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ARTICLE

Progranulin mutations in Dutch familial frontotemporal lobar degeneration

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Mutations in the *progranulin* (*PGRN*) gene have recently been identified in frontotemporal lobar degeneration with ubiquitin inclusions linked to chromosome 17q21. We report here the finding of two novel frameshift mutations and three possible pathogenic missense mutations in the *PGRN* gene. Furthermore, we determined the frequency of *PGRN* mutations in familial cases recruited from a large population-based study of frontotemporal lobar degeneration carried out in The Netherlands.
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Introduction

The term frontotemporal lobar degeneration (FTLD) refers to an heterogeneous group of neurodegenerative disorders clinically characterized by progressive behavioral changes and cognitive dysfunctions, including executive and language functions.[1] Sometimes, language impairment presents as an initial symptom sub-classifying this FTLD group into progressive nonfluent aphasia and semantic dementia. Additionally, the clinical picture can be complicated by motor symptoms such as motor neuron disease (MND) or parkinsonism.[2]

Two main pathological FTLD subtypes are recognized based on the presence of tau-positive inclusions (tauopathies) or tau-negative ubiquitin-positive neuronal inclusions (FTLD-U).[3] Characteristically the ubiquitin immunoreactive inclusions (ub-i) are observed in the dentate gyrus of the hippocampus and in the superficial layers of the frontal and temporal cortex.[4]

A positive family history is found in approximately 40% of FTLD cases, and linkage studies have shown that FTLD is genetically heterogeneous with loci and genes identified on chromosomes 3 (FTD3),[5] 9p,[6] 9q[7] and 17q (FTDP-17[8] and FTDU-17[9,10]). Recently, mutations in the *PGRN* gene were found in several families with FTDU-17.[9,10] *PGRN* encodes a biologically active precursor glycoprotein described previously as a multifunctional growth factor involved in development, inflammation and wound repair.[11]

In the present study, we report the finding of two novel frameshift mutations and three possible pathogenic missense mutations in the *progranulinv* (*PGRN*) gene. In addition, we describe the genetic contribution of *PGRN* to FTLD in a series of familial cases recruited from a large cohort of FTLD patients.

Materials and methods

Patients

Three hundred and thirty-eight patients with FTLD (182 females and 156 males) with mean age at onset of

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57.4±9.3 years were identified in a genetic–epidemiological study in the Netherlands. The clinical diagnosis in all patients was established according to international consensus criteria.[12] Clinical family history was positive in 166 patients (59%) and among them DNA was available in 137 cases. Eighty-seven of these 137 patients came from independent families: 10 families presented *MAPT* mutations,[13] two large families showed FTLD-U with definite linkage to chromosome 17q21–22, six smaller families had multiple (>2) affecteds and 69 had two affecteds.

DNA study

The 13 exons of *PGRN* including intron/exons boundaries were amplified from genomic DNA by PCR and directly sequenced in both strands. Novel sequence variants were analyzed in a minimum of 380 chromosomes from healthy individuals of matched ethnicity.

Immunohistochemistry

Immunohistochemistry experiments were performed on eight available brains as described previously.[14]

Results

To determine the possible involvement of the newly found *PGRN* gene in our cohort, we systematically screened for mutations in 77 cases with positive family history of dementia consistent with autosomal dominant pattern of inheritance and with no *MAPT* and *CHMP2B* mutations. The mean age at onset in this group was 59.3±9.1 years.

We identified two novel frameshift mutations Ser82-Valfs174X and Val411Sfr1X (Table 1) predicted to cause premature termination of the coding sequence likely leading to loss of functional PGRN protein similar to previous reports. One nonsense mutation (Gln125X) was also observed in a independently ascertained member of the 1083 FTLD-U family already described.[10] Furthermore, we identified five novel coding sequence variants (three missense and two silent mutations), two intronic sequence changes in intron 2 and 7 and the previously reported missense mutation Gly414Val.[15] The frameshift mutations and the GGG93GGA, Thr182Met, Pro233His, CAC447CAT and Trp541Cys mutations were not found in controls; in contrast, the two intronic variants were also present in healthy individuals suggesting they are not pathogenic. Moreover, the GGG93GGA silent mutation was detected in co-occurrence with the Pro233His.

The Ser82Valfs174X mutation was found in a 69-year-old woman, member of the HFTD3 family previously linked to 17q21–22[4] (Figure 1a). A large variation in age at onset (between 45 and 75 years) was observed between affected individual from this family.

Sequencing of 13 additional DNA samples from affected family members showed complete segregation of the mutation with the disease. The clinical symptoms in this family consisted of apathy, loss of initiative and interest, roaming behaviour and word finding difficulties. Two patients developed parkinsonism early in the course of the disease, which moderately responded to levodopa treatment. Signs of motor neuron disease were not observed. Extensive neuropsychological testing in six patients revealed impaired naming with normal comprehension of language.

The Val411Serfr1X mutation was identified in a 66-year-old woman, who presented with speech, and writing errors, and word finding difficulties. The patient showed social inappropriate behaviour and emotional bluntness. Magnetic resonance imaging showed asymmetric right-sided frontotemporal atrophy. The patient developed loss of initiative, and died from bronchopneumonia. Her mother and grandmother suffered from identical symptoms, whereas her uncle and a nephew were diagnosed as Pick's disease.

Table 1 *PGRN* mutations identified in FTLD patients and healthy control individuals

Location	*Genomic*[a]	*Predicted cDNA*[b]	*Protein*[c]	*Rs number*	*Patients (N)*	*Controls (N)*
Exon 2	g.4407delC	c.243delC	Ser82ValfsX174		HFTD3	—
Intron 2	g.4436G>A				1	4
Intron 2	g.4445G>A			rs9897526	19	35
Exon 3	g.4559G>A	c.279G>A	Gly93Gly		1	—
Intron3	g.4661G>C				—	1
Exon 4	g.5129C>T	c.592C>T	Gln125X		1	—
Exon 5	g.5402C>T	c.545C>T	Thr182Met		1	—
Exon 6	g.5667>A	c.698C>A	Pro233His		1	—
Intron 7	g.6048G>A				22	18
Exon 10	g.6944_6945 delGT	c.1231_1232delGT	Val411SerfsX1		1	—
Exon10	g.6954G>T	c.1241G>T	Gly414Val		1	1
Exon10	g.7054C>T	c.1341C>T	His447His		1	—
Exon10	g.6966G>A	c.1253G>A	Arg418Gln		—	2
Exon11	g.7428G>C	c.1623G>C	Trp541Cys		1	—

[a]Numbering relative to NC_000017.9 Genbank Accession Number and starting at nucleotide 1.
[b]Numbering relative to NM_002087.2 starting at the ATG.
[c]Numbering relative to NP_002087.1.

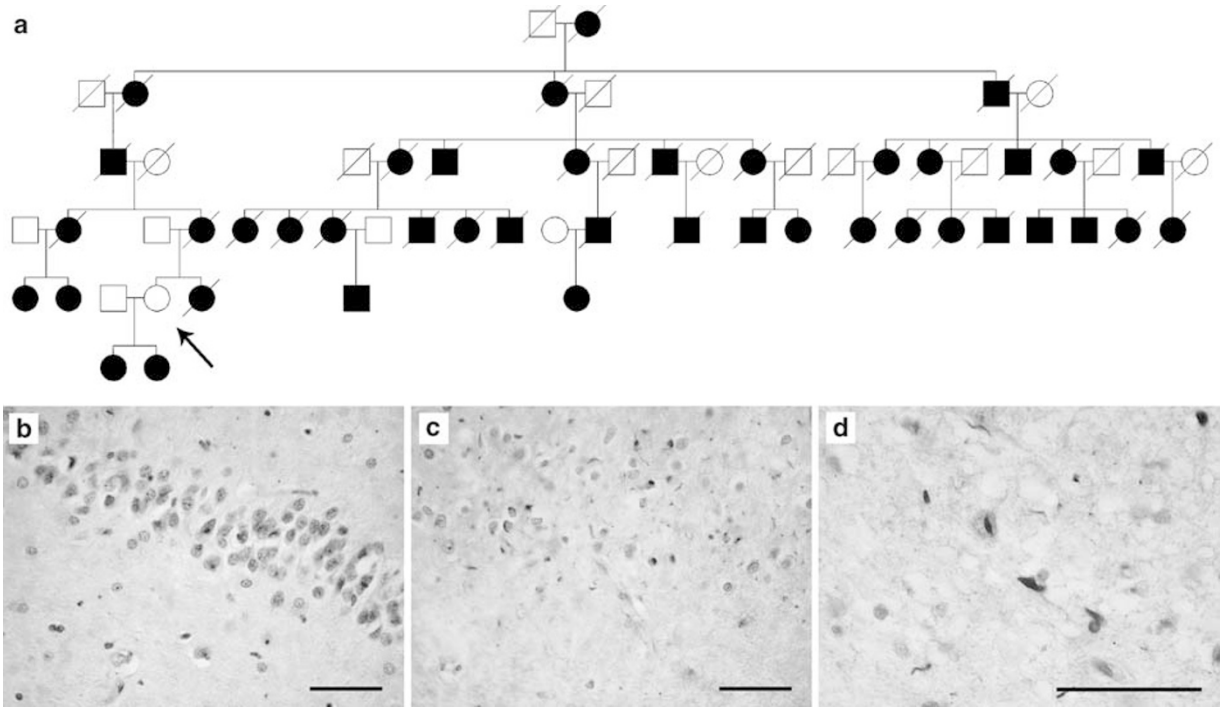

Figure 1 Pedigree and ubiquitin pathology (**a**). Pedigree of family HFTD3; only affected individuals are shown. This large family consists of 42 affected and 102 unaffected members. Arrow indicates a healthy carrier: a 72-year-old mother of two affected sisters who carried the mutation and did not have any cognitive complaints and behavioural changes as confirmed by reports of other family members and by neurological examination. (**b**–**d**) Ubiquitin staining; ubiquitin-positive neuronal cytoplasmatic inclusions in granular cells of the dentate gyrus (**b**) and in the superficial layers of the frontal neocortex (**c**) with ubiquitin-positive dystrophic neurites (**c**) in Ser82Valfs174X brain. Lentiform ubiquitin-positive neuronal intranuclear inclusion in one FTLD case with no *PGRN* mutations (**d**). Scale bars: 100 μm.

The Gln125X mutation was found in a 60-year-old woman, who came from the family 1083, described previously.[10] She presented with memory problems and word findings difficulties.

Neuropathological examination showed ub-i in dentate gyrus, neocortex and/or striatum (Figure 1b and c). Ubiquitin inclusions were also present in six additional brains from FTLD cases with no *PGRN* mutations with the distinct morphology and distribution pattern characteristic of FTLD-U type 2,[16] including several neuronal intranuclear inclusions in the frontal neocortex in one case (Figure 1d).

Discussion

The present study report the identification of three pathogenic (Val411Serfr1X and Ser82Valfs174X as novel) *PGRN* mutations that account for $\sim$4% of the independent familial FTLD cases.

Similar to previous *PGRN* studies the two novel mutations determine a frameshift, which results in the generation of premature termination codons. Eukaryotic cells are capable to detect and degrade transcripts harbouring premature signals for the termination of translation through the nonsense-mediated mRNA decay (NMD) pathway. Degradation of mutant mRNAs results in null alleles[9,15] with loss of functional PGRN.

Several rare missense and silent mutations were detected in patients but not in controls. Segregation studies could not be performed in these cases, as DNA from affected family members was not available.

Although it cannot be excluded that these changes are benign variants as they are located in granulin domains each composed of 7,5 tandem repeats of highly conserved motifs of 12 cystein residues suggested to be functional redundant,[15] several studies have shown that separate repeats may have alternative binding capacities and therefore different functions,[17] highlighting the possibility that these variants are pathogenic. The Pro233 and the Trp541, in particular, are highly conserved among species (Figure 2a) and in the granulin domains (Figure 2b). Furthermore, previous reports have suggested these amino acids are essential for the proper folding of the protein.[18–20] The Trp residue is likely involved in the hydrophobic packaging of the beta-sheet and substitution with the cys residue, which has the ability to form disulfide bridges, might affect PGRN 3D structure. The Pro residue is part of an antiparallel beta-sheet, and might be important for stacking multiple repeats,

a GRANULIN MOTIF II

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 1 HOMO SAPIENS               IQCPDSQFECPDFST CCVMVDGSWGCCPMP QASCCEDRVHCCPHG AFCDLVHTRCITPT- -GTHPLAKKLPAQRT NRAV----------    200

 2 PAN TROGLODYTES            ---------------- ---------------- -ASCCEDRVHCCPHG AFCDLVHTRCITPT- -GTHPLAKKLPAQRT NRAV----------     46
 3 MACACA MULATTA             VQCPDSHFECPDLST CCVMVDGSWGCCPMP QASCCEDRVHCCPHG AFCDLVHTRCITPT- -GTHPLAKKIPAQRS NRAV----------    200
 4 BOS TAURUS                 VQCPDKQFQCPNSST CCTMPDGSWGCCPMP QASCCEDKIHCCPHG TSCDLARGRCLSAT- -GTHPLAKKMPAHKT KSSA----------    213
 5 CANIS FAMILIARIS           IQCPDSQFLCPNSST CCTMLDGSWGCCPMP QASCCEDKVHCCPHG TSCDLAHARCLTAT- -GSHPLAKKIPAQRS NR----------    199
 6 RATTUS NORVEGICUS          VQCPGSQFECPDSAT CCIMIDGSWGCCPMP QASCCEDRVHCCPHG ASCDLVHTRCISPT- -GTHPLLKKFPAQRT NRAV----------    199
 7 MUS MUSCULUS               VQCPGSQFECPDSAT CCIMVDGSWGCCPMP QASCCEDRVHCCPHG ASCDLVHTRCVSPT- -GTHTLLKKFPAQKT NRAV----------    212
 8 DASYPUS NOVEMCINCTU        IQCPDSQFECPDFST CCVMVDGSWGCCPMP QASCCEDRVHCCPHG AFCDLVHTRCITPT- -GTHPLAKKLPAQRT NRAV----------    200
 9 MONODELPHIS DOMESTICA      VKCPDSEFECPDEST CCMMQDGSWGCCPMP KASCCEDKVHCCPQG SVCDLAHSRCLTSG- -GTYPLAQKNPAKKI QQEKD---------    201
10 DANIO RERIO GRNA           DVACNDTAACPDGST CCKTKDGGWACCPLP EAVCCEDFIHCCPHG KKCDVAAGSCEDPS- -GSVSWVEKVPVRPI RKQK----------    364
11 DANIO RERIO GRNB           VICPDKISKCPEDTT CCLLETGSYGCCPMP KAVCCSDQKHCCPEG TTCDLIHSTCLSAN- -GVSEMAIKIPAVT- ----------    256
12 TAKIFUGU RUBRIPES          IICPDGKSRCQLGHT CCQLASGAYGCCPLQ QAVCCSDHERCCPAG TRCDLEHDACVSG-- -ATPVPMLRIAAVPG EGTRVFAAT--AAET    256
13 TETRAODON NIGROVIRIDIS     VICPDGKSSCSEGAT CCQLTSGEYGCCPYP QAVCCSDHLHCCPTG TRCDLALSVCVAGP- -GGPSPASKIIAALG PEPKSSGQV--FAGV    215
14 GASTEROSTEUS ACULEATUS GRNA VLCKDGVSECPDGTT CCENPDGKWACCPLP KAVCCEDKTHCCPEG TTCDVEHSKCISLFT KQELPMWAKSPARLR ADWENPKDRFPLSEQ    259
15 GASTEROSTEUS ACULEATUS GRNB VSCPGGKSSCPDSYT CCLLASGDYGCCPYS QAMCCSDHLHCCPSN TICDLAHGVCKDGE- -AIFPLLKKIAAVPN DVTCPDETS--SCPD    264
16 CIONA INTESTINALIS         VQCPDGRSACPDGNT CCKLASGAYGCCPQP KAVCCSDHVHCCPQG YSCNVGSGTCLKQDS LSVVPWMEKQEAVTL NVGMVQCPDGHSACP    331
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GRANULIN MOTIF III

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 1 HOMO SAPIENS               ------------AL S-SSVMCPDARSRCP DGSTCCELPSGKYGC CPMPNATCCSDHLHC CPQDTVCDLIQSKCL SKENATTDLLTKLPA    276

 2 PAN TROGLODYTES            ------------AL S-SSVMCPDARSQCP DGSTCCELPSGKYGC CPMPNATCCSDHLHC CPQDTVCDLVQSKCL SKENATTDLLTKLPA    122
 3 MACACA MULATTA             ------------AL S-SSVMCPDARSQCP DGSTCCELPSGKYGC CPMPNAMCCSDHLHC CPQDTVCDLIQSKCL SKENTTMDLLTKLPA    276
 4 BOS TAURUS                 ------------FF PLPVILCPDGQSQCP DGSTCCKLPTGKYGC CPMPNAICCSDHLHC CPQNTVCDLTQSKCL SKE-NATDLLTKLPA    289
 5 CANIS FAMILIARIS           ------------- --AGVICPDGRSQCP DGSTCCELPSGKYGC CPMPHAICCSDHLHC CPQDTVCDLVRSKCL SKE-NATDLLTKLPA    271
 6 RATTUS NORVEGICUS          ------------AF P-FSVVCPDAKTQCP DDSTCCELPTGKYGC CPMPNAICCSDHLHC CPQDTVCDLIQSKCI SKD-YTTDLMTKLPG    274
 7 MUS MUSCULUS               ------------SL P-FSVVCPDAKTQCP DDSTCCELPTGKYGC CPMPNAICCSDHLHC CPQDTVCDLIQSKCL SKN-YTTDLLTKLPG    287
 8 DASYPUS NOVEMCINCTUS       ------------AL S-SSVMCPDARSRCP DGSTCCELPSGKYGC CPMPNATCCSDHLHC CPQDTVCDLIQSKCL SKENATTDLLTKLPA    276
 9 MONODELPHIS DOMESTICA      ------------VT VTTNRLCPDGRSQCS DGTTCCQLPSGSYGC CPLPNAICCPDHMHC CPQNTVCDLEKSECL SKNGSASGLFVKLPA    278
10 DANIO RERIO GRNA           ----------VAVTQVS SVSSDVPCNDTAACA DGTTCCKTKEGDWAC CPLPEAVCCEDFVHC CPKGKKCNIAAMKCE DPLCTEEPLVKQTPV    446
11 DANIO RERIO GRNB           -----------VLK PKEEVVPCNETVACS SGTTCCKTPEGSWAC CPLPKAVCCEDHIHC CPEGTLCNVAASSCD DPTELSVSVPWMEKV    334
12 TAKIFUGU RUBRIPES          ----------ATPVVP IKIDNNKCDESTTCP GDSTCCRTLEGGWAC CPLAQAVCCDDHVHC CPHDTICNLETQTCD GQSGGRPPLRWVEKV    353
13 TETRAODON NIGROVIRIDIS     ----SPGVATTPVLP FLPDDTKCDDTASCP GDYTCCRTLKGGWAC CPLAQAVCCSDHTHC CPHNTICNLQERTCN SQSGGRPPLRWVEKV    318
14 GASTEROSTEUS ACULEATUS GRNA VPRPGFRHEGNFAVV FAGVSVACDATEACA GNSTCCMTPEGGWSC CPLPEAVDCEDSVHC CPKGRKCNPATQACD SEGCSVPWLQKVPTI    439
15 GASTEROSTEUS ACULEATUS GRNB AALTVVAVATPTEVQ KKVSVLPCNDSVACA EGSTCCGLVEGGWAC CPLPKAVCCEDHQHC CPHGTVCDLEASTCV DSSAG-TSTPWFDKS    402
16 CIONA INTESTINALIS         LTVVPWMEKQDSVAF NVGMVQCPDGRSACP DGNTCCKLASGAYGC CPQPKAVCCSDHVHC CPQGYSCNVGSGTCL KQD-SLSVVPWMEKQ    469
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GRANULIN MOTIF V-a

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 1 HOMO SAPIENS               LSLPD---------- --PQ----ALKRDVP CDNVSSCPSSDTCCQ LTSG-EWGCCPIP-- ---------------- ----------------    392

 2 PAN TROGLODYTES            LSLPD---------- --PQ----ALKRDVP CDNVSSCPSSDTCCQ LMSG-EWGCCPIP-- ---------------- ----------------    238
 3 MACACA MULATTA             LSLPD---------- --PQ----ALKRDVP CHIVS---------- ---------------- ----------------    370
 4 BOS TAURUS                 LSLLD---------- --LG----AVEGDVP CDNVTSCPSSTTCCR LKSG-EWACCPAP-- ---------------- ----------------    405
 5 CANIS FAMILIARIS           LQLLN---------- --PQ----ATENDVP CDNVTSCPSSNTCCR LMSG-EWGCCPAP-- ---------------- ----------------    387
 6 RATTUS NORVEGICUS          LSLPD---------- --PQ----ILKNDVP CDDFSSCPSNNTCCR LSSG-DWGCCPIP-- ---------------- ----------------    390
 7 MUS MUSCULUS               LRLPD---------- --PQ----ILKSDTP CDDFTRCPTNNTCCK LNSG-DWGCCPIP-- ---------------- ----------------    403
 8 DASYPUS NOVEMCINCTUS       LSLPD---------- --PQ----ALKRDVP CDNVSSCPSSDTCCQ LTSG-EWGCCPIP-- ---------------- ----------------    392
 9 MONODELPHIS DOMESTICA      LAAVG---------- ----------NVP CDNTTSCPSETTCCV LESG-AWGCCPAP-- ---------------- ----------------    388
10 DANIO RERIO GRNA           PIRKQKV--------- --AVTTASSASSDVP CNDTAACPDGSTCCK TKDG-GWACCPLP-- ---------------- ----------------    578
11 DANIO RERIO GRNB           ATR------------ ---AVAMPTLPARNM CDAQTSCPRDTTCCF MDQTRKWGCCPLP-- ---------------- ----------------    525
12 TAKIFUGU RUBRIPES          QEEPS---------- --GAASGPARPAGVM CDDRTSCPRDTSCCF MQETRRWGCCPVP-- ---------------- ----------------    476
13 TETRAODON NIGROVIRIDIS     VEP------------ ---SPALPAQLAEVV CDNQTSCPSHTTCCF VQKWQKFGCCPVPNV GPAVQRHRRRGQALT F----------------    459
14 GASTEROSTEUS ACULEATUS GRNA VTAGPFPQSRATITK GEEATKAPEEDEVVQ CDSRTSCPQSNTCCF MAESQKWGCCPLPKT LCHTHCSHCRIDRFC TNHISAVKQPAEISD    606
15 GASTEROSTEUS ACULEATUS GRNB ATR------------ ---AVAMPTLPARNM CDAQTSCPRDTTCCF MDQTRKWGCCPLP-- ---------------- ----------------    525
16 CIONA INTESTINALIS         TFN------------ ---------VGMVQC PDGRSACPDGNTCCK LASG-AYGCCPQP-- ---------------- ----------------    587
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GRANULIN MOTIF V-b

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 1 HOMO SAPIENS               ---------------- ---------------- ---------------- ---EAVCCSDHQHCC PQGYTCVAEQQ-CQR -GSEIVA-GLEKMPA    431

 2 PAN TROGLODYTES            ---------------- ---------------- ---------------- ---EAVCCSDHQHCC PQGYTCVAEQQ-CQR -GSEIVA-GLEKMPA    277
 3 MACACA MULATTA             ---------------- ---------------- ---------------- ---------------- ---------------- ----------------    370
 4 BOS TAURUS                 ---------------- ---------------- ---------------- ---EAVCCSDHQHCC PKGYTCVARRH-CKR -GKQVVT-GLDKVPA    444
 5 CANIS FAMILIARIS           ---------------- ---------------- ---------------- ---EAVCCSDHQHCC PHGYTCLDDQH-CQR -GSKVVS-GLEKMPA    426
 6 RATTUS NORVEGICUS          ---------------- ---------------- ---------------- ---EAVCCLDHQHCC PQGFKCMDEQY-CQK -GDRMVA-GLEKMPV    429
 7 MUS MUSCULUS               ---------------- ---------------- ---------------- ---EAVCCSDNQHCC PQGFTCLAQQY-CQK -GDTMVA-GLEKIPA    442
 8 DASYPUS NOVEMCINCTUS       ---------------- ---------------- ---------------- ---EAVCCSDHQHCC PQGYTCVAEQQ-CQR -GSEIVA-GLEKMPA    431
 9 MONODELPHIS DOMESTICA      ---------------- ---------------- ---------------- ---QAVCCPDHKHCC PHGFVCSPDQQ--CKS -GQKAVP-WLEKIAA    426
10 DANIO RERIO GRNA           ---------------- ---------------- ---------------- ---EAVCCEDFIHCC PHGKKCNVAAGSCDD --PSGSVPWVEKVPV    618
11 DANIO RERIO GRNB           ---------------- ---------------- ---------------- ---QAVCCADQEHCC PQGYTCDLAQSSCVR -SGLPSMAWFRKEPA    495
12 TAKIFUGU RUBRIPES          ---------------- ---------------- ---------------- ---NAVCCEDGDHCC PRGHRCDPHRRSCSK -GPLVTP-WFTKLSA    516
13 TETRAODON NIGROVIRIDIS     ---------------- ---------------- ---------------- ARPQAVCCEGGSHCC PMGHRCDPYRSSCSK -GPLVTP-WFTKLSA    502
14 GASTEROSTEUS ACULEATUS GRNA FGNRLPFTSRTCSNT KINALRCLHSPSFEE LRNGIADVKLALPPP PPPQAVCCSDGNHCC PTDYTCDVEKTTCTK G--EVVIPWVTTKLPA    694
15 GASTEROSTEUS ACULEATUS GRNB ---------------- ---------------- ---------------- ---KAVCCHDGDHCC PSGHTCEPHRSSCSK -GPLVLVPWFSKLSA    566
16 CIONA INTESTINALIS         ---------------- ---------------- ---------------- ---KAVCCSDHVHCC PQGYSCNVGSGTCLK QDSLSVVPWMEKQGE    629
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GRANULIN MOTIF VII

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 1 HOMO SAPIENS               ---------VKDVEC G--EGHFCHDNQTCC RDNRQGWACCPYRQG VCCADRRHCCPAGFR CAARGTKCLRREAPR WDAPLRDPALRQLL-    593

 2 PAN TROGLODYTES            ---------VKDVEC G--EGHFCHDNQTCC RDNRQGWACCPYRQG VCCADRRHCCPAGFR CAARGTKCLRREAPR WDAPLRDPALRQLL-    439
 3 MACACA MULATTA             ------------SW -----HFCHDNQTCC RDNRGGWACCPYRQG ICCADRRHCCPAGFR CAARGAKCLRREALR WDAPLRDPALRQLL-    441
 4 BOS TAURUS                 ---------MGNVEC G--ARHFCHDNQTCC PDSQGGWACCPYRKG TCCGDKHHCCP----- ---------------- ----------------    573
 5 CANIS FAMILIARIS           ---------VGNVEC G--EGHFCHDNQTCC RDSRGGWACCPYHQG VCCADQRHCCPTGFH CGAKGTKCLRRESLR WDMPLRDPAPRPLL-    588
 6 RATTUS NORVEGICUS          ----------GNVEC G--AGHFCHDNQSCC KDSQGGWACCPYVKG VCCRDGRHCCPIGFH CSAKGTKCLRKKTPR WDILLRDPAPRPLL-    589
 7 MUS MUSCULUS               ----------GNVEC G--EGHFCHDNQTCC KDSAGVWACCPYLKG VCCRDGRHCCPGGFH CSARGTKCLRKKIPR WDMFLRDPVPRPLL-    602
 8 DASYPUS NOVEMCINCTUS       ---------VKDVEC G--EGHFCHDNQTCC RDNRQGWACCPYRQG VCCADRRHCCPAGFR CAARGTKCLRREAPR WDAPLRDPALRQLL-    593
 9 MONODELPHIS DOMESTICA      ----------KGDIQC D--GKHFCHSHQTCC LARGGRWACCPLDKG VCCADGQHCCPNGFH CRAKGTKCLRRKNLR WDVLWELL--------    580
10 DANIO RERIO GRNA           P-KL----DLGVVKC D--EQSSCSADSTCC LLSKDETGCCPFPEA VCCPDQKHCCPEGYR CDLRRRSCVKTTRLY VEITQLTHIRSNKPQ    786
11 DANIO RERIO GRNB           -SDE----LLGHEDV KCDSSTSCPSGSTCC ILPTGQWGCCPLVKA VCCKDMKHCCPMGYK CDPKVQGCTKSSS-- ----------------    648
12 TAKIFUGU RUBRIPES          RSRG----AQGVLSC GGTGEFHCPKEDTCC PTSATEWACCPSPRA VCCSDQKHCCPAGFS CDPSGG-CVQDLSSW DAWFDRS--------    676
13 TETRAODON NIGROVIRIDIS     GAGR----PEGLAPC GGTGEFHCPKEDTCC PTSATEWACCPSPGA VCCSDHKHCCPAGFS CDLKAGGCVRDPSPW DAWFHRPVRSRL---    668
14 GASTEROSTEUS ACULEATUS GRNA AEPTSSPSEQGDVVC D--DQTSCPDGQTCC RTSATTWGCCPAPNA VCCSDMQHCCPEGHT CTETGG-CTGNNVPH WHKWQVFFSNKKRSL    865
15 GASTEROSTEUS ACULEATUS GRNB PVDH----EDVACDV G--GEFRCPGRATCC RVSASEWGCCPSPQA VCCPDSKHCCPAGYS CDPKAGGCSRPQLTW DGKSDFVPHGL-----    732
16 CIONA INTESTINALIS         N--------VGMVQC P-DGRSACPDGNTCC KLASGAYGCCPQPKA VCCSDHVHCCPQGYS CNVGSGTCLKQDSLS VVPWMEKQILTKEKC    799
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b HUMAN PROGRANULIN

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   1                                MWTLVSWVALTAGLVAGTRCPDGQFCPVACCLDPGGASYSCCRPLLDKWPTTLSRHLG  58
  59 -GPCQVDAHCSAGHSCIFTVSGTSSCCPFPEAVACGDGHHCCPRGFHCSADGRSCFQRSGNNSVGA ---------------- 123
 124 IQCPDSQFECPDFSTCCVMVDGSWGCCPMPQASCCEDRVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPAQRTNRAVALSSS- 205
 206 VMCPDARSRCPDGSTCCELPSGKYGCCPMPNATCCSDHLHCCPQDTVCDLIQSKCLSKENATTDLLTKLPAHTVGD------- 281
 282 -VKCDMEVSCPDGYTCCRLQSGAWGCCPFTQAVCCEDHIHCCPAGFTCDTQKGTCEQGPHQVPWMEKAPAHLSLPDPQALKRD 363
 464 -VPCDNVSSCPSSDTCCQLTSGEWGCCPIPEAVCCSDHQHCCPQGYTCVAEQQCQRGSEIVAGLEKMPARRASLSHPRD---- 441
 542 -IGCDQHTSCPVGQTCCPSLGGSWACCQLPHAVCCEDRQHCCPAGYTCNVKARSCEKEVVSAQPATFLARSPHVGVKD----- 518
 619 -VECGEGHFCHDNQTCCRDNRQGWACCPYRQGVCCADRRHCCPAGFRCAARGTKCLRREAPRWDAPLRDPALRQLL      593

CONSENSUS --C-D----CPD--TCC----G-WGCCP-----CC-D--HCCP----CD--G--C---T-----------------------
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necessary for the proper protein conformation. Therefore, his substitution with the His residue may also change PGRN 3D structure with consequences at functional level. The effect of the Thr182Met mutation is less clear since it is just outside the granulin motif. This amino acid is conserved between mammals and was not detected in controls.

Consistent with other *PGRN* studies, the clinical presentation in patients carrying the pathogenic mutations is characterized by a large variation in age at onset and by occurrence of symptoms of nonfluent aphasia, whereas semantic deficits were more often seen in patients with missense and intronic *MAPT* mutations within the same cohort.[21] The HTFD3 family, in particular, shows a large variation in age at onset. The high variability is further confirmed by the presence of a 72-year-old healthy carrier. Our and other findings show that a significant proportion of patients remain unaffected until old age suggesting therefore an interplay of several genetic and/or environmental factors in the disease development.

The percentage of *PGRN* mutations detected in our familial FTLD cohort (up to ~7% by including the two highly conserved missense mutations) is lower compared to the much higher frequency observed in other studies where *PGRN* mutations explain up to ~25% of familial FTLD.[10,15] The lower frequency of *PGRN* mutations in our group might reflect differences in patients recruitment methods, as the *MAPT* mutations in the Belgian cohort account for only to 7% of all familial cases compared to 14% detected in this cohort.[9,10,14] In addition, in the studies by Cruts *et al*.[10] and Baker *et al*.[9] a strong founder effect among probands carrying the IVS0+5G>C and Arg493X was observed, whereas we restricted our estimation of mutation frequency to independent patients only.

In addition, geographical differences in frequencies may also play a role, as seen in *MAPT* studies, and they cannot be ruled out until more reports will allow a better estimate of *PGRN* mutation frequency in familial FTLD.

In summary, mutations in *PGRN* explain only part of FTLD in our cohort and they are absent in ~80% of cases including familial FTLD+MND as well as FTLD-U without MND strongly suggesting that we are only beginning to unravel the molecular pathways leading to FTLD and that additional genes contribute to the disease pathogenesis.

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References

1 Neary D, Snowden JS, Gustafson L *et al*: Frontotemporal lobar degeneration – a consensus on clinical diagnostic criteria. *Neurology* 1998; **51**: 1546–1554.
2 Forman MS, Farmer J, Johnson JK *et al*: Frontotemporal dementia: clinicopathological correlations. *Ann Neurol* 2006; **59**: 952–962.
3 McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ: Clinical and pathological diagnosis of frontotemporal dementia: report of the Work Group on Frontotemporal Dementia and Pick's Disease. *Arch Neurol* 2001; **58**: 1803–1809.
4 Rosso SM, Kamphorst W, de Graaf B *et al*: Familial frontotemporal dementia with ubiquitin-positive inclusions is linked to chromosome 17q2l-22. *Brain* 2001; **124**: 1948–1957.
5 Skibinski G, Parkinson NJ, Brown JM *et al*: Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* 2005; **37**: 806–808.
6 Vance C, Al-Chalabi A, Ruddy D *et al*: Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. *Brain* 2006; **129**: 868–876.
7 Hosler BA, Siddique T, Sapp PC *et al*: Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. *JAMA* 2000; **284**: 1664–1669.
8 Hutton M, Lendon CL, Rizzu P *et al*: Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 1998; **393**: 702–705.
9 Baker M, Mackenzie IR, Pickering-Brown SM *et al*: Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 2006; **442**: 916–919.
10 Cruts M, Gijselinck I, van der Zee J *et al*: Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 2006; **442**: 920–924.
11 He Z, Bateman A: Progranulin (granulin–epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. *J Mol Med* 2003; **81**: 600–612.
12 The Lund and Manchester Groups: Clinical and neuropathological criteria for frontotemporal dementia. *J Neurol Neurosurg Psychiatry* 1994; **57**: 416–418.
13 Rizzu P, van Mil SE, Anar B *et al*: CHMP2B mutations are not a cause of dementia in Dutch patients with familial and sporadic frontotemporal dementia. *Am J Med Genet B Neuropsychiatr Genet* 2006; **141**: 944–946.
14 Rosso SM, Kaat LD, Baks T *et al*: Frontotemporal dementia in The Netherlands: patient characteristics and prevalence estimates from a population-based study. *Brain* 2003; **126**: 2016–2022.
15 Gass J, Cannon A, Mackenzie IR *et al*: Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Hum Mol Genet* 2006; **15**: 2988–3001.
16 Sampathu DM, Neumann M, Kwong LK *et al*: Pathological heterogeneity of frontotemporal lobar degeneration with

Figure 2 (**a**) Progranulin motif alignment between species. Human progranulin sequence is given in blue. Only progranulin motifs containing mutations are shown. Progranulin motifs are underlined. Conserved amino acids described by He and Bateman[11] are turquoise and nonconserved amino acids are shown in yellow. The possible Thr182Met, Pro233His and Trp541Cis mutations are shown in red. The Gly414Val polymorphism is given in green. (**b**) Conservation of amino acids between progranulin motifs. The longest human progranulin isoform is shown. All human progranulin motifs are aligned. Conserved amino acids described by He and Bateman[11] are turquoise and non-conserved amino acids are shown in yellow. Consensus depicts the consensus motif adapted from He and Bateman.[11] The possible Thr182Met, Pro233His and Trp541Cys mutations are shown in red. The Gly414Val polymorphism is given in green.

ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. *Am J Pathol* 2006; **169**: 1343–1352.
17 Hoque M, Tian B, Mathews MB, Pe'ery T: Granulin and granulin repeats interact with the Tat.P-TEFb complex and inhibit Tat transactivation. *J Biol Chem* 2005; **280**: 13648–13657.
18 Vranken WF, Chen ZG, Xu P, James S, Bennett HP, Ni F: A 30-residue fragment of the carp granulin-1 protein folds into a stack of two beta-hairpins similar to that found in the native protein. *J Pept Res* 1999; **53**: 590–597.
19 Tolkatchev D, Ng A, Vranken W, Ni F: Design and solution structure of a well-folded stack of two beta-hairpins based on the amino-terminal fragment of human granulin A. *Biochemistry* 2000; **39**: 2878–2886.
20 Vranken WF, James S, Bennett HP, Ni F: Solution structures of a 30-residue amino-terminal domain of the carp granulin-1 protein and its amino-terminally truncated 3–30 subfragment: implications for the conformational stability of the stack of two beta-hairpins. *Proteins* 2002; **47**: 14–24.
21 Neary D, Snowden J, Mann D: Frontotemporal dementia. *Lancet Neurol* 2005; **4**: 771–780.