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 |
| Case      | Sex | Diagnosis | Subtype | Age | C9orf72 Expansion |
|-----------|-----|-----------|---------|-----|------------------|
| DR680.1*  | F   | FTD       |         | 56  |                  |
| DR681.1*  | M   | FTD-ALS   | bvFTD   | 50  |                  |
| DR835.1*  | M   | FTD       | bvFTD   | 54  |                  |
| DR1199.1* | M   | FTD       | U       | 58  |                  |
| DR1200.1* | M   | FTD       | PNFA    | 61  |                  |
| DR1201.1* | M   | FTD       | bvFTD   | 54  |                  |
| DR898.2   | F   | FTD       | bvFTD   | 61  |                  |
| DR679.1*  | M   | FTD-ALS   | PNFA    | 53  |                  |
| DR598.4   | M   | FTD-ALS   | bvFTD   | 46  |                  |
| DR1202.1* | M   | FTD       | bvFTD   | 54  |                  |
| DR52.2    | F   | FTD       | bvFTD   | 75  |                  |
| DR393.1*  | F   | FTD-ALS   | bvFTD   | 66  |                  |
| DR393.2   | M   | FTD-ALS   | bvFTD   | 55  |                  |
| DR396.1*  | F   | FTD-ALS   | bvFTD   | 60  |                  |
| DR454.1*  | F   | FTD-ALS   | bvFTD   | 69  |                  |
| DR489.4   | M   | FTD-ALS   | bvFTD   | 53  |                  |
| DR598.1*  | F   | FTD-ALS   | PNFA    | 65  |                  |
| DR1203.1* | F   | FTD-ALS   | PNFA    | 1   |                  |
| DR390.1*  | M   | FTD-ALS   | bvFTD   | 49  |                  |
| DR682.1*  | F   | FTD-ALS   | U       |     |                  |
| DR212.1*  | M   | FTD       | bvFTD   | 68  |                  |
| DR1204.1* | F   | FTD       | bvFTD   | 46  |                  |
| Case No.     | Gender | Clinical Diagnosis | Neuroradiological Diagnosis | Age at Onset | Duration | Genotype  |
|-------------|--------|--------------------|------------------------------|--------------|----------|-----------|
| DR1205.1*  | M      | FTD                | bvFTD                        |              |          |           |
| DR2.1*      | M      | FTD                | bvFTD                        |              |          | C9orf72 repeat expansion |
| DR31.1      | M      | FTD                | PNFA                         |              |          |           |
| DR1206.1    | F      | FTD                | bvFTD                        |              |          | GRN IVS1+5G>C |
| DR1207.1    | F      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR1208.1    | F      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR686.1     | F      | FTD                |                              |              |          | GRN IVS1+5G>C |
| DR27.1      | F      | FTD                | bvFTD                        |              |          | GRN IVS1+5G>C |
| DR8.2       | M      | FTD                |                              |              |          | GRN IVS1+5G>C |
| DR25.1      | F      | FTD                | bvFTD                        |              |          | GRN IVS1+5G>C |
| DR1209.1    | M      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR1210.1    | M      | FTD                | bvFTD                        |              |          | GRN IVS1+5G>C |
| DR2.17      | M      | FTD                | MXD                          |              |          | GRN IVS1+5G>C |
| DR2.18      | M      | FTD                | bvFTD                        |              |          | GRN IVS1+5G>C |
| DR792.1     | F      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR8.15      | F      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR1211.1    | M      | FTD                |                              |              |          | GRN IVS1+5G>C |
| DR1212.1    | F      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR1213.1    | M      | FTD                |                              |              |          | GRN IVS1+5G>C |
| DR1194.1    | F      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR1214.1    | M      | FTD                |                              |              |          | GRN IVS1+5G>C |
| DR28.1      | M      | FTD                | PNFA                         |              |          | GRN IVS1+5G>C |
| DR     | Sex | Diagnosis | Condition | Age | Year | Mutation          |
|--------|-----|-----------|-----------|-----|------|-------------------|
| DR8.1  | F   | FTD       | bvFTD     | F-AD| 62   | 6GRN IVS1+5G>C    |
| DR2.3  | F   | FTD       | PNFA      | F-AD| 63   | 8GRN IVS1+5G>C    |
| DR2.15 | F   | FTD       | MXD       | F-AD| 68   | 5GRN IVS1+5G>C    |
| DR26.1 | M   | FTD       | PNFA      | F   | 65   | 3GRN IVS1+5G>C    |
| DR404.1| F   | FTD       | SD        | F   | 55   | 7GRN IVS1+5G>C    |
| DR25.5 | M   | FTD       | bvFTD     | F-AD| 70   | 3GRN IVS1+5G>C    |
| DR119.1| F   | FTD       | PNFA      | F   | 45   | 5GRN IVS1+5G>C    |
| DR28.3 | M   | FTD       | bvFTD     | F-AD| 46   | 4GRN IVS1+5G>C    |
| DR8.40 | F   | FTD       | bvFTD     | F-AD| 52   | 4GRN IVS1+5G>C    |
| DR8.42 | M   | FTD       | bvFTD     | F-AD| 62   | 7GRN IVS1+5G>C    |
| DR91.1*| F   | FTD       | bvFTD     | U   | 66   | 6GRN Ala237TrpfsX4|
| DR184.1*| F | FTD       | SD        | S   | 70   | 4GRN del         |
| DR118.1| F   | FTD       | bvFTD     | F   | 63   | 8GRN Met1Ile     |
| DR609.1*| M | FTD       | bvFTD     | F   | 52   | 9GRN Glu498X    |
| DR120.1*| F | FTD       | PNFA      | F-AD| 55   | GRN Pro127ArgfsX2 |
| DR403.1*| M | FTD       | PNFA      | F   | 53   | 7GRN Val279GlyfsX5|
| DR510.1*| F | FTD       | U         |     |      | GRN Asn118PhefsX4|
| DR698.1*| M | FTD       | SD        | F   | 57   | 4GRN Ala237TrpfsX4|
| DR554.1*| F | FTD       | bvFTD     | F   | 56   | 4GRN Trp304X    |
| DR1215.1*| F | FTD       | U         |     |      | GRN Pro127ArgfsX2|
| DR287.1*| F | FTD       | PNFA      | F   | 65   | 6GRN Ala89ValfsX41|
| DR529.1*| M | FTD       | bvFTD     | F   |      | 4GRN 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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