

**Supplementary information**

Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia  
linked to chromosome 17q21

**Supplementary tables**

**Supplementary table 1:** Clinicopathological findings of index patients from the Belgian founder family. Detailed description of the clinical phenotype of the patients, and the brain pathology of patient DR31 II-1, has been published <sup>1</sup>.

<b>Index patient</b>	<b>Gender</b>	<b>Age at Onset (years)</b>	<b>Disease Duration (years)</b>	<b>Clinical diagnosis and most prominent presenting symptoms</b>	<b>Structural Neuroimaging (CT/MRI)</b>	<b>Functional Neuroimaging (PET/SPECT)</b>	<b>Pathological diagnosis and most prominent features</b>
DR8 III-28	F	62	6	FTD; Personality changes (apathy), behavioral disturbances (psychosis, disinhibition), word finding difficulties and impaired memory	Frontotemporoparietal cortical and subcortical atrophy, left>right (MRI)	Relative bilateral frontal HP, left>right (SPECT)	FTDU; Very severe frontal- and less severe temporal lobe atrophy. Frequent ubiquitin-positive, cat-eye, nuclear and cytoplasmic neuronal and glial inclusions in neocortex, especially in frontal cortex.
DR25 III-17	F	69	7	FTD; Behavioral disturbances, personality changes, reduced spontaneous speech	Cortical and subcortical frontal atrophy; PWML (CT)	Severe relative bilateral frontal, parietal and temporal HP. Scintigraphic indications of subcortical loss (SPECT)	FTDU; Very severe frontal lobe atrophy and less severe temporal and parietal lobe atrophy. Frequent ubiquitin-positive, cat-eye, nuclear and cytoplasmic neuronal inclusions in neocortex and CA1.
DR27 III-4	F	58	6	FTD; Behavioral disturbances, personality changes	Cortical and subcortical atrophy, maximal frontotemporally, right>left; PWML (MRI)	Bilateral frontal, temporal and parietal HP, right>left Right HP at parieto-occipital transition (PET)	FTDU; Very severe frontotemporal atrophy with ubiquitin-positive, cat-eye, nuclear and cytoplasmic neuronal inclusions in neocortex and CA regions.
DR28 III-3	M	57	5	FTD; Non-fluent aphasia	NA	Relative frontal, temporal and parietal HP, left>right (SPECT)	Preliminary diagnosis of FTDU with ubiquitin-positive, cat-eye, nuclear and cytoplasmic neuronal inclusions in neocortex
DR31 II-1	M	66	4	FTD; Non-fluent aphasia	Global cortical and minor subcortical temporal atrophy, left>right (MRI)	Marked relative bilateral frontal and temporal HP, left>right. Diastasis of frontal cortical activity (SPECT)	FTDU; Severe frontal lobe atrophy and less severe temporal lobe atrophy. Rare, ubiquitin-positive, cat-eye, nuclear inclusions in frontotemporal cortex.

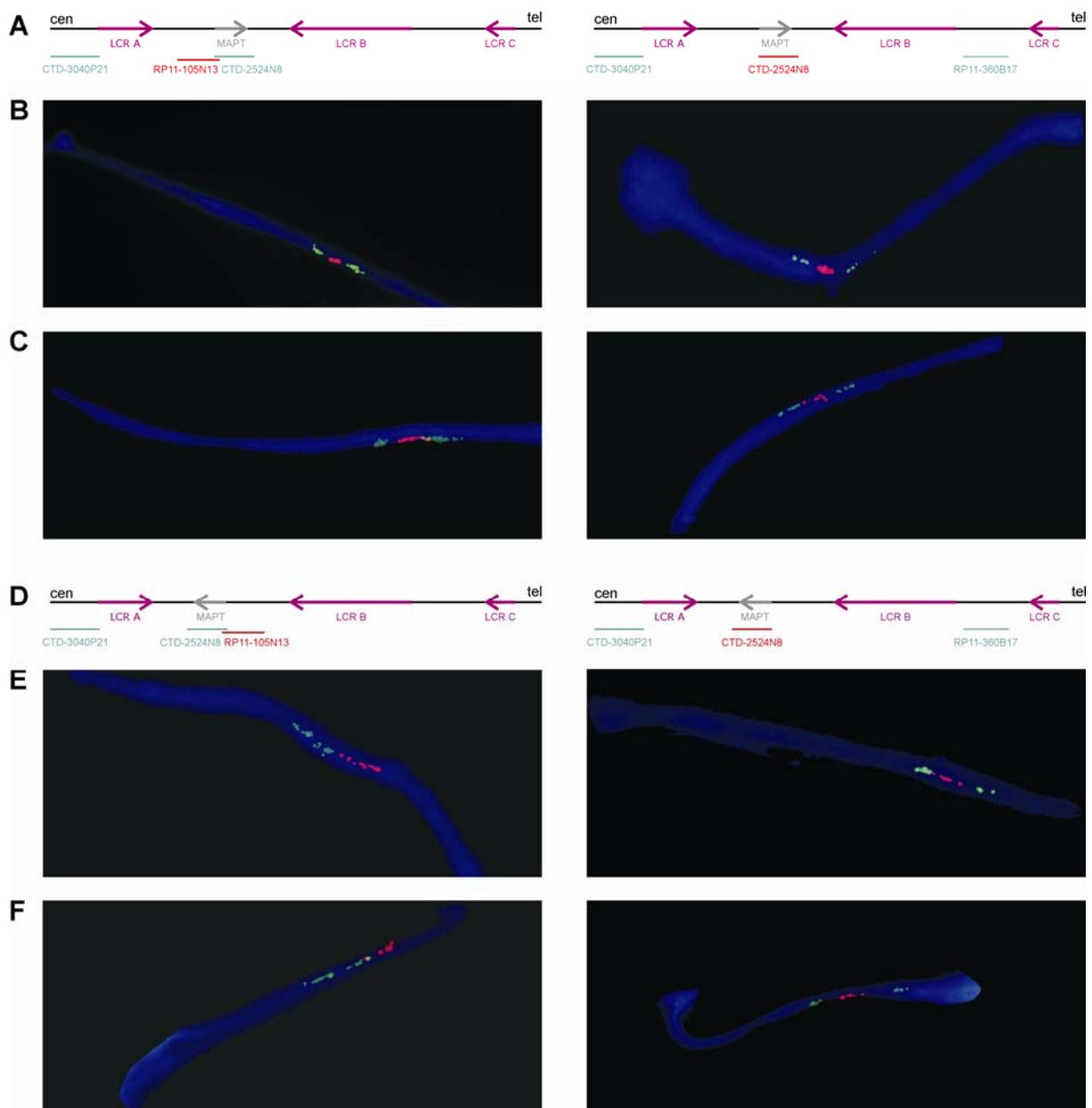
Abbreviations: CA= Corpora ammonii, HP = hypoperfusion, NA = not available, PWML = periventricular white matter lesions; FTD = frontotemporal dementia, FTDU = FTD with ubiquitin-immunoreactive inclusions; MRI = magnetic resonance imaging, PET = positron emission tomography, SPECT = single photon emission computed tomography.

**Supplementary table 2.** PCR and sequencing primers of *PGRN* genomic and cDNA.

Region	PCR primers		Size in bp
	Name <sup>1</sup>	Sequence	
<b>gDNA</b>			
EX 0	ex0F	CTGTCAATGCCCCAGACACG	499
	ex0R	CCCCAAGGAGTTTCAGTAAGC	
EX 1	ex1F	TTGAGAAGGCTCAGGCAGTC	400
	ex1R	GGCCATTTGTCCTAGAAAGACAGG	
	ex1Fseq	GGGCTAGGGTACTGAGTGAC	
EX 2+3	ex2+3F	TGGGTTTTCCCAAAGGGTCA	516
	ex2+3R	GCACAAGGGCAGGAATCAGG	
EX 4+5	ex4+5F	GCCACCAGCTCCTTGTGTGA	545
	ex4+5R	GGCCACTGGAAGAGGAGCAA	
	ex4+5Fseq	CCTTCCCTGAGTGGGCTGGTA	
EX 6	ex6F	TGAGGAGGTGGGAGAGCATC	307
	ex6R	CCATGCCACAGAGCCCC	
	ex6Rseq	TGTAAGGTGCGTGTCAGG	
EX 7	ex6F	ACAGGGCAGGTGAGGAGGTG	733
	ex7R	CCTTTGCCGGCTCCACAG	
	Ex7Fseq	GGCTGATGCAGGGTTCATGC	
EX 8	ex8F	GGCCTGGCCTTAGGATCACTG	698
	ex9R	CCAGCTGGGGACGAATCTGT	
	Ex8Fseq	TACCCTCCATCTTCAACAC	
	ex8R	CGCGGGACAGCAGTGTATGT	
EX 9	ex8F	GGCCTGGCCTTAGGATCACTG	698
	ex9R	CCAGCTGGGGACGAATCTGT	
	ex9Fseq	ATACCTGCTGCCGTCTAC	
EX 10	ex10F	TCCGCATAGCCCATAGGTGA	499
	ex10R	GCGATCCTCGCAGCACAC	
EX 11	ex11F	TGGACTGGAGAAGATGCC	574
	ex11R	CGATCAGCACAAACAGACG	
EX 12	ex12.F1	CAGACCTGCTGCCGAGACAA	736
	ex12R2	CGATGTGGGCAGCAGCAAAT	
	ex12R1seq	GGAGGGGATGGCAGCTTGTA	
	ex12F2seq	TGGGACGCCCTTTGAGG	
<b>cDNA</b>			
EX 0-12	c.ex0-12F	CCAAGGACCGCGGAG	1891
	c.ex0-12R	AGGTGCTAGGGAGGCCTGA	
	c.ex0-4Rseq	TCGACCATAACACAGCACGTG	
	c.ex2-F	TGCTGCCCTTCCCAGAG	
EX 5/6-12	c.ex5/6-12F	GCAGTGGCCTTGTCCAGCTC	1370
	c.ex5/6-12R	CTTTTGCCACAACCCCTTCT	
	c.ex11-Fseq	ACCTGCTGCCGAGACAACC	

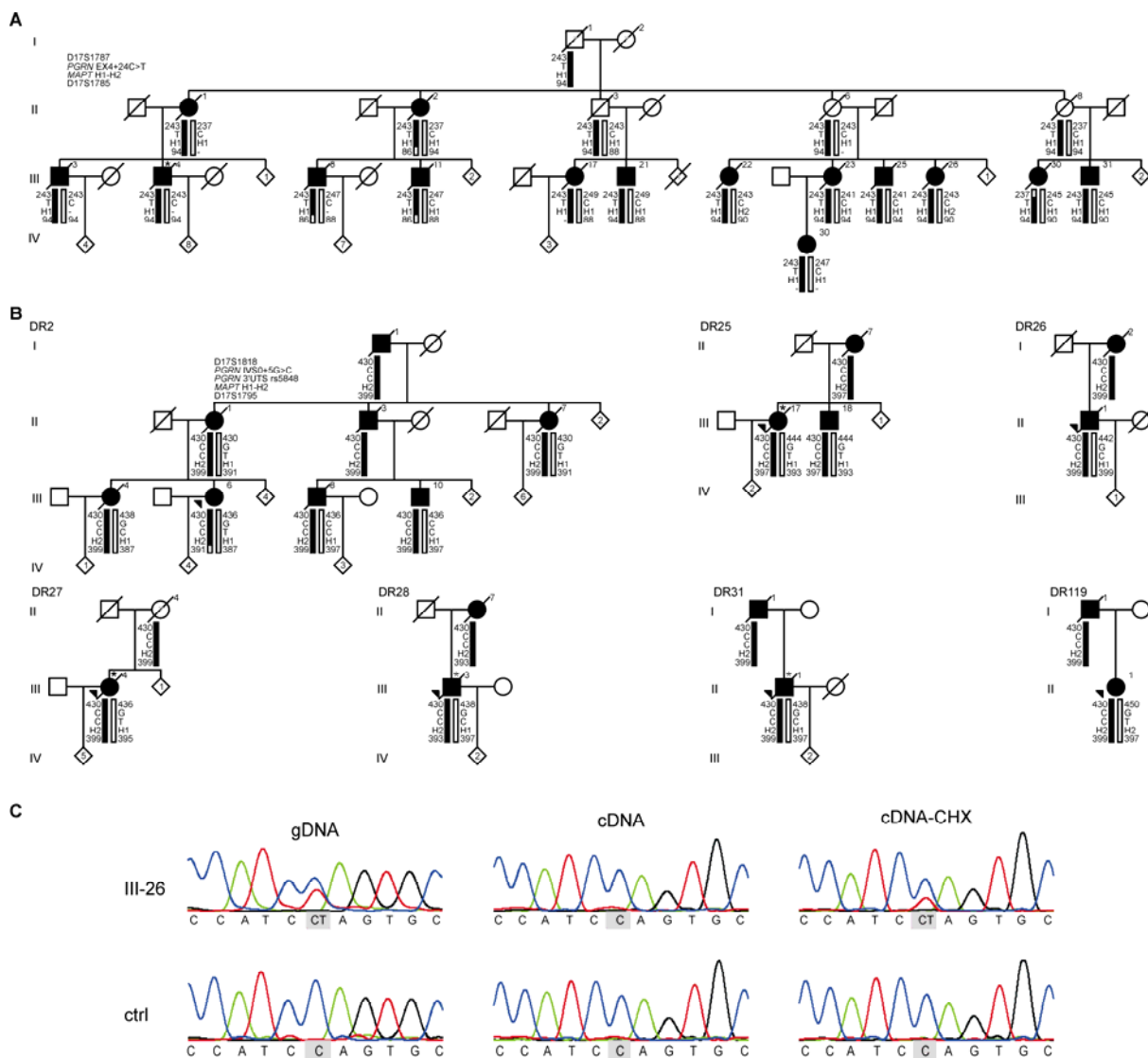
Note: <sup>1</sup>PCR primers were also used for sequencing. Additional sequencing primers were named with suffix “seq”.

## Supplementary figures



**Supplementary figure 1: FISH of the *MAPT* genomic region on mechanically stretched chromosomes.** The *MAPT* locus contains three highly homologous low-copy repeats (LCRs A, B and C) that during evolution have mediated an inversion producing the common *MAPT* inversion polymorphism H1-H2<sup>2</sup>. Here left and right panes show FISH data using different BAC hybridization probe combinations schematically represented in **A** (H1) and **D** (H2) showing the positions of the BAC clones relative to *MAPT* and surrounding LCR A, LCR B, LCR C<sup>2</sup>, with BAC clones colored in accordance with the hybridization signals. (**B-C**) FISH

patterns obtained on mechanically stretched chromosomes of patient 1083 III-26 (Supplementary fig. 2A), heterozygous for the *MAPT* H1-H2 inversion polymorphism (B) and an unaffected relative, homozygous for *MAPT* H1 (C). (E-F) FISH patterns obtained on mechanically stretched chromosomes of patient DR8 III-32 (Fig. 2), heterozygous for the *MAPT* H1-H2 inversion polymorphism (E) and an unaffected relative, homozygous for *MAPT* H2 (F). FISH of mechanically stretched chromosomes was performed on lymphoblast cells as described<sup>3</sup>. Cells were arrested in metaphase by colcemid treatment, incubated in a hypotonic solution, cytocentrifuged (Cytospin 4, Thermo Shandon, Waltham, MA USA) to silanized glass slides resulting in mechanical stretching of chromosomes, and fixed. BAC clones were cultured and DNA was prepared according to standard procedures and their inserts validated by PCR amplification of STS markers. Probes were labeled with biotin-11-dUTP or digoxigenin-11-dUTP by nick translation and detected by a 3-layer immunofluorescence detection system of respectively Texas Red-conjugated avidin and FITC-conjugated anti-digoxigenin. Chromosomal DNA was counterstained with DAPI and hybridization signals were imaged using a Zeiss Axioskop 50 fluorescent microscope (Carl Zeiss NV, Zaventem, Brussels).



**Supplementary figure 2: Segregation data of *PGRN* mutations in FTDU1-17 families**

**1083 and DR8.** Black bars represent the disease haplotype of patients and obligate carriers.

Numbers within each diamond are unaffected at-risk individuals that were included in the

genotyping. Arrowheads identify probands. **(A)** Segregation in Dutch family 1083<sup>4</sup> is shown

for the *PGRN* Gln125X mutation, the *MAPT* H1-H2 inversion haplotype analyzed in the

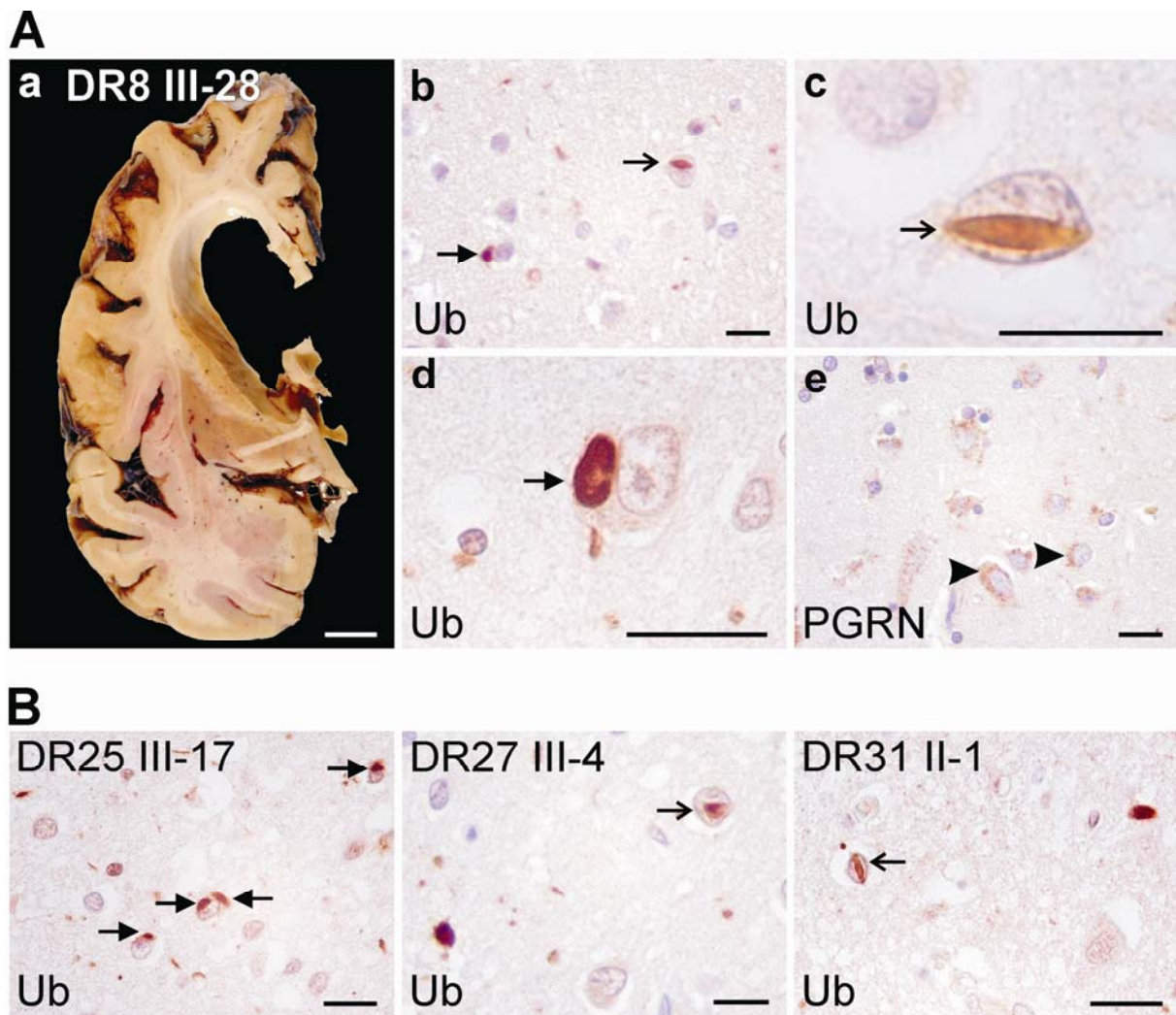
chromosomal FISH analysis (Supplementary fig. 1B-C), and microsatellite markers

D17S1787 and D17S1785 flanking the linked region (Fig. 1A). The asterisk indicates that a

pathological diagnosis of FDTU was available for patient III-4, showing moderate frontal

lobe atrophy with gliosis and nuclear and cytoplasmic neuronal inclusions in neocortex

compatible with FTDU<sup>4</sup>. **(B)** Segregation data is shown in the seven branches of the Belgian founder family DR8 sharing the disease haplotype<sup>1</sup> for the microsatellite markers D17S1818 and D17S1795 flanking the founder haplotype (Fig. 1A), the *PGRN* IVS0+5G>C mutation, the *PGRN* 3'UTS SNP rs5848 used in cDNA analyses (Fig. 3B), and the *MAPT* H1-H2 inversion haplotype. Since the previous report, an additional branch of the founder family DR8 was identified (DR119) sharing the complete founder haplotype ranging from D17S1818 to D17S1795<sup>1</sup>. Asterisks indicate that a pathological diagnosis of FDTU was available for patients DR25 III-17, DR27 III-4, DR28 III-3 and DR31 II-1 summarized in Supplementary table 2 and Supplementary fig. 3B. **(C)** Nonsense-mediated mRNA decay analysis of the *PGRN* Gln125X mutation in patient III-26 and an unaffected relative of family 1083. Sequence traces are shown of genomic DNA, cDNA prepared from lymphoblast cells treated (cDNA-CHX) and not treated (cDNA) with 100 µg/ml cycloheximid for 4 hours prior to mRNA isolation.



**Supplementary figure 3: FTDU neuropathology of patients from the Belgian founder**

**family: A.** *PGRN* IVS0+5G>C index patient DR8 III-28 of the Belgian founder family showed severe cortical atrophy with narrowing of gyri in frontal lobe (a). The temporal and parietal lobes were less affected. Histologic sections showed widespread, superficial laminar spongiosis and gliosis that was most remarkable in the frontal lobe. The motor neurons in the cortex and brain stem were preserved. Except for occasional, age-related, neurofibrillary tangles and vascular amyloidosis, brain sections were negative for hyperphosphorylated tau and A $\beta$  immunoreactivity. Ubiquitin immunostaining utilizing a polyclonal anti-ubiquitin antibody (DAKO, Denmark) showed besides presence of dystrophic neurites, typical ubiquitin-immunoreactive neuronal intranuclear (open arrow in b and c) and cytoplasmic

(closed arrow in b and d) inclusions in the frontal cortices, and to some extent, also in the temporal cortex. Intranuclear neuronal inclusions were often cat-eye shaped.

Immunoreactivity with a polyclonal antibody against PGRN holoprotein (anti-human Progranulin; R&D Systems, Minneapolis, MN) showed PGRN immunostaining in neuronal perikaryon (e, arrowheads) and also in activated glial cells, however, no immunoreactivity was observed for the neuronal and glial inclusions. These results were confirmed by polyclonal antibodies directed against the amino- or carboxyl-terminus of PGRN (N-19 and S-15, Santa Cruz Biotechnology, Santa Cruz, CA). **B.** The characteristic intranuclear (open arrow) and cytoplasmic (closed arrow) inclusions in frontal and temporal cortical regions were also observed in index patients of 3 different branches, DR25, DR27 and DR31, of the Belgian founder family (Supplementary fig. 2B). For immunohistochemistry, sections were antigen retrieved by microwaving in citrate buffer (pH 6) and stained using streptavidin-biotin-horse radish peroxidase (ABC/HRP) and 3'3'diaminobenzidine (DAB, Roche, New Jersey, NJ) as described previously<sup>5</sup>. Scale bars in A (a) represents 1 cm; in A (b) through (e) and in B, 20  $\mu$ m.

### Supplementary references

1. van der Zee, J. *et al.* A Belgian ancestral haplotype harbours a highly prevalent mutation for 17q21-linked tau-negative FTL. *Brain* **129**, 841-852 (2006).
2. Cruts, M. *et al.* Genomic architecture of human 17q21 linked to frontotemporal dementia uncovers a highly homologous family of low copy repeats in the tau region. *Hum. Mol. Genet.* **14**, 1753-1762 (2005).
3. Gijselinck, I. *et al.* Visualization of *MAPT* inversion on stretched chromosomes of tau-negative frontotemporal dementia patients. *Human Mutation* . 2006. In Press.
4. Rademakers, R. *et al.* Tau negative frontal lobe dementia at 17q21: significant finemapping of the candidate region to a 4.8 cM interval. *Mol. Psychiatry* **7**, 1064-1074 (2002).
5. Kumar-Singh, S. *et al.* Dense-core plaques in Tg2576 and PSAPP mouse models of Alzheimer's disease are centered on vessel walls. *Am. J. Pathol.* **167**, 527-543 (2005).