Clinical features of *TBK1* carriers compared with *C9orf72*, *GRN* and nonmutation carriers in a Belgian patient cohort.

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SUPPLEMENTARY MATERIAL

Clinical evaluation and technical investigations

Clinical evaluation was performed by the treating neurologist. Neuropsychological testing was performed by a trained and experienced neuropsychologist affiliated with the neurological department where the patient was treated. All the available medical records were reviewed and clinical characteristics were described in a standardized way. Age at onset was defined as the age at which first clinical symptoms were reported by hetero-anamnesis. Disease duration was measured from onset of clinical symptoms until death.

Neuroimaging was performed in the center where the patient was treated.
Interpretation of images was done by the treating neurologist and the radiologist or nuclear radiologist of the center where neuroimaging was performed. The FDG PET images were stereotactically realigned to a normalized FDG template in commercial MIMVISTA software. The voxel values (in Standardized Uptake value (SUV)) were then activity normalized to the mean gray matter value in the brain, and therefore represent relative glucose metabolism. The figures are stereotactic surface projections, with values in (negative) standard deviations from normality (z-scores). The Z maps of the FDG were calculated by comparing the individual glucose metabolic pattern to the normal age-matched control database using MIMVISTA, with a range between 50-80 years. The PET images were not corrected for brain volume loss and therefore reflect the combined effect of volume loss and hypometabolism. However, the particular method of analysis and display in a stereotactic surface projection method with a radial ray tracing approach diminishes any atrophy effects as it takes into consideration the maximum pixel value in a radial line of view, which is not as much influenced by cortical thickness (Burdette *et al.*, 1996).When the images were available, we reviewed them as well.

Cerebrospinal fluid biomarker profiles were analyzed at the Central Laboratory, UZ Leuven or at BIODEM, IBB, University of Antwerp, as previously described (Engelborghs *et al.*, 2008).

Massive parallel sequencing of the coding region of *TBK1*

19 coding exons of *TBK1* were amplified in multiplex PCR reactions using the Multiplex Amplification of Specific Targets for Resequencing (MASTR) (Multiplicom N.V., Niel, Belgium) technology. Primers for multiplexing were designed using the mpcr primer design tool (Multiplicom N.V.). Targets were amplified in highly multiplexed PCR reactions and unique dual indices were added to these amplicons, resulting in up to 1536 uniquely barcoded samples (Lange *et al.*, 2014). After equimolar pooling, the barcoded samples were sequenced on the MiSeq platform using the MiSeq V2 chemistry (250 bp paired-end reads, Illumina, San Diego, CA, USA). After sample demultiplexing, sequence reads were mapped using the Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2010) to a minigenome consisting of the combined amplicon sequences extracted from the human genome reference sequence hg19. Sequence variants were called using the Genome Analysis Toolkit (GATK) (DePristo *et al.*, 2011; McKenna *et al.*, 2010) and SAM (Sequence Alignment/Map) tools (Li *et al.*, 2009) annotated using the GenomeComb variant annotation pipeline (Reumers *et al.*, 2011) and visualized in IGV (Robinson *et al.*, 2011; Thorvaldsdóttir *et al.*, 2013). Exon 4, which failed in this setup, was PCR amplified in simplex followed by Sanger sequencing using the BigDye® Terminator Cycle Sequencing kit v3.1 (Applied Biosystems) on an ABI3730 automated sequencer (Applied

Biosystems). Variants identified in the mutation analysis were validated by PCR–based amplification of genomic DNA followed by Sanger sequencing.

We predicted the pathogenic effect of rare coding variants using two conservation scoring programs SIFT (Sorting Intolerable From Tolerable) (Ng and Henikoff, 2001) and ConSurf (Goldenberg *et al.*, 2009). The effect on stability and dimerization was evaluated in silico by FoldX (Guerois *et al.*, 2002; J. Schymkowitz *et al.*, 2005a; J. Schymkowitz *et al.*, 2005b).

Neuropathological and immunohistochemical analysis

Autopsied brains of two *TBK1* patients DR1124 and DR189 were obtained using informed consents and protocols that were approved by the Ethical Committee of University of Antwerp and Antwerp University Hospital and stored in The Antwerp Biobank of the Institute Born-Bunge. After a fixation period of 8 to 16 weeks in 10% buffered formalin, 5µm slices were cut. Samples were obtained from frontal cortex, temporal neocortex (superior temporal gyrus), hippocampus, area striata, neostriatum, basal ganglia, substantia nigra, thalamus, mesencephalon, pons, medulla oblongata, cerebellum and in addition spinal cord of DR1124. Sections were deparaffinized, rehydrated and pretreated with citric acid 0.1M. Immunohistochemical analysis was performed with anti-ubiquitin antibody (Dako, Glostrup, Denmark), AT8 against hyperphosphorylated tau (Innogenetics, Zwijnaarde, Belgium), 4G8 against β-amyloid (Signet, Dedham, Massachusetts), anti-FUS antibody (Sigma Aldrich, St Louis), anti-TDP-43 antibody (Proteintech Group Inc, Chicago, Illinois). Additionally, immunohistochemistry was performed with anti-p62 antibody (DB Transduction Laboratories). Sections were counterstained with hematoxylin and images were taken on an Axioskop 50 light microscope (Zeiss) equipped with a CCD UC30 camera (Olympus Inc.).

Supplementary table 1. Demographic characteristics and clinical diagnosis of FTD patients with a *C9orf72* repeat expansion or a *GRN* LOF mutation

Patient	Gender	Clinical diagnosis	Diagnosis subtype	Family history	AAO	DD	Mutation
DR14.5	M	FTD	MXD	F-AD	65	7	*C9orf72* repeat expansion
DR1146.1*	M	FTD-ALS	bvFTD	F-AD	55		*C9orf72* repeat expansion
DR710.1*	F	FTD-ALS	bvFTD	F	70		*C9orf72* repeat expansion
DR1195.1*	M	FTD-ALS	MXD	F	55		*C9orf72* repeat expansion
DR439.5	M	FTD	bvFTD	F	52		*C9orf72* repeat expansion
DR10.1*	F	FTD	bvFTD	F-AD	54	11	*C9orf72* repeat expansion
DR14.1*	M	FTD	bvFTD	F-AD	56	4	*C9orf72* repeat expansion
DR14.57	F	FTD	bvFTD	F-AD			*C9orf72* repeat expansion
DR29.1*	F	FTD	bvFTD	F-AD	50	5	*C9orf72* repeat expansion
DR29.12	F	FTD	bvFTD	F-AD	64	2	*C9orf72* repeat expansion
DR439.1*	M	FTD	bvFTD	F	54	15	*C9orf72* repeat expansion
DR439.6	M	FTD	bvFTD	F	69		*C9orf72* repeat expansion
DR489.1*	F	FTD	PNFA	F-AD	45	7	*C9orf72* repeat expansion
DR52.1*	M	FTD	bvFTD	F-AD	51	7	*C9orf72* repeat expansion
DR55.1*	F	FTD	bvFTD	U	42	6	*C9orf72* repeat expansion
DR575.1*	M	FTD	bvFTD	F-AD	45	6	*C9orf72* repeat expansion
DR659.1*	M	FTD	bvFTD	F-AD	38		*C9orf72* repeat expansion
DR659.2	M	FTD	SD	F-AD	63	11	*C9orf72* repeat expansion
DR672.1*	F	FTD	bvFTD	F-AD	50		*C9orf72* repeat expansion
DR715.1*	M	FTD	bvFTD	F-AD	52		*C9orf72* repeat expansion

DR715.8	F	FTD	bvFTD	F-AD	49		*C9orf72* repeat expansion
DR819.1*	M	FTD	bvFTD	F	49	2	*C9orf72* repeat expansion
DR830.1*	M	FTD	bvFTD	F-AD	29		*C9orf72* repeat expansion
DR911.1*	F	FTD	bvFTD	F-AD	72		*C9orf72* repeat expansion
DR912.1*	F	FTD	bvFTD	F-AD	45		*C9orf72* repeat expansion
DR912.6	M	FTD	bvFTD	F-AD	49		*C9orf72* repeat expansion
DR1196.1*	F	FTD	bvFTD	F	52		*C9orf72* repeat expansion
DR1053.1*	M	FTD	bvFTD	F	55		*C9orf72* repeat expansion
DR212.4	F	FTD	bvFTD	F			*C9orf72* repeat expansion
DR1085.1*	F	FTD	bvFTD	F-AD	42	12	*C9orf72* repeat expansion
DR1119.1*	F	FTD	bvFTD	S	47	19	*C9orf72* repeat expansion
DR1197.1*	F	FTD	bvFTD	F-AD	35		*C9orf72* repeat expansion
DR1198.1*	M	FTD	bvFTD	F	52		*C9orf72* repeat expansion
DR194.1*	M	FTD	bvFTD	S	49	18	*C9orf72* repeat expansion
DR389.1*	F	FTD	bvFTD	F-AD	46		*C9orf72* repeat expansion
DR660.1*	M	FTD	bvFTD	S	58	8	*C9orf72* repeat expansion
DR661.1*	M	FTD	bvFTD	F-AD	53		*C9orf72* repeat expansion
DR673.1*	F	FTD	SD	F-AD	57	7	*C9orf72* repeat expansion
DR674.1*	F	FTD		U			*C9orf72* repeat expansion
DR676.1*	M	FTD		U			*C9orf72* repeat expansion
DR677.1*	M	FTD	bvFTD	F	60	7	*C9orf72* repeat expansion
DR678.1*	M	FTD	SD	F-AD	65	4	*C9orf72* repeat expansion

DR680.1*	F	FTD		F	56		*C9orf72* repeat expansion
DR681.1*	M	FTD-ALS	bvFTD	F	50	3	*C9orf72* repeat expansion
DR835.1*	M	FTD	bvFTD	F-AD	54		*C9orf72* repeat expansion
DR1199.1*	M	FTD	bvFTD	U	58	3	*C9orf72* repeat expansion
DR1200.1*	M	FTD	PNFA	F-AD	61	2	*C9orf72* repeat expansion
DR1201.1*	M	FTD	bvFTD	F-AD	54		*C9orf72* repeat expansion
DR898.2	F	FTD	bvFTD	F	61		*C9orf72* repeat expansion
DR679.1*	M	FTD-ALS	PNFA	F	53	2	*C9orf72* repeat expansion
DR598.4	M	FTD-ALS	bvFTD	F-AD	46		*C9orf72* repeat expansion
DR1202.1*	M	FTD	bvFTD	F	54		*C9orf72* repeat expansion
DR52.2	F	FTD	bvFTD	F-AD	75		*C9orf72* repeat expansion
DR393.1*	F	FTD-ALS	bvFTD	F	66	3	*C9orf72* repeat expansion
DR393.2	M	FTD-ALS	bvFTD	F	55	1	*C9orf72* repeat expansion
DR396.1*	F	FTD-ALS	bvFTD	F-AD	60	2	*C9orf72* repeat expansion
DR454.1*	F	FTD-ALS	bvFTD	F-AD	69	2	*C9orf72* repeat expansion
DR489.4	M	FTD-ALS	bvFTD	F-AD	53	2	*C9orf72* repeat expansion
DR598.1*	F	FTD-ALS	PNFA	F-AD	65	3	*C9orf72* repeat expansion
DR1203.1*	F	FTD-ALS	PNFA	F-AD		1	*C9orf72* repeat expansion
DR390.1*	M	FTD-ALS	bvFTD	F	49	5	*C9orf72* repeat expansion
DR682.1*	F	FTD-ALS		U			*C9orf72* repeat expansion
DR212.1*	M	FTD	bvFTD	F	68	3	*C9orf72* repeat expansion
DR1204.1*	F	FTD	bvFTD	F	46		*C9orf72* repeat expansion

DR1205.1*	M	FTD	bvFTD	U			*C9orf72* repeat expansion
DR2.1*	M	FTD	bvFTD	F-AD	67	4	*GRN* IVS1+5G>C
DR31.1	M	FTD	PNFA	F	65	6	*GRN* IVS1+5G>C
DR1206.1	F	FTD	bvFTD	F			*GRN* IVS1+5G>C
DR1207.1	F	FTD	PNFA	S	62	4	*GRN* IVS1+5G>C
DR1208.1	F	FTD	PNFA	S	63		*GRN* IVS1+5G>C
DR686.1	F	FTD		U			*GRN* IVS1+5G>C
DR27.1	F	FTD	bvFTD	F-AD	58	4	*GRN* IVS1+5G>C
DR8.2	M	FTD		U			*GRN* IVS1+5G>C
DR25.1	F	FTD	bvFTD	F-AD	69	5	*GRN* IVS1+5G>C
DR1209.1	M	FTD	PNFA	S	60		*GRN* IVS1+5G>C
DR1210.1	M	FTD	bvFTD	S	70		*GRN* IVS1+5G>C
DR2.17	M	FTD	MXD	F-AD	69		*GRN* IVS1+5G>C
DR2.18	M	FTD	bvFTD	F-AD	70		*GRN* IVS1+5G>C
DR792.1	F	FTD	PNFA	U	69		*GRN* IVS1+5G>C
DR8.15	F	FTD	PNFA	F-AD	61	3	*GRN* IVS1+5G>C
DR1211.1	M	FTD		U	56		*GRN* IVS1+5G>C
DR1212.1	F	FTD	PNFA	U	63	5	*GRN* IVS1+5G>C
DR1213.1	M	FTD		U	58	2	*GRN* IVS1+5G>C
DR1194.1	F	FTD	PNFA	F-AD	62		*GRN* IVS1+5G>C
DR1214.1	M	FTD		U			*GRN* IVS1+5G>C
DR28.1	M	FTD	PNFA	F-AD	56	6	*GRN* IVS1+5G>C

DR8.1	F	FTD	bvFTD	F-AD	62	6	*GRN* IVS1+5G>C
DR2.3	F	FTD	PNFA	F-AD	63	8	*GRN* IVS1+5G>C
DR2.15	F	FTD	MXD	F-AD	68	5	*GRN* IVS1+5G>C
DR26.1	M	FTD	PNFA	F	65	3	*GRN* IVS1+5G>C
DR404.1	F	FTD	SD	F	55	7	*GRN* IVS1+5G>C
DR25.5	M	FTD	bvFTD	F-AD	70	3	*GRN* IVS1+5G>C
DR119.1	F	FTD	PNFA	F	45	5	*GRN* IVS1+5G>C
DR28.3	M	FTD	bvFTD	F-AD	46		*GRN* IVS1+5G>C
DR8.40	F	FTD	bvFTD	F-AD	52	4	*GRN* IVS1+5G>C
DR8.42	M	FTD	bvFTD	F-AD	62	7	*GRN* IVS1+5G>C
DR91.1*	F	FTD	bvFTD	U	66	6	*GRN* Ala237TrpfsX4
DR184.1*	F	FTD	SD	S	70	4	*GRN* del
DR118.1	F	FTD	bvFTD	F	63	8	*GRN* Met1Ile
DR609.1*	M	FTD	bvFTD	F	52	9	*GRN* Glu498X
DR120.1*	F	FTD	PNFA	F-AD	55		GRN Pro127ArgfsX2
DR403.1*	M	FTD	PNFA	F	53	7	*GRN* Val279GlyfsX5
DR510.1*	F	FTD		U			*GRN* Asn118PhefsX4
DR698.1*	M	FTD	SD	F	57		*GRN* Ala237TrpfsX4
DR554.1*	F	FTD	bvFTD	F	56		*GRN* Trp304X
DR1215.1*	F	FTD		U			*GRN* Pro127ArgfsX2
DR287.1*	F	FTD	PNFA	F	65	6	*GRN* Ala89ValfsX41
DR529.1*	M	FTD	bvFTD	F			*GRN* Ala303GlyfsX14

DR701.1*	F	FTD	PNFA	F	53		*GRN* Ala303GlyfsX14
DR1216.1*	M	FTD	bvFTD	F			*GRN* Tyr294* c.882T>G
DR1084.1*	F	FTD	bvFTD	F-AD	58		*GRN* Ala237Trpfs*4
DR1103.1*	M	FTD	bvFTD	F-AD	58		*GRN* Met1?
DR1217.1*	M	FTD		U			*GRN* Trp304CysfsX58
DR1218.1*	F	FTD		U			*GRN* Ala303Profs*58
DR1219.1*	F	FTD		U			*GRN* Ala303Profs*58
DR1220.1*	M	FTD	MXD	F	62		*GRN* Thr330Alafs*6
DR737.1*	F	FTD	SD	F	68	11	*GRN* Gln249ProfsX6

*Abbreviations: *Indexpatient; ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant frontotemporal dementia; F (gender), female; F (familial history), familial; F-AD, familial with autosomal dominant pattern; FTD, frontotemporal dementia; M, male; MXD, mixed frontotemporal dementia (behavioral as well as language features); PNFA, progressive nonfluent aphasia; S, sporadic; SD, semantic dementia; U, unknown*

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