [56/3582], RAF in controls 1.0% [47/4454] [Supplementary Table S5]), compared to the other 2 SORT1 domains (10CC *p*-value 0.75 RAF in patients 0.1% [5/3582], RAF in controls 0.1% [6/4454]) and transmembrane domain *p*-value 0.21 (RAF in patients 0.05% [2/3582], RAF in controls 0% [0/4454]) (Supplementary Table S5).

3.3. Clinical characteristics of *SORT1* patient-only variant carriers

The *SORT1* carriers that were identified in the Belgian and the European patient cohorts (Table 1) had a clinical diagnosis of FTD and a subtype diagnosis of bvFTD (n = 12), PNFA (n = 2), SD (n = 1), or PPA not further specified (n = 1) (Table 1). For 1 patient, the clinical subtype diagnosis was not defined. The average age at onset of all patients was 64.6 ± 8.9 years (range 48–77). Eight of the 17 *SORT1* variant carriers had a known positive familial history of neurodegenerative disease. Previous genetic profiling of common FTD-amyotrophic lateral sclerosis genes (*MAPT, GRN, C9orf72, TBK1,*

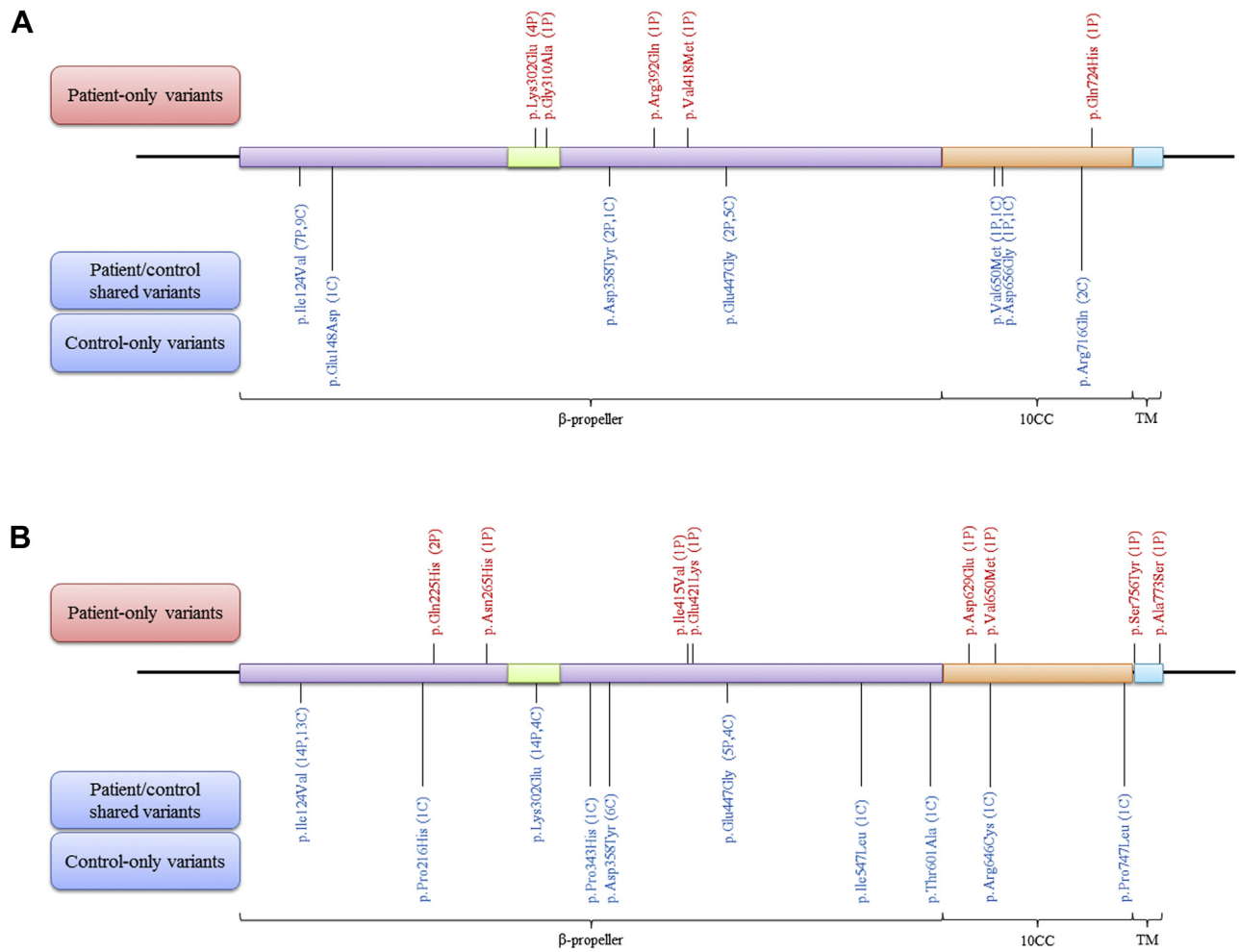

Fig. 1. Schematic presentation of the SORT1 protein domains and genetic variants. The known domains of SORT1, β-propeller (aa 78–611), 10 CC module (aa 612–755), and transmembrane domain (TM, aa 756–778) are indicated below the diagram and are visualized by the different colors, purple, orange, and blue. The predicted binding region of GRN within the β-propeller domain is indicated in green (aa 281–320). The area variants (MAF ≤1%), observed in patients only are indicated in red above the protein diagram and those present in patients and controls or in controls only in blue below the protein diagram. (A) Variants observed in the Belgian FTLD and control cohorts. (B) Variants observed in the European patient and cohorts. Abbreviations: FTLD, Frontotemporal lobar degeneration; MAF, minor allele frequency. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

SOD1, *VCP*, *FUS*, and *TARDBP*) and AD genes (*APP*, *PSEN1*, *PSEN2*) in patients and control persons were negative.

For 6/8 Belgian and 7/9 European carriers, we were able to retrieve detailed clinical information from their medical record (Table 4), which was compatible with an FTD phenotype. The bvFTD carriers had various behavioral problems ranging from apathy, loss of empathy, loss of interest, loss of hygiene, social withdrawal and decreased spontaneous speech to disinhibition, mental rigidity, hyperorality, perseveration, and compulsive or stereotyped behavior. Also attention deficits and executive dysfunctions were present and, in a few patients, memory complaints and decreased orientation. Word retrieval deficits were the most frequent reported language problem. Extrapyramidal rigidity and/or hypokinesia or bradykinesia were reported in 3 patients. Structural and functional neuroimaging findings were variable, but compatible

Table 2
SORT1 rare variants present in patients and controls

SORT1 mutation[a]	gDNA[b]	cDNA[c]	No. of FTD (%)	No. of controls (%)
Belgian cohort				
p.Ile124Val	g.35464A>G	c.370A>G	7 (1.0)	9 (0.8)
p.Asp358Tyr	g.60892G>T	c.1072G>T	2 (0.3)	1 (0.1)
p.Glu447Gly	g.66671A>G	c.1340A>G	2 (0.3)	5 (0.5)
p.Val650Met	g.79934G>A	c.1948G>A	1 (0.2)	1 (0.1)
p.Asp656Gly	g.79953A>G	c.1967A>G	1 (0.2)	1 (0.1)
European cohort				
p.Ile124Val	g.35464A>G	c.370A>G	14 (1.2)	13 (1.1)
p.Lys302Glu	g.57132A>G	c.904A>G	14 (1.2)	4 (0.3)
p.Glu447Gly	g.66671A>G	c.1340A>G	5 (0.4)	4 (0.3)

Key: FTD, frontotemporal dementia.
[a] According to NP_002950.3.
[b] Relative to nucleotide 1 in NG_028280.1.
[c] Relative to the translation initiation codon in NM_002959.5.

Table 3
SKAT-O meta-analysis of rare variant burden

Country of origin	Rare/total alleles in patients (%)	Rare/total alleles in controls (%)	SKAT-O *p*-value
Belgium	21/1272 (1.6)	20/2132 (0.9)	**0.04**
Spain	19/876 (2.2)	14/1004 (1.4)	**0.02**
Italy	15/972 (1.5)	15/1074 (1.4)	0.51
Portugal	8/462 (1.7)	4/244 (1.6)	0.80
Meta-analysis	63/3582 (1.7)	53/4454 (1.2)	**0.006**

Key: SKAT-O, optimized sequence kernel association test. The bold entries in the table represent the SKAT-O p-values below 0.05 (statistical significant).

Table 4
Clinical features, neuroimaging, and CSF biomarker data of *SORT1* carriers of patient-only variants

Identifier	Clinical features	Structural neuroimaging[a]	Functional neuroimaging[b]	CSF biomarkers
DR1247	Word retrieval deficits, executive dysfunction, dysgraphia; mild extrapyramidal rigidity	NI	Bilat. F and anteroT ↓	NI
DR1149	Memory complaints, decreased orientation in time, word retrieval deficits, hyperorality (sweet food preference), verbal disinhibition (sexually explicit remarks)	CSCA; severe leukoaraiosis Ri > Le	Mild to moderate ↓ bilat. F; relative ↓ P Ri and thalamus Ri	Normal $A\beta_{1-42}$, P-tau and T-tau
DR1193	Apathy, loss of empathy, disinhibition, agitation, loss of hygiene, mental rigidity, hyperorality (sweet food and alcohol preference), economy of speech	Mild atrophy F > T, P, O	Moderate ↓ FT, Le > Ri	NI
DR1116	Apathy, loss of interest, attention deficit, executive dysfunction; perioral dyskinesia	No significant changes	LateroF ↓, Ri > Le inferoP Ri ↓	NI
DR732	Inertia, loss of interest, loss of empathy, loss of hygiene, mental rigidity, perseveration, compulsive behavior, anosognosia, hyperorality (sweet food preference), delusions; hypomimia, dysdiadochokinesia Ri > Le	Moderate atrophy FT	Bilat. anteroT ↓, lateroT Ri ↓	Normal $A\beta_{1-42}$, P-tau and T-tau
DR1252	Loss of interest, disinhibition, impulsivity, loss of hygiene, anosognosia, echolalia, verbal stereotypes, attention deficit, executive dysfunction	Atrophy bilat. basoF, F Le > Ri, anteroT Le	AnteroT ↓, Le > Ri	NI
EOD-P51	Apathy, disinhibition, compulsive behavior; later language, memory, and visuospatial alterations	Atrophy FT	NI	NI
EOD-P52	Attention deficit, language alteration, delusions	Atrophy FP Le > Ri	T and anteroF Le ↓	NI
EOD-P53	Apathy, abulia; parkinonism	Bilat. atrophy T and F; severe supratentorial leukoaraiosis	Bilat. FT and P ↓	↓$A\beta_{1-42}$, normal P-tau and T-tau
EOD-P54	Apathy, loss of interest, attention deficit, executive dysfunction	Atrophy FT	NI	NI
EOD-P55	NI	NI	NI	NI
EOD-P56	Memory complaints, decreased orientation, word retrieval deficits, depression (at onset), epileptic seizures	("compatible with FTD")	Bilateral F and lateroT ↓, Le > Ri	NI
EOD-P57	Apathy, loss of interest, anomic aphasia, verbal stereotypes, mild constructive apraxia, depression	Atrophy bilat. F (pred. supero- and basoF), DLPFC Le > Ri, anteroT and insulae Le > Ri; leukoaraiosis	LateroT Le, basoF, anterior cingulate and DLPFC ↓	NI
EOD-P58	Word retrieval deficits, dysgraphia, memory complaints, executive dysfunction, impulsivity, inertia, loss of empathy	No significant changes	No significant changes	NI
EOD-P59	NI	NI	NI	NI

Key: bilat, bilateral(ly); CSCA, corticosubcortical atrophy; CSF, cerebrospinal fluid; F, frontal; Le, left; NI, no information; O, occipital; P, parietal; pred., predominant; Ri, right; T, temporal; DLPFC, dorsolateral prefontral cortex; CT, computed tomography; MRI, magnetic resonance imaging; SPECT, single-photon emission computed tomography; FDG-PET, fluorodeoxyglucose-positron emission tomography.
[a] CT or MRI.
[b] SPECT or FDG-PET.

with FTD. In 3 patients, leukoaraiosis was present, potentially contributing to the cognitive decline.

4. Discussion

In this study, we investigated whether genetic variation in *SORT1* might contribute to the genetic etiology of FTD by sequencing the complete coding region of *SORT1* in independent patient/control cohorts. We identified multiple rare non-synonymous variants that were present in either patients only, controls only, or both patients and controls. A burden analysis collapsing all rare variants across the protein showed an excess of coding *SORT1* variants in patients compared to controls in both the Belgian ($p = 0.04$) and Spanish ($p = 0.02$) cohorts. This association was largely driven by variants located in the β-propeller domain of SORT1 in both populations (Belgium $p = 0.04$ and Spain $p = 0.02$). No significant enrichment of rare nonsynonymous variants could be observed in the Italian ($p = 0.51$) and Portuguese ($p = 0.80$) patient/control cohorts. A possible explanation for the lack of replication in these 2 populations might be population specificity of our associated variants. Recently, multiple studies on rare variant analysis showed the difficulty of replicating their findings in different populations, which led them to conclude that rare variants are rather private compared to common variants (Dopazo et al., 2016; Gaastra et al., 2016).

SORT1 is composed of 3 protein domains, a β-propeller, a 10 CC module, and a transmembrane domain (Fig. 1). The propeller region is composed of 10 blades and forms a large tunnel in which the ligands, including GRN, can bind (Zheng et al., 2011). The 10 CC module comprises 10 cysteine residues that form 5 disulfide bonds. Based on the crystal structure of SORT1, it is believed that the 10 CC module is important for the stabilization of the β-propeller by interacting with its outer surface (Quistgaard et al., 2009).

Of the 11 patient-only variants in *SORT1*, 7 received a pathogenicity CADD_Phred score above 20, indicating that these rare variants are among the 1% most deleterious substitutions in the human genome (Kircher et al., 2014). Furthermore, 7 patient-only variants are located in the β-propeller, 2 in the 10 CC module, and another 2 in the transmembrane domain. Considering the functions of these domains, one can speculate that the mutations in the β-propeller domain have an effect on ligand binding and those located in the 10 CC module and transmembrane domain on protein stability. Two of the variants (p.Lys302Glu and p.Gly310Ala) are located in the predicted binding site for GRN (Fig. 1). Based on their localization, it can be hypothesized that these variants might abolish binding between GRN and SORT1. However, a functional follow-up study will be needed to confirm these hypotheses.

Previous reports have shown genome-wide association between common variants, located in the proximity of *SORT1*, and GRN plasma levels in control persons, FTD patients and patients carrying

a *GRN* mutation (Carrasquillo et al., 2010). In addition, using an unbiased ligand binding assay, SORT1 was identified as a neuronal receptor for GRN (Hu et al., 2010). It was shown that, in a stressed central nervous system, GRN is secreted by activated microglia and undergoes rapid endocytosis upon binding to SORT1, which is expressed on the neuronal cell surface. These findings make SORT1 one of the most important neuronal receptors to regulate extracellular GRN levels (Hu et al., 2010). However, it was shown that the neurotrophic effects of GRN are not dependent on the binding between GRN and SORT1, indicating that SORT1 may not be the only neuronal receptor for GRN (De Muynck et al., 2013; Gass et al., 2012).

Here, we reported for the first time a genetic association of rare nonsynonymous variants in *SORT1* in FTD patients. Because of the link between GRN and SORT1 and because neuropathology of FTD patients with a *GRN* mutation is consistent with FTLD-TDP, these *SORT1* mutation carriers are more likely to also have neuropathological findings consistent with FTLD-TDP. Unfortunately, we could not confirm this hypothesis because autopsy brain was not available for any of the patients carrying a *SORT1* variant. Nevertheless, none of the rare nonsynonymous *SORT1* variants in this study was identified in *MAPT* mutation carriers or FTLD-tau patients, which supports this hypothesis. In conclusion, our data implicate that *SORT1* contributes to the genetic etiology of FTD with rare nonsynonymous variants increasing risk for FTD potentially by interfering with the binding of GRN to its receptor. Additional genetic studies of *SORT1* in FTD patient cohorts and functional studies of the potential impairment of SORT1 receptor activities by genetic variations are essential to understand the underlying biological mechanism of increased risk for FTD.

Disclosure statement

The authors declare that they have no conflicts of interest.

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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.2018.02.011.

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