Loss of *TBK1* is a frequent cause of frontotemporal dementia in a Belgian cohort

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Supplemental data at Neurology.org

ABSTRACT

Objective: To assess the genetic contribution of *TBK1*, a gene implicated in amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and FTD-ALS, in Belgian FTD and ALS patient cohorts containing a significant part of genetically unresolved patients.

Methods: We sequenced *TBK1* in a hospital-based cohort of 482 unrelated patients with FTD and FTD-ALS and 147 patients with ALS and an extended Belgian FTD-ALS family DR158. We followed up mutation carriers by segregation studies, transcript and protein expression analysis, and immunohistochemistry.

Results: We identified 11 patients carrying a loss-of-function (LOF) mutation resulting in an overall mutation frequency of 1.7% (11/629), 1.1% in patients with FTD (5/460), 3.4% in patients with ALS (5/147), and 4.5% in patients with FTD-ALS (1/22). We found 1 LOF mutation, p.Glu643del, in 6 unrelated patients segregating with disease in family DR158. Of 2 mutation carriers, brain and spinal cord was characterized by TDP-43-positive pathology. The LOF mutations including the p.Glu643del mutation led to loss of transcript or protein in blood and brain.

Conclusions: *TBK1* LOF mutations are the third most frequent cause of clinical FTD in the Belgian clinically based patient cohort, after *C9orf72* and *GRN*, and the second most common cause of clinical ALS after *C9orf72*. These findings reinforce that FTD and ALS belong to the same disease continuum. ***Neurology*® 2015;85:2116–2125**

GLOSSARY

ALS = amyotrophic lateral sclerosis; **bvFTD** = behavioral variant frontotemporal dementia; **FTD** = frontotemporal dementia; **FTLD** = frontotemporal lobar degeneration; **KD** = kinase domain; **LOF** = loss of function; **NCI** = neuronal cytoplasmic inclusions; **Ser172** = serine 172.

Frontotemporal lobar degeneration (FTLD) is a heterogeneous neurodegenerative disorder associated with amyotrophic lateral sclerosis (ALS) in approximately 10%–15% of patients with frontotemporal dementia (FTD).[1] Evidence that common disease pathways are involved in FTD and ALS stems from the observation of families and individual patients in which both diseases occur (FTD-ALS),[1] and the TDP-43 inclusions in both patient groups.[2]

Nearly 50% of FTD cases and 10% of ALS cases aggregate in families, suggesting a strong genetic component. The most convincing genetic evidence for a common pathomechanism is provided by the repeat expansion mutations in *C9orf72* in patients with FTD, ALS, and FTD-ALS.[3–5] Recently, *TBK1* loss-of-function (LOF) mutations were identified in patients with ALS[6,7] and FTLD-TDP.[8]

In the Belgian FTD cohort, mutations in known genes accounted for 30% of familial FTD and 75% of familial FTD-ALS, with several families with autosomal dominant inheritance

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Belgium Neurology (BELNEU) consortium members are listed on the *Neurology*® Web site at Neurology.org.

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remaining genetically unresolved.[5,9] Here, we investigated the genetic role of *TBK1* in a Belgian cohort of 629 patients with FTD, FTD-ALS, and ALS.

METHODS

Subjects. Our study population consisted of 482 patients with FTD, 22 of whom had concomitant ALS (FTD-ALS), and 147 patients with ALS ascertained in Belgium through an ongoing multicenter collaboration of neurology departments and memory clinics partnering in the Belgian Neurology (BELNEU) consortium. Additional patients were included who had initially been referred to the Diagnostic Service Facility for medical genetic testing. Patients were diagnosed using a standard protocol and established clinical criteria.[10–12] Postmortem neuropathologic analysis confirmed diagnosis in 25 patients with FTD, 3 patients with FTD-ALS, and 6 patients with ALS. A positive familial history, i.e., at least one first-degree relative with a FTD-ALS spectrum disease, was recorded in 132 patients with FTD (28.7%), 4 patients with FTD-ALS (36.4%), and 18 patients with ALS (12.2%). Of these familial index patients, 93 cases of FTD (70.5%), 1 case of FTD-ALS (25.0%) and 7 cases of ALS (38.9%) were not explained by mutations in the known FTLD and ALS genes (*MAPT*, *GRN*, *C9orf72*, *VCP*, *CHMP2B*, *FUS*, *TARDBP*, *SOD1*), in the AD genes (*PSEN1*, *PSEN2*, *APP*), or in the prion gene (*PRNP*). One patient with unexplained FTD-ALS was the index patient of a 4-generation family (family DR158), of which we collected genomic DNA and generated lymphoblast cell lines of 38 individuals including 4 patients in generation III (figure 1 and table 1). A Belgian control cohort of 1,044 persons free of personal and familial history of neurodegenerative or psychiatric diseases and with a Mini-Mental State Examination score >26 was also analyzed.

Standard protocol approvals, registrations, and patient consents. All participants provided written informed consent for participation in clinical, pathologic, and genetic studies. Clinical study protocols and informed consent forms were approved by the local medical ethics committees of the collaborating clinical centers. Genetics study protocol and informed consent forms were approved by the medical ethics committees of the University Hospital Antwerp and the University of Antwerp.

Experimental procedures. Nineteen coding *TBK1* exons were amplified in multiplex PCR reactions using MASTR technology (http://www.multiplicom.com) and sequenced on a MiSeq platform (Illumina; San Diego, CA) and 1 exon was analyzed by Sanger sequencing. Identified variants were validated and relatives of mutation carriers were analyzed using Sanger sequencing.

TBK1 transcripts were measured in lymphoblast cells of 8 mutation carriers and frontal cortex of 2 carriers using real-time PCR amplification of a *TBK1*amplicon, quantified against 2 housekeeping genes. We sequenced real-time PCR products to establish the transcribed alleles based on the coding mutation or the coding polymorphism rs7486100. We used western blotting of protein lysates of lymphoblast cells and brain with a monoclonal antibody against TBK1 and quantified against GAPDH. We performed neuropathologic analysis of brain and spinal cord of 2 *TBK1* mutation carriers.

Appendix e-1 on the *Neurology*® Web site at Neurology.org contains further technical details.

RESULTS

***TBK1* mutation analysis and the effect on transcript and protein expression.** We screened the coding region of *TBK1* and identified 2 frameshift, 1 nonsense, 1 splice site mutation, and 2 single amino acid deletions in 11 unrelated index patients (table 1, table e-1, and figure e-1). Further, we identified 5 missense mutations in 5 patients. Copy number variation analysis of all *TBK1* exons did not reveal exonic or whole gene deletions or duplications.

We predicted that the nonsense and frameshift mutations resulted in a premature termination codon leading to mRNA degradation by nonsense-mediated decay. cDNA sequencing of *TBK1* in lymphoblast cells of the Ser398Profs*11 carrier, and in brain of the p.Ser518Leufs*32 carrier, did not identify the mutant allele, suggesting a complete loss of the mutant transcript. Quantification of *TBK1* transcripts demonstrated a highly reduced gene expression that was restored to normal levels using an inhibitor of protein synthesis (figure 2). Western blot analysis demonstrated near 50% reduction of protein expression in lymphoblast cells and in brain (figure 3).

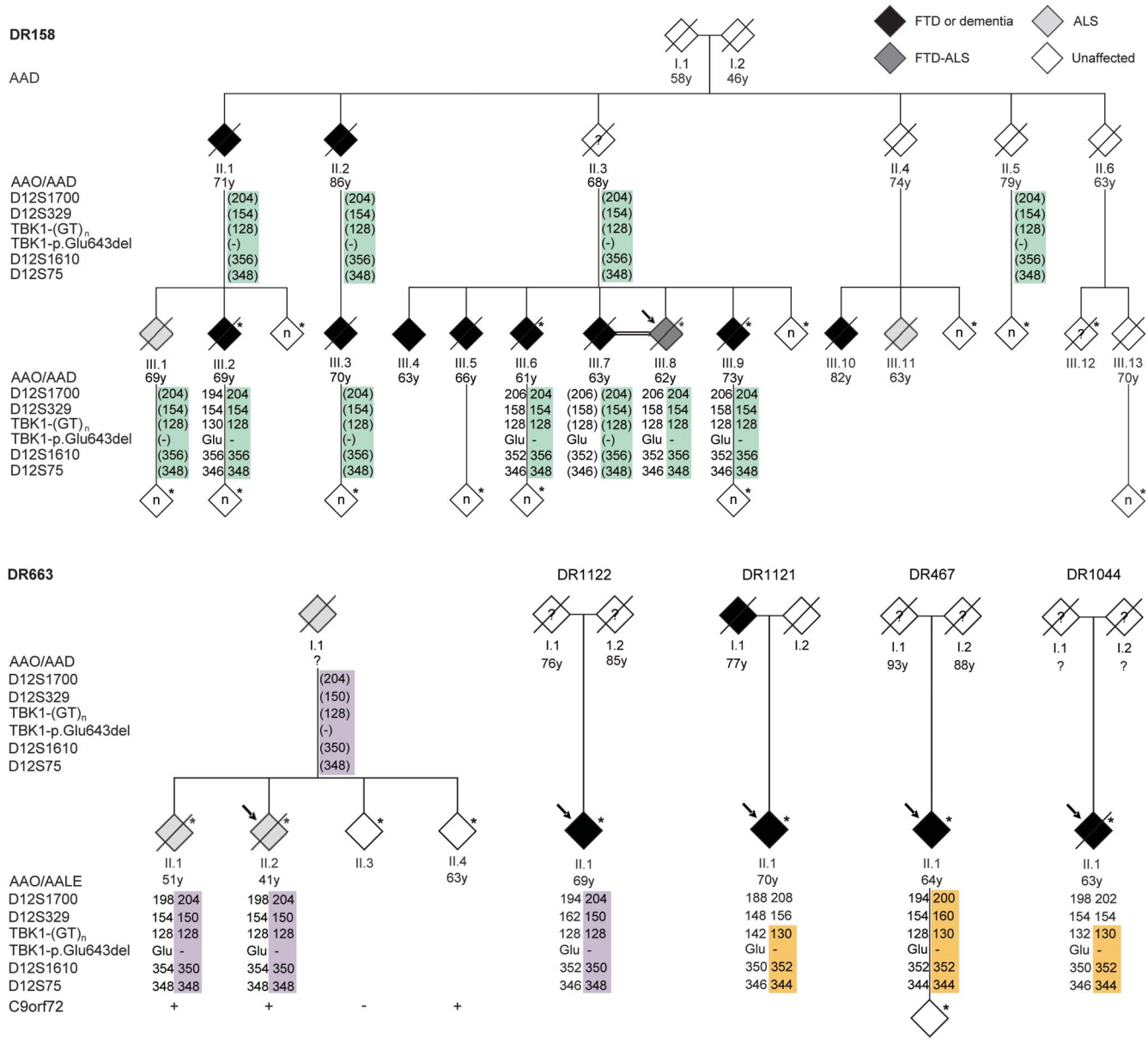
In DR189, a mutation affecting the intron 8 splice donor site resulted in in-frame skipping of exon 8 in lymphoblast cells and brain. In addition, use of a cryptic splice site in exon 8 resulted in an out-of-frame transcript (figure 2). Quantitative PCR analysis showed 30% reduced expression in lymphoblast cells and 50% reduced expression in brain (figure 2). Western blot analysis detected only the normal-sized protein band with 45% reduced expression in lymphoblast cells and 34% in brain lysates, suggesting that no stable protein was produced from the mutant transcripts (figure 3).

In lymphoblast cells of patients carrying the in-frame deletion mutations, p.Asp167del and p.Glu643del (n = 5), *TBK1* transcript expression was not reduced. Also, cDNA sequencing showed the presence of both alleles. However, we observed a significantly reduced protein level of 44% in the p.Glu643del carriers ($p = 0.0017$). In the p.Asp167del carrier, protein expression was not altered (figure 3).

The 5 missense mutations were scattered over the 4 functional domains (table e-1 and figure e-1). Three missense mutations—p.Arg271Leu, p.His322Tyr, and p.Ile515Thr—were absent from 1,044 control individuals. We observed p.Ala535Thr in 1 male control (age at inclusion 63 years), and p.Lys291Glu in a male control and a female control (age at inclusion 67 and 69 years). Two mutations, p.Arg271Leu and p.Ala535Thr, were predicted neutral, while the other 3 mutations received variable effects using 3 prediction algorithms. Missense mutation carriers did not show reduced transcript and protein expression levels (figure e-2).

DR158 family, segregation, and sharing of p.Glu643del. Six unrelated index patients carried the p.Glu643del mutation (table 1), among them index patient III-8 of autosomal dominant FTD-ALS family DR158,

Figure 1 Pedigrees show segregation and disease haplotype sharing in patients and families with the TBK1 mutation p.Glu643del



To protect the privacy of the participants, we masked the sex of each person, scrambled the pedigree of family DR158, and did not specify the number of at-risk individuals tested shown in the white diamonds. The index patient is indicated with an arrow. Filled symbols represent patients, with their age at onset (AAO) in years below their symbol. Age at death (AAD) is shown for individuals who died in old age without symptoms. In family DR663, 3 of the 4 children also carry a repeat expansion mutation in *C9orf72*, 2 affected (I-1 and II-2) and 1 asymptomatic (II-4), for whom the age at last examination (AALE) is given. An asterisk identifies the family members for whom genomic DNA was available. The Glu643del mutation is shown as "–." The 5 polymorphic markers, including the TBK1-$(GT)_n$ repeat polymorphism located 400 kb upstream of *TBK1*, are indicated at the left and flank the *TBK1* mutation p.Glu643del at both sites. Genotypes of additional polymorphic markers can be found in table e-3. The 3 different disease haplotypes are shown with colored bars: green haplotype of at least 7.5 Mb, purple haplotype of at least 8.7 Mb, and orange haplotype of at least 3.0 Mb. Haplotypes based on segregation data are indicated in regular font, those based on allele sharing are in italics, and those that were inferred are between brackets. ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia.

containing 13 patients (table 1 and figure 1). Ten patients of family DR158 were diagnosed with a dementia syndrome, i.e., FTD (n = 4) or unspecified dementia (n = 6), while 2 patients had a diagnosis of ALS. The mean onset age was 69.1 ± 7.7 years, with a mean disease duration of 6.4 ± 3.9 years. The 5 patients with FTD or FTD-ALS presented with bvFTD (table e-2). Remarkably, memory loss and disorientation were present relatively early in the disease in most of the p.Glu643del mutation carriers (table e-2). However, most of these patients also developed early behavioral problems. One patient with ALS (III-1) and 1 patient with FTD-ALS (III-8) presented with ALS with spinal onset (table e-2), both showing signs of motor neuron disease on EMG. Analysis of

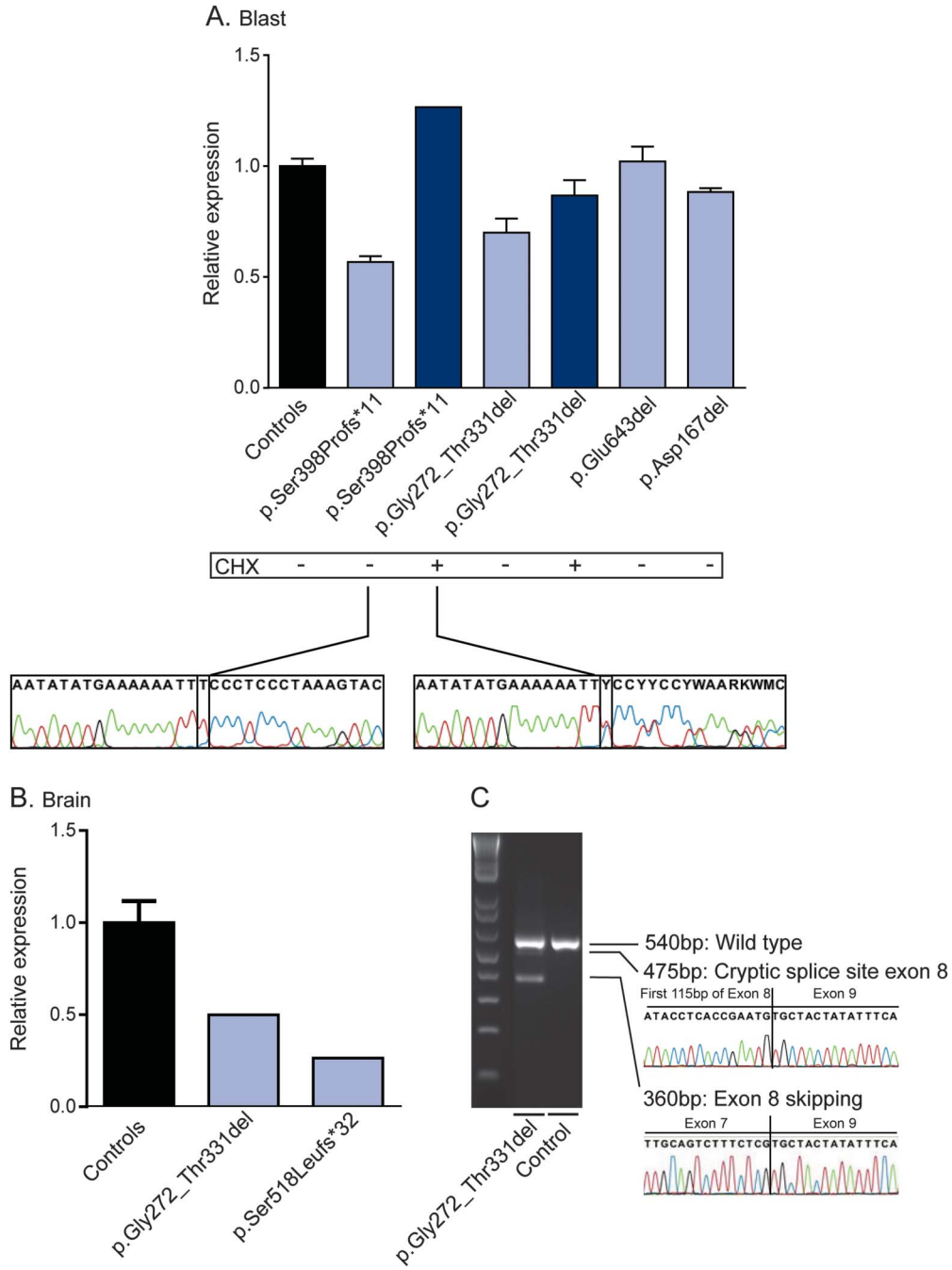
Table 1 Clinical characteristics of patients of family DR158 and *TBK1* mutation carriers

Patient ID	Sex	AAO, y	AAD, y	DD, y	Clinical diagnosis	Family history	Predicted protein[a]
Family DR158							
II-1	F	71	81	10	D	F	p.Glu643del
II-2	F	86	90	4	D	F	p.Glu643del
III-1	M	69	72	3	ALS	F	p.Glu643del
III-2	M	69	75	6	FTD	F	p.Glu643del
III-3	M	70	73	3	D	F	p.Glu643del
III-4	M	63	—	>3	FTD	F	NA
III-5	F	66	71	6	FTD	F	NA
III-6	F	61	74	13	FTD	F	p.Glu643del
III-7	F	63	69	6	D	F	p.Glu643del
III-8[b]	F	62	74	11	FTD-ALS	F	p.Glu643del
III-9	F	73	84	11	D	F	p.Glu643del
III-10	F	82	86	4	D	F	NA
III-11	M	63	64	1	ALS	F	NA
Unrelated index patients							
DR1120	F	56	60	4	FTD	U	p.Gln2*
DR1127	M	60	61	1	ALS	S	p.Asp167del
DR189	M	48	50	2	FTLD[c]	F	p.Gly272_Thr331del
DR1123	M	59	—	>5	ALS	U	p.Ser398Profs*11
DR1124	F	64	64	<1	ALS[c]	U	p.Ser518Leufs*32
DR158	F	62	74	11	FTD-ALS	F	p.Glu643del
DR467	F	64	—	>9	FTD	F	p.Glu643del
DR1121	M	70	—	>6	FTD	F	p.Glu643del
DR1122	F	69	—	>7	FTD	S	p.Glu643del
DR1044	M	63	66	3	ALS	S	p.Glu643del
DR663	M	41	41	<1	ALS	F	p.Glu643del
DR1132	M	80	87	7	FTD	F	p.Arg271Leu
DR609	M	52	62	10	FTD	F	p.Ala535Thr
DR1133	M	60	64	4	FTD	F	p.Lys291Glu
DR1134	M	64	66	2	ALS	S	p.His322Tyr
DR1135	F	59	—	>10	ALS	U	p.Ile515Thr

Abbreviations: AAD = age at death; AAO = age at onset; ALS = amyotrophic lateral sclerosis; D = dementia; DD = disease duration; F = familial; FTD = frontotemporal dementia; FTLD = frontotemporal lobar degeneration; NA = not analyzed; S = sporadic; U = unknown.
[a] Protein numbering according to NP_037386.1 isoform.
[b] Index patient of family DR158.
[c] TDP-43-positive pathology in brain or spinal cord.

all family members indicated that the disease segregated with the p.Glu643del mutation on a disease haplotype of minimal 7.5 Mb (figure 1). Seven mutation carriers were without symptoms at age ≥70 years, including obligate carrier II-5 and 2 children of II-5 who, at their last clinical evaluation, were 79, 81, and 82 years of age, respectively (figure 1). Given the frequent occurrence of psychiatric disease in family DR158, it cannot be excluded that symptoms of FTD were not recognized (table e-2). Carrier II-5 and one of her children with a current age of 81 years had repeated depression. Carrier II-3 died at age 68 years and was diagnosed with schizophrenia. One of the asymptomatic children of patient II-1 had alcohol abuse and has a current age of 70 years. Among the other 5 carriers of the p.Glu643del mutation and their relatives, we identified 2 distinct disease haplotypes that differed from that in DR158 (figure 1 and table e-3). In family DR663, ALS patient II-1 and II-2 carried, besides the *TBK1* mutation, a *C9orf72* repeat

Figure 2 Transcript analysis of TBK1 loss-of-function mutations

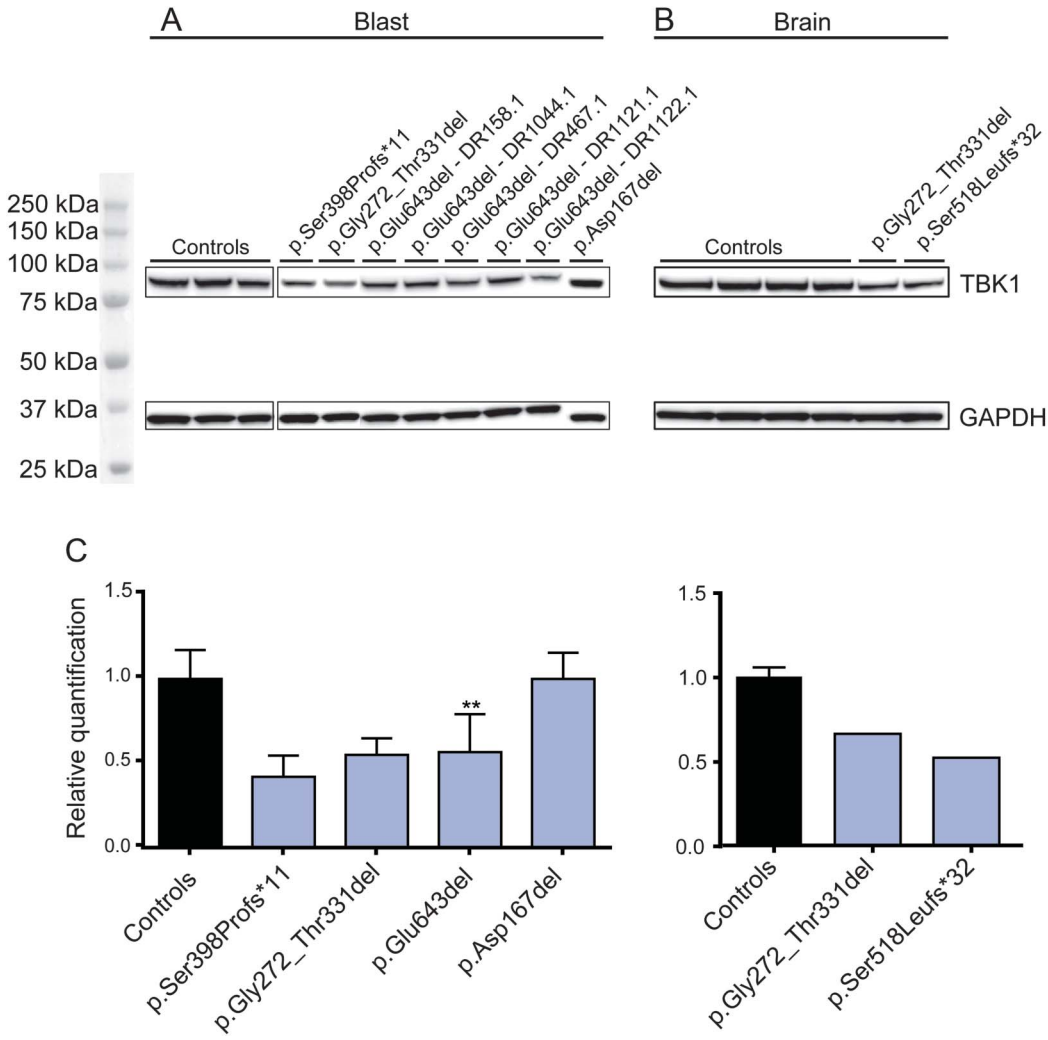


Quantitative PCR quantification of *TBK1* transcripts from lymphoblast cells (A) and frontal cortex (B) of LOF mutation carriers (blue bars) compared to control persons without mutation (black bars) normalized to different housekeeping genes. Five unrelated p.Glu643del mutation carriers were included. Error bars represent the SD. Below the chart is indicated if cells were treated (+, dark blue bars) or not treated (−, light blue bars) with cycloheximide (CHX), a protein synthesis inhibitor. Sequence traces are obtained by real-time PCR from the lymphoblast cells of the p.Ser398Profs*11 mutation carrier treated or not treated with CHX using primers in exon 6 and exon 21. (C) Real-time PCR with primers in exon 6 and 10 of cDNA of brain of the p.Gly272_Thr331del mutation carriers is shown on agarose gel, with the respective sequence traces from the aberrant transcripts.

expansion, as did the asymptomatic sib II-4 without the *TBK1* mutation, age 63 years (figure 1).

Genotype–phenotype correlations. The mean onset age in the 11 patients carrying a LOF mutation (table 1) was 59.6 ± 8.6 years. In 4 p.Glu643del carriers, excluding the 2 patients carrying a *C9orf72* repeat expansion, we calculated a mean onset age of 66.3 ± 3.9 years, which was significantly later ($p = 0.04$) than in the 5 patients with another LOF mutation, with a mean onset age of 57.4 ± 6.0 years. When we included all affected carriers of family DR158, the

Figure 3 Protein analysis of TBK1 loss-of-function mutations



Western blot analysis of protein extracts from lymphoblast cells (A) and frontal cortex (B) of loss-of-function (LOF) mutation carriers compared to control persons without mutation. Of the p.Glu643del mutation, 5 unrelated index patients were included. The upper band represents TBK1 (84 kDa) and the lower band the housekeeping protein GAPDH (37 kDa). The graphs below (C) show the quantification in control samples (black) and patient samples (blue) of the TBK1 signal normalized to the signal of GAPDH. Error bars represent the SD; ** indicates a significant p value of <0.01 compared to controls.

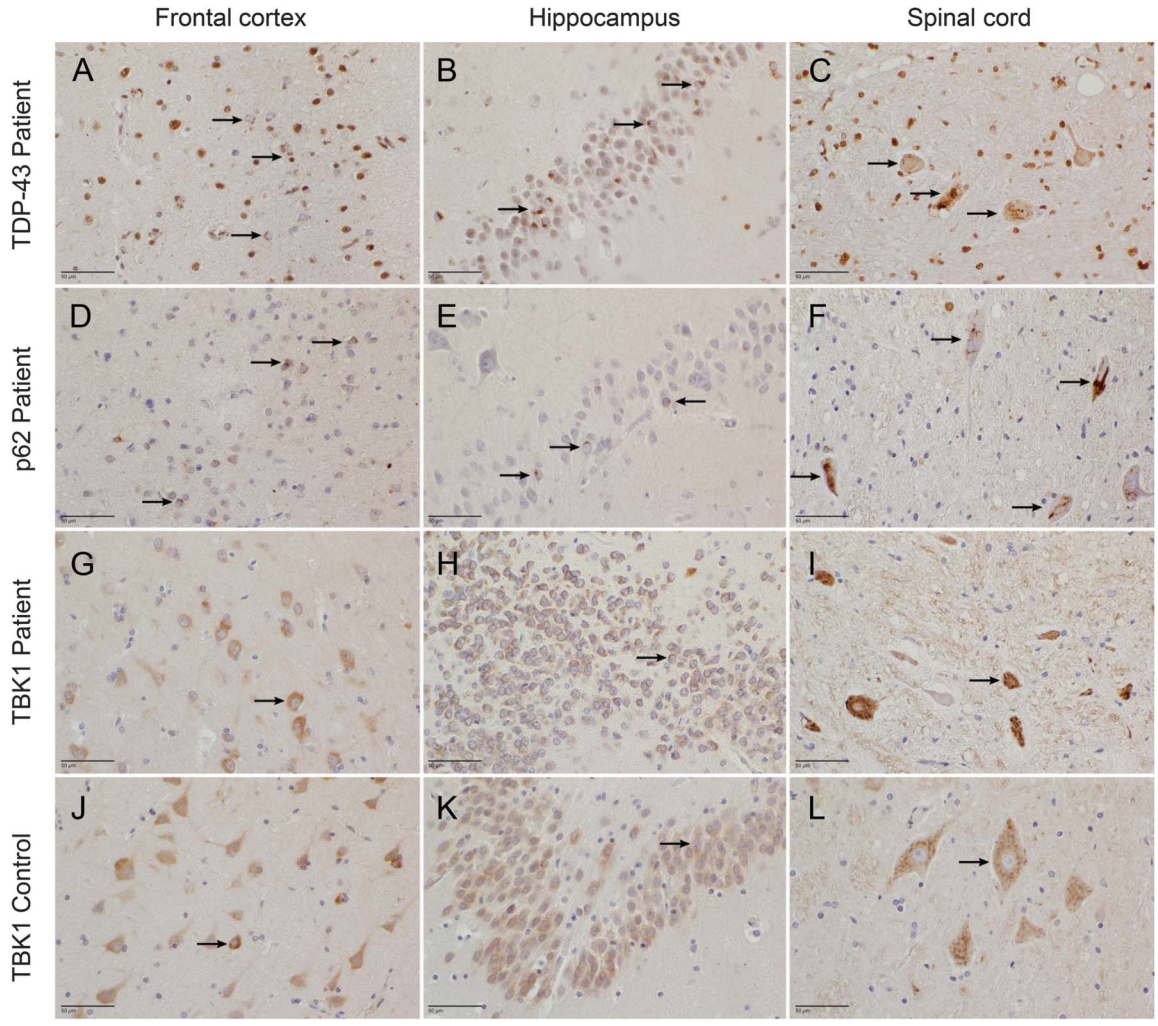
significance became stronger ($p = 0.004$). Also, the mean disease duration of 8.3 ± 1.8 years was significantly longer ($p = 0.016$) than the mean disease duration of 2.6 ± 2.2 years in the patients with another LOF mutation with an increasing effect if all DR158 patients were included ($p = 0.002$). Four of 5 FTD mutation carriers and the patient with FTD-ALS had behavioral variant FTD (bvFTD) (table e-2). The 3 patients with FTD with a p.Glu643del mutation expressed extrapyramidal symptoms, while the patients with FTD with another *TBK1* mutation did not (table e-2). In 1 patient with bvFTD (DR1120), muscular fasciculations were observed in 1 limb (table e-2). The proband of DR158 presented with spinal onset ALS; in the 3 other documented patients with ALS (DR663, DR1123, DR1124), the site of onset was bulbar (table e-2). Of 3 *TBK1* mutation carriers with ALS or FTD-ALS (DR158.III-8, DR1123, DR1124), we had EMG reports, while of 3 other patients with ALS (DR663, DR1044, DR1127), no detailed information was available. Based on a clinical examination, there was no cognitive evidence for FTD in the majority of ALS cases. However, in ALS patient DR1124 memory deficits and the impression of behavioral disinhibition were reported (table e-2), but we did not have sufficient information to determine if the criteria for possible FTLD were fulfilled.

Neuropathologic brain examination of FTD patient DR189 (p.Gly272_Thr331del) showed mild neuronal loss of the frontal cortices. We found a moderate amount of TDP-43 and p62 neuronal cytoplasmic inclusions (NCI) and short dystrophic neurites

but no intraneuronal intranuclear inclusions (figure 4, A, B, D, and E) compatible with a TDP type B proteinopathy.[13] In ALS patient DR1124 (p.Ser518Leufs*32), pathologic signs of lower and upper motor neuron disease were apparent. The TDP-43 positive NCI were mainly restricted to the hypoglossal nucleus and the ventral horn neurons of cervical and thoraco-lumbar spinal cord (figure 4, C, F). Staining with TBK1 showed variable cytoplasmic neuronal immunoreactivity. The Nissl substance of the neurons in the ventral horn of the spinal cord was stained. We did not observe differences between patients and controls (figure 4, G–L).

DISCUSSION The overall *TBK1* LOF mutation frequency is 1.7% (11/629), with 1.1% (5/460) in FTD, 4.5% (1/22) in FTD-ALS, and 3.4% (5/147) in ALS. In patients with FTD, *TBK1* LOF mutations explained 3.2% of the unexplained familial FTD cases and were the third most common genetic cause after *C9orf72* and *GRN* mutations (figure e-3), consistent with the findings in a FTLD-TDP

Figure 4 TDP-43, p62, and TBK1 immunohistochemistry



Immunohistochemistry of frontal cortex (A, D, G) and hippocampus (B, E, H) of 1 patient with frontotemporal lobar degeneration with the p.Gly272_Thr331del mutation (DR189) and spinal cord (C, F, I) of 1 patient with amyotrophic lateral sclerosis (ALS) with the p.Ser518Leufs*32 mutation (DR1124) compared to a control individual without mutation (J–L). In frontotemporal dementia patient DR189, a mild neuronal loss of the frontal cortices and to a lesser extent of the temporal neocortex and the hippocampus was shown. A moderate amount of TDP-43 and p62 neuronal cytoplasmic inclusions (NCI) (arrows) and short dystrophic neurites, but no intraneuronal intranuclear inclusions, were found throughout all layers of the frontal (A, D) and temporal neocortices, in the dentate gyrus of the hippocampus (B, E), the parahippocampal transentorhinal cortex, the neostriatum, and the pallidum, but not in the cerebellum. In ALS patient DR1124, no explicit cortical abnormalities were present, but pathologic signs of lower and upper motor neuron disease were apparent. Severe demyelination of the pyramidal tract in mesencephalon, pons, medulla oblongata, and the entire spinal cord and severe neuronal loss in the hypoglossal nucleus, as well as in the ventral horns of the cervical and thoracic spinal cord, were observed. The TDP-43-positive (C) and p62-positive (F) NCI were mainly restricted to the hypoglossal nucleus and the ventral horn neurons of cervical and thoraco-lumbar spinal cord (arrows). Cytoplasmic TBK1 immunoreactivity (arrows) in cortical neurons of the frontal cortex (G, J), in the dentate gyrus of the hippocampus (H, K), and in the cervical ventral horn neurons (I, L) showed no differences between patients and controls. The TDP-43-positive inclusions were not stained with TBK1.

cohort.[8] In the ALS cohort, *TBK1* mutations were the second most common after *C9orf72* mutations (figure e-3).

All described LOF mutations, except 1, are different from those in the other published reports. The p.Glu643del mutation that was present in 6/629 (1%) unrelated Belgian patients was also found in 2/1,010 German patients with sporadic ALS.[7] We showed segregation of the p.Glu643del mutation with disease. The presence of 3 different disease haplotypes of at least 3 Mb among the p.Glu643del carriers might be explained by a mutation hotspot due to a repetitive $(GAA)_3$ sequence encoding 3 Glu residues (codons 641–643) or because of an extreme distant common founder. Expression studies in lymphoblast cells and in brain of 3 LOF mutation carriers confirmed the loss of transcript and of protein leading to 50% reduced protein levels, as previously reported.[7,8] A novel finding is that the p.Glu643del mutation had no effect on transcript levels but resulted in a near 50% reduced protein expression in blood, possibly due to loss of protein stability. The p.Asp167del mutation and the missense mutations did not show reduced transcript or protein levels, but we cannot exclude their pathogenicity by mechanisms like reduced kinase activity or hampered protein dimerization. TBK1 has a 4-domain structure with an N-terminal kinase domain (KD), a ubiquitin-like domain, an α-helical scaffold dimerization domain, and a C-terminal domain[14] (figure e-1). The correct interplay among these domains is essential for proper kinase function. TBK1 is activated by trans-autophosphorylation of serine 172 (Ser172), for which homodimerization is required.[15,16] Ser172 is located in a kinase activation loop of the KD starting at the conserved DFG motif (residues 157–159) and extending to residue 198.[15] Interestingly, the p.Asp167del mutation is located in this activation loop and hence might hamper the conversion of TBK1 to the active state. In other *TBK1* reports, some missense mutations were found to be pathogenic.[7,8] Since at least one of the used prediction algorithms predicted that some of our missense mutations are pathogenic, we expect that some might hamper proper protein functioning. Further experiments are necessary to confirm these predictions.

Clinical heterogeneity among the *TBK1* mutation carriers was apparent since all different phenotypes of the FTD-ALS spectrum and a wide range in age at onset and disease duration were present, comparable to what was observed in *C9orf72* expansion carriers.[17] However, remarkably, 5 of 6 FTD or FTD-ALS index patients with a *TBK1* mutation presented with bvFTD. The later onset age and longer disease duration associated with the p.Glu643del mutation suggested that this mutation has a milder pathogenic effect. Since amnestic deficits and orientation problems were apparent early in the disease process, it might be interesting to screen *TBK1* in patients with clinically diagnosed AD, also since in another study 2 of 4 patients with FTLD-TDP with a *TBK1* mutation presented with clinical AD.[8] In ALS family DR663, we found both the *TBK1* p.Glu643del mutation and a *C9orf72* repeat expansion (>80 repeat units) segregating with disease. The 2 sibs who carried both mutations had a remarkably younger onset age (41 and 51 years) and a shorter disease duration (<1 and 2 years) compared to the other p.Glu643del mutation carriers. Also, one other index patient of the ALS cohort, DR1044, carried both the *TBK1* p.Glu643del mutation and a *C9orf72* repeat expansion of only 59 repeat units. This patient had later onset age of 63 years, corroborating our recent findings that patients with a short repeat expansion (45–80 units) have a later age at onset compared to patients with a longer expansion.[18] Further, the disease penetrance in p.Glu643del mutation is highly variable, with 7 carriers of family DR158 still unaffected at age ≥70 years, with 2 at age 81 and 82 years, nearly 2 SDs beyond the mean onset age. The 7 unaffected carriers were not homozygous for the minor protective allele of *TMEM106B* rs1990622.[19] The observed TDP-43 type B pathology in brain or spinal cord could be distinguished from that in patients with a *C9orf72* mutation by the absence of p62-positive inclusions containing dipeptide repeats.[20,21] This pathology was previously reported in 1 patient with FTD-ALS[7] while another study reported FTLD-TDP type A in 3 mutation carriers.[8]

The observation that *TBK1* mutations were present in a significant part of sporadic and late-onset patients is not uncommon in adult-onset neurodegenerative diseases because of reduced penetrance, similar to what is described for *C9orf72* mutations and to a lesser extent for *GRN* mutations.[9] A relatively high de novo mutation rate may be another explanation, supported by the observation that the p.Glu643del mutation occurred on 3 different haplotype backgrounds. Also, as proposed by others,[8] more than 1 gene might be involved in some patients, as observed in family DR663 segregating both a *TBK1* and a *C9orf72* mutation.

TBK1 is an important serine/threonine kinase of the IKK family phosphorylating a wide range of substrates involved in several cellular processes, including innate immune response/inflammation,[22] autophagy,[23,24] and cell proliferation.[25] Substrates of TBK1 include optineurin (*OPTN*),[26,27] another gene with LOF mutations in ALS,[28] and p62, a major component of pathologic depositions and showing mutations in FTLD and ALS.[29,30] p62 and OPTN are 2

autophagic adapters controlling protein degradation by selective autophagy.[31,32] Moreover, TBK1 targets the VPS37C protein of the endosomal sorting complex required for transport-I,[33] thereby regulating the vesicular retroviral budding system, which is also involved in neurodegeneration, e.g., CHMP2B.[34] A dysfunctional vesicular transport system might in turn induce autophagy defects.[35] As other FTD/ALS genes, including VCP,[36] are also involved in autophagy, and since mutations in *OPTN* and *TBK1* are also involved in glaucoma,[37,38] our findings emphasize the major role of autophagic defects in neurodegeneration.[39] Also, in the antiviral innate immune response both TBK1 and OPTN are involved through the NF-KB complex pathway.[40]

In this study, we demonstrated that LOF mutations in *TBK1* are associated with FTD-ALS spectrum disorders in a Belgian clinical patient cohort, can segregate in families according to an autosomal dominant pattern, and are present in a significant part of sporadic cases. The identification of *TBK1* emphasizes the convergence of FTD and ALS in 1 continuum and will accelerate effective drug development.

AUTHOR CONTRIBUTIONS

Ilse Gijselinck: literature search, figures, study design, genetic data collection, data analysis, data interpretation, writing. Sara Van Mossevelde: patient samples collection, clinical data collection, data analysis, data interpretation, writing. Julie van der Zee: patient data collection, genetic data collection, data analysis, data interpretation. Anne Sieben: patient samples collection, clinical data collection, neuropathology data collection, figures, data analysis, data interpretation, writing. Stéphanie Philtjens: genetic data collection, data analysis. Bavo Heeman: literature search, experimental data collection, data analysis, data interpretation. Sebastiaan Engelborghs: patient samples collection, clinical data collection, data analysis, data interpretation. Mathieu Vandenbulcke: patient samples collection, clinical data collection, data analysis, data interpretation. Greet De Baets: literature search, figures, protein structure data collection, data analysis, data interpretation. Veerle Bäumer: data collection, data analysis, data interpretation. Ivy Cuijt: genetic data collection, data analysis. Marleen Van den Broeck: genetic data collection, data analysis, data interpretation. Karin Peeters: patient samples collection, genealogy data collection, data analysis. Maria Mattheijssens: patient samples collection, genealogy data collection, data analysis. Frederic Rousseau: data analysis, data interpretation. Rik Vandenberghe: patient samples collection, clinical data collection, data analysis, data interpretation. Peter De Jonghe: patient samples collection, clinical data collection, data analysis, data interpretation. Patrick Cras: patient samples collection, clinical data collection, data analysis, data interpretation. Peter P. De Deyn: patient samples collection, clinical data collection, data analysis, data interpretation. Jean-Jacques Martin: patient samples collection, neuropathology data collection, data analysis, data interpretation, writing. Marc Cruts: literature search, study design, data interpretation, writing, study supervision. Christine Van Broeckhoven: literature search, figures, study design, genetic data collection, genealogy data collection, data analysis, data interpretation, writing, study supervision.

ACKNOWLEDGMENT

The authors thank the neurologists Marie-Claire De Poorter (AZ Oudenaarde), Frederik Clement (AZ Roeselare), and Marc Bruyland (AZ Glorieux Ronse) for their contribution to the clinical evaluation of patients, the personnel of the Genetic Service Facility of the VIB Department of Molecular Genetics (http://www.vibgeneticservicefacility.be), and the Antwerp Biobank of the Institute Born-Bunge for their support.

STUDY FUNDING

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The research was funded in part by the Belgian Science Policy Office Interuniversity program; the Flemish government-supported European Initiative on Centers of Excellence in Neurodegeneration (CoEN); the Flemish government-initiated Methusalem excellence program; the Flemish government-initiated Flanders Impulse Program on Networks for Dementia Research (VIND); the Alzheimer Research Foundation (SAO-FRA); the Medical Foundation Queen Elisabeth (QEMF); the Research Foundation Flanders (FWO); the Agency for Innovation by Science and Technology Flanders (IWT) and the University of Antwerp Research Fund, Belgium; and the MetLife Foundation Award for Medical Research to C.V.B. The FWO provided a PhD fellowship to S.P. and a postdoctoral fellowship to I.G. The Article Processing Charge was paid by the Flanders Impulse Program on Networks for Dementia Research (VIND).

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received May 19, 2015. Accepted in final form August 18, 2015.

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Loss of *TBK1* is a frequent cause of frontotemporal dementia in a Belgian cohort

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Neurology 2015;85;2116-2125 Published Online before print November 18, 2015
DOI 10.1212/WNL.0000000000002220