Alzheimer and Parkinson Diagnoses in Progranulin Null Mutation Carriers in an Extended Founder Family

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Background: Progranulin gene (PGRN) haploinsufficiency was recently associated with ubiquitin-positive frontotemporal lobar degeneration linked to chromosome 17q21 (FTLDU-17).

Objective: To assess whether PGRN genetic variability contributed to other common neurodegenerative brain diseases, such as Alzheimer disease (AD) or Parkinson disease (PD).

Design: Mutation analysis of PGRN.

Setting: Memory Clinic of the Middelheim General Hospital.

Patients: We analyzed 666 Belgian patients with AD and 255 with PD.

Main Outcome Measures: Results of PGRN sequencing, PGRN transcript analysis, short tandem repeat genotyping, and neuropathologic analysis.

Results: We identified 2 patients with AD and 1 patient with PD who carried the null mutation IVS0+5G>C, which we reported earlier in an extensively characterized Belgian founder family, DR8, segregating FTLDU. Postmortem pathologic diagnosis of the patient with PD revealed both FTLDU and Lewy body pathologic features. In addition, we identified in PGRN only 1 other null mutation, the nonsense mutation p.Arg535X, in 1 patient with probable AD. However, in vitro analysis predicted a PGRN C-truncated protein, although it remains to be elucidated if this shortened transcript leads to haploinsufficiency.

Conclusions: Our mutation data indicated that null mutations are rare in patients with AD (3/666=0.45%) and PD (1/255=0.39%). Also, AD and PD clinical diagnoses in patients who carry PGRN null mutations likely result from etiologic heterogeneity rather than PGRN haploinsufficiency.

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In families segregating ubiquitin-positive frontotemporal lobar degeneration linked to chromosome 17q21 (FTLDU-17), null mutations have been identified in the progranulin gene (PGRN), resulting in haploinsufficiency. Most PGRN mutations result in loss of the PGRN transcript after nonsense-mediated decay (NMD) or nuclear retention and degradation of messenger RNA (mRNA). Two PGRN mutations affect the translation initiation codon, preventing translation. Apart from null mutations, missense mutations scattered throughout the protein have been identified in patients with frontotemporal lobar degeneration (FTLD) and control individuals. The signal peptide mutation, p.Ala9Asp, is expected to lead to loss of protein due to protein mislocalization and subsequent degradation. Also, reduced levels of the respective mutant transcript have been observed that might contribute to haploinsufficiency. We recently provided in silico evidence that at least some of the missense mutations observed in patients with FTLD affect PGRN structure and/or stability, again resulting in loss of functional protein by protein degradation.

Frontotemporal lobar degeneration marks a subtype of neurodegenerative dementia in which the presenting clinical features consist of behavioral dysfunction and personality changes, often with language impairments. Despite the fact that these symptoms are noticeable early in disease in most patients with FTLD, a differential diagnosis of FTLD or Alzheimer disease (AD) often remains difficult to estab-
lish. Furthermore, patients with FTLD and AD often develop parkinsonian symptoms later in their disease and in PGRN null mutation carriers, parkinsonism has already been reported. On the other hand, dementia is common in patients with Parkinson disease (PD). The PGRN gene encodes a precursor protein, PGRN, which contains a secretory signal peptide and 7.5 tandemly repeated granulin domains (GRN A through G and paragranulin), which are characterized by a highly conserved motif of 12 cysteine residues that form 6 disulfide bridges. The PGRN gene is expressed in a wide variety of tissues, including neurons in the cerebral cortex, the Purkinje cells in the cerebellum, and pyramidal cells in the hippocampus. PGRN and at least some of the GRNs have been shown to have growth modulatory properties, and PGRN transcripts have been found up-regulated in neurodegenerative disorders, such as Creutzfeld-Jakob disease and amyotrophic lateral sclerosis. These characteristics, along with the fact that PGRN null mutations cause FTLD, support a role for PGRN in neuronal survival.

Because patients with FTLD, AD, and PD display a broad spectrum of overlapping clinical symptoms and PGRN potentially exerts a role as a neuronal survival factor, we argued that PGRN mutations might potentially be implicated in the etiology of AD and PD. Consequently, we performed an extensive mutation analysis of PGRN in large groups of well-characterized patients with AD and PD.

METHODS

STUDY GROUPS

The AD group consisted of 666 patients (mean±SD age at onset, 74.6±8.8 years; 65.5% female) derived from a prospective study of neurodegenerative and vascular dementia in Belgium. All patients were examined at the Memory Clinic of the Middelheim General Hospital, Antwerp, Belgium (by P.P.D.D., S.E., and B.A.P.), and underwent diagnostic neuropsychological examination (including Middelheim Frontality Score [MFS]) and structural (computed tomography and magnetic resonance imaging) and functional neuroimaging (single-photon emission computed tomography). All patients and control individuals.20

PGRN SEQUENCING

In all patients and control individuals, we performed a mutation analysis of all coding exons, 1 to 12, and noncoding exon 0, as previously described. A total of 20 ng of genomic DNA (gDNA) was amplified by polymerase chain reaction (PCR), and amplification products were sequenced (Applied Biosystems 3730xl DNA Analyzer using the Big Dye Terminator v3.1 Cycle Sequencing kit; Applied Biosystems, Foster City, California). Sequences were analyzed using novoSNP.

PGRN TRANSCRIPT ANALYSIS

Lymphocytes were isolated from total blood with Ficoll density gradient centrifugation (Greiner Bio-One, Wemmel, Belgium), and mRNA was isolated from lymphocytes using the Chemagic mRNA Direct Kit (Chemagen, Leiden, the Netherlands). First-strand complementary DNA (cDNA) was synthesized using the SuperScript III First-Strand Synthesis System for RT (reverse transcription)—PCR kit (Invitrogen, Carlsbad, California) and random hexamer primers. After PCR amplification of part of the transcript (exons 5-6 to 3′ untranslated region), genotypes at the mutated position were generated by sequencing as described.

SHORT TANDEM REPEAT GENOTYPING

In patients carrying IVS0 + 5G>C and their family members, we genotyped 14 STR markers located in and flanking the 8-CM ancestral DR8 haplotype, as previously described. Allele frequencies for each STR marker were estimated in 102 unrelated Belgian control individuals.

NEUROPATHOLOGIC ANALYSIS

Brain autopsy was performed on patient DR205.1, who had given prior informed consent, at the University Hospital Gasthuisberg. Brain hemispheres were fixed in buffered formalin and tissue was further processed for paraffin, embedding from the right and left frontal and parietal cortices, hippocampus, basal ganglia, midbrain,pons, medulla oblongata, and cerebellum. Sections 10 µm thick were sliced and stained with hematoxylin-eosin, cresyl violet, Bodian, and Gallyas. Sections 5 µm thick were also sliced from all brain regions, and immunohistochemical analysis was performed with the following antibodies: 4G8 (anti-Aβ; Senetek, Maryland Heights, Missouri), AT8 (against abnormally phosphorylated paired helical filament-tau; Innogenetics, Zwijnaarde, Belgium), ubiquitin (UBI) (Dako, Glostrup, Denmark), α-synuclein (SNCA) (Dako), anti-glial fibrillary acidic protein (GFAP) (Dako), and rabbit TAR DNA-binding protein 43 antisera (TDP-43) (Proteintech Group Inc, Chicago, Illinois). Antigen retrieval for Aβ immu-
no histochemical analysis was performed by treating sections with 98% formic acid for 5 minutes at room temperature for 4G8, SNCA, and TDP-43 and by boiling in citrate buffer (pH = 6) for GFAP and UBI. All dilutions were made in 0.1M phosphate-buffered saline with 0.1% bovine serum albumin. Staining was performed with appropriate secondary antibodies and streptavidin–biotin–horseradish peroxidase with chromogen 3,3'-diaminobenzidine (Roche, Nutley, New Jersey), as previously described.21

RESULTS

PGRN MUTATION ANALYSES

Direct exonic sequencing of PGRN identified 1 patient with AD who carried a novel nonsense mutation and 2 patients with AD and 1 with PD with a known splice-donor site mutation in intron 0 (Table 1). The g.103432C>T mutation is located in exon 11 of PGRN (EX11) and predicts a premature termination codon (PTC) at position 535 and a C-truncated protein, p.Arg535X. Comparison of sequences obtained from the patient’s cDNA and from gDNA (Figure 1) showed equal amounts of wild-type and mutant alleles, indicating that the mutant transcript escaped NMD.

The g.96241G>C mutation identified in 2 patients with AD and 1 with PD is located close to the splice-donor site of intron 0 (IVS0) and is known to prevent splicing of intron 0, leading to loss of the mutant transcript.2 Haplotype sharing analyses using 14 STR markers flanking PGRN indicated that all 3 carriers shared 1.61 cM (4.37 Mb) of the 8.0-cM disease haplotype of founder family DR8.20 (Table 2). Pedigree analysis could not identify other living affected relatives of AD patient DR142.1 and PD patient DR205.1, preventing cosegregation studies of IVS0 g.96241G>C with disease. The other patient with AD was the first cousin of the index patient DR25.1 of 1 known branch of the Belgian DR8 founder family and was subsequently labeled DR25.14.

CLINICAL CHARACTERISTICS

Patient DR181.1, who carried p.Arg535X, was clinically diagnosed as having probable AD 1 year after disease onset. She presented with impairment of recent memory and attention deficits (Table 3). Neuropsychological examination revealed a Mini-Mental State Examination score of 19/30 with severe recent memory deficits and moderate impairment of praxis, concentration, long-term memory, and orientation in time. Three years later, her cognitive

<table>
<thead>
<tr>
<th>Patient</th>
<th>Presentation</th>
<th>Age at Onset, y</th>
<th>Family Historya</th>
<th>Locationb</th>
<th>Mutation</th>
<th>Alias</th>
<th>Genomicc</th>
<th>Predicted RNA</th>
<th>Predicted Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR142.1</td>
<td>AD</td>
<td>66</td>
<td>?</td>
<td>IVS 0</td>
<td>IVS0 + 5G&gt;C</td>
<td>g.96241G&gt;C</td>
<td>...</td>
<td>p.0</td>
<td></td>
</tr>
<tr>
<td>DR25.14</td>
<td>AD</td>
<td>76</td>
<td>+</td>
<td>IVS 0</td>
<td>IVS0 + 5G&gt;C</td>
<td>g.96241G&gt;C</td>
<td>...</td>
<td>p.0</td>
<td></td>
</tr>
<tr>
<td>DR205.1</td>
<td>PD</td>
<td>54</td>
<td>+</td>
<td>IVS 0</td>
<td>IVS0 + 5G&gt;C</td>
<td>g.96241G&gt;C</td>
<td>...</td>
<td>p.0</td>
<td></td>
</tr>
<tr>
<td>DR181.1</td>
<td>AD</td>
<td>72</td>
<td>?</td>
<td>EX 11</td>
<td>EX11 + 190C&gt;T</td>
<td>g.103432C&gt;T</td>
<td>c.1603C&gt;T</td>
<td>p.Arg535X</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; EX, exon; IVS, intron; PD, Parkinson disease.  

a Family history of dementia: plus sign indicates that patient had first-degree family members who were affected; question mark, unknown.  
b Exon numbering starts with noncoding first exon EX 0.  
c Numbering relative to the reverse complement of GenBank Accession No. AC003043.1 and starting at nucleotide 1.  
d Numbering according to the largest PGRN transcript (GenBank Accession No. NM_002087.2) and starting at the translation initiation codon.  
e Numbering according to the largest PGRN isoform (GenPept Accession No. NP_002078.1).
state had severely deteriorated (Mini-Mental State Examination score of 3/30). The patient also displayed severe frontal lobe signs (MFS of 7/10). A CSF biomarker analysis showed decreased Aβ42 and increased total tau levels, a finding compatible with AD.22 However, CSF P-tau181P levels were within normal limits. Although patient DR181.1 had no relatives with dementia, a sibling of the patient was diagnosed as having PD.

In Table 3 we summarize the medical information of 16 patients of the DR8 founder family who carried the IVS0 + 5G>C null mutation. Both AD patients DR25.14 and DR142.1 presented with symptoms of forgetfulness. In addition to severe impairment of recent memory, which first became apparent at the age of 76 years, patient DR25.14 had deficits of long-term memory. She became apathetic, displaying lack of initiative. Furthermore, reduced verbal fluency was noted, but naming was unaffected. The patient was disoriented in both time and space and displayed impaired problem solving. The CSF biomarker profile was typical for AD, with decreased levels of Aβ42 and increased levels of total tau and P-tau181P. Patient DR25.14 had a family history of dementia, with a mother, a maternal aunt, and a grandmother who had late-onset dementia and 2 first cousins who were diagnosed with FTLD (Figure 2). For patient DR142.1, early symptoms included repetitive telephone calls and repetition of stories and questions at the age of 66 years. Because of disorientation in space, she got lost on several occasions. Toward later stages of disease, a loss of decorum became obvious. A year before her death, spontaneous speech was impaired, and she displayed verbal and motor stereotypes. Age at onset varied considerably between these patients with AD (ie, 66 and 76 years), which is in accordance with the wide onset range noted in patients with FTD/amygdala. Both patients had an MFS score of 4/10.

The PD patient DR205.1 had his condition diagnosed at the age of 56 years, 1 year after the onset of symptoms. Symptoms included global and cogwheel rigidity, hypomimia, bradykinesia, shuffling gait, postural instability, and a discrete resting tremor. The patient responded well to levodopa treatment. The patient reported loss of concentration, but a neuropsychological examination revealed no abnormalities. Two years later, however, progressive memory problems were recorded, accompanied by apathy, hypophonia, and reduced verbal expression. Behavioral observation and neuropsychological testing revealed loss of insight and judgment, changes in sexual behavior, impaired control of emotions, mutism, and echolalia, with comparatively spared memory and spatial abilities. The cognitive problems were not typical for Lewy body disease or PD with dementia but were compatible with pronounced frontal dysfunction in light of PD or with FTLD. Patient DR205.1 had an MFS of 6/10. The mother and 2 maternal aunts of patient DR205.1 had late-onset dementia, but there were no relatives with PD.

Since the identification of the DR8 founder mutation and subsequent PGRN screening in a series of patients with FTLD, we have identified 1 other patient with FTLD (DR119.1) who had the IVS0 + 5G>C mutation, defining a novel branch of founder family DR8.2 Patient DR119.1 presented with word-finding difficulties at the age of 45 years and social withdrawal.23 Memory was preserved. Her speech was characterized by shortening and simplification of sentences and phonemic paraphasia, which led to a diagnosis of progressive nonfluent aphasia. The patient’s father died at the age of 65 years after a 5-year period of progressive language impairment and behavioral changes. Furthermore, patient DR8.15, a first cousin of the index patient DR8.1, developed primary progressive aphasia at the age of 63 years. With unimpaired

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Table 2. Haplotype Sharing of STR Markers in IVS0 + 5G>C Mutation Carriers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genetic Location, cM</th>
<th>Physical Location, Mb</th>
<th>Linked Allele</th>
<th>Frequency Linked Allele, %</th>
<th>DR142.1</th>
<th>DR25.14</th>
<th>DR205.1</th>
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<tbody>
<tr>
<td>D17S1818</td>
<td>6.04</td>
<td>34.42</td>
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<td>...</td>
<td>446</td>
<td>430</td>
<td>430</td>
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<tr>
<td>D17S1814</td>
<td>6.48</td>
<td>35.37</td>
<td>465</td>
<td>19</td>
<td>465</td>
<td>465</td>
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<td>D17S800</td>
<td>6.01</td>
<td>36.31</td>
<td>367</td>
<td>10</td>
<td>361</td>
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<td>D17S1787</td>
<td>6.01</td>
<td>36.98</td>
<td>181</td>
<td>35</td>
<td>181</td>
<td>181</td>
<td>181</td>
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<td>D17S1793</td>
<td>6.09</td>
<td>37.61</td>
<td>392</td>
<td>81</td>
<td>392</td>
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<tr>
<td>D17S951</td>
<td>6.62</td>
<td>39.18</td>
<td>143</td>
<td>23</td>
<td>143</td>
<td>143</td>
<td>143</td>
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<td>PGRN</td>
<td>...</td>
<td>...</td>
<td>39.78</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>D17S1861</td>
<td>6.62</td>
<td>40.16</td>
<td>278</td>
<td>6</td>
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<td>D17S934</td>
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<td>359</td>
<td>27</td>
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<tr>
<td>Chr17-16</td>
<td>...</td>
<td>40.68</td>
<td>401</td>
<td>22</td>
<td>401</td>
<td>401</td>
<td>399</td>
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<tr>
<td>D17S810</td>
<td>6.62</td>
<td>40.84</td>
<td>186</td>
<td>30</td>
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<tr>
<td>MAPT</td>
<td>...</td>
<td>41.33</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>D17S920</td>
<td>6.14</td>
<td>42.17</td>
<td>326</td>
<td>64</td>
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<td>326</td>
<td>326-332</td>
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<td>277</td>
<td>265</td>
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<td>Chr17-43</td>
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<td>233</td>
<td>47</td>
<td>233</td>
<td>233</td>
<td>239</td>
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<tr>
<td>D17S1795</td>
<td>6.44</td>
<td>45.28</td>
<td>...</td>
<td>...</td>
<td>399</td>
<td>397</td>
<td>391</td>
</tr>
</tbody>
</table>

Abbreviations: MAPT, microtubule-associated protein tau; STR, short tandem repeat.

a Bold indicates minimal founder haplotype (1.61 cM).
b Ancestral haplotype identified in the DR8 founder family.
c Allele frequencies were calculated in 92 control chromosomes. Genetic locations of the STR markers were obtained from the Marshfield gender-averaged map.

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Table 3. Overview of Clinical and Pathologic Characteristics of Mutation Carriers

<table>
<thead>
<tr>
<th>Patient/ Sex</th>
<th>Clinical Diagnosis</th>
<th>Age at Onset, y</th>
<th>Presenting Impairments</th>
<th>Additional Features</th>
<th>Structural Neuroimaging (CT/MRI)</th>
<th>Functional Neuroimaging (SPECT/PET)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR181.1/F</td>
<td>AD</td>
<td>72</td>
<td>Memory; concentration</td>
<td>CSF A1-42 and total tau typical for AD, P-tau normal for age</td>
<td>Cortical and subcortical atrophy; cerebellar atrophy; discrete PWML</td>
<td>Relative parietotemporal HP with involvement of both frontal lobes; diastasis of frontal cortical activity</td>
<td>NA</td>
</tr>
<tr>
<td>DR2.1 (DR2 III-8)/M</td>
<td>FTD</td>
<td>66</td>
<td>Memory, concentration</td>
<td>Disinhibition, verbal fluency</td>
<td>Global mainly subcortical atrophy (CT)</td>
<td>Relative bilateral frontal, parietal and temporal HP, L&gt;R (SPECT)</td>
<td>NA</td>
</tr>
<tr>
<td>DR2.3 (DR2 III-6)/F</td>
<td>PNFA</td>
<td>63</td>
<td>PNFA, apathy</td>
<td>Global corticocortical atrophy R&gt;L; PWML (MRI)</td>
<td>Relative frontal and frontoparietal HP, L&gt;R; relative HP of left thalamus; diastasis of frontal cortical activity (SPECT)</td>
<td>Frontotemporal atrophy with FTDU; concurrent AD (Braak A-II)</td>
<td>NA</td>
</tr>
<tr>
<td>DR2.17 (DR2 III-10)/M</td>
<td>FTD</td>
<td>69</td>
<td>Spontaneous speech, perseveration, dysarthria, apathy</td>
<td>Global moderate cortical and subcortical atrophy (MRI)</td>
<td>Frontotemporal atrophy and subcortical atrophy L&gt;R (MRI)</td>
<td>Relative bilateral frontal HP, L&gt;R (SPECT)</td>
<td>FTDU; frontal atrophy &gt; temporal; marked neuronal loss in 2C of the SN; rare brainstem Lewy bodies</td>
</tr>
<tr>
<td>DR8.1 (DR8 III-28)/F</td>
<td>FTD</td>
<td>62</td>
<td>Word finding, apathy, disinhibition, Memory</td>
<td>Spontaneous speech, echolalia, apathy, As behavior</td>
<td>Global cortical and subcortical atrophy (CT)</td>
<td>Severe relative bifrontal HP, L&gt;R (SPECT)</td>
<td>NA</td>
</tr>
<tr>
<td>DR8 III-18 (DR8 III-18)/F</td>
<td>FTD</td>
<td>51</td>
<td>Spontaneous speech, echolalia, apathy, As behavior</td>
<td>Memory, control of emotions, loss of decorum</td>
<td>Frontotemporal bilateral cortical and subcortical atrophy (MRI)</td>
<td>Postcontusional bilateral frontal and R temporal (MRI)</td>
<td>NA</td>
</tr>
<tr>
<td>DR8.15/F</td>
<td>PPA</td>
<td>63</td>
<td>Aspraxia, verbal apraxia</td>
<td>Repetitive movement of the hands</td>
<td>Cortical and subcortical frontal atrophy; periventricular white matter lesions (CT)</td>
<td>Severe relative bilateral frontal, parietal, and temporal HP; scintigraphic indications of subcortical loss (SPECT).</td>
<td>FTDU; frontal atrophy &gt; temporal; marked neuronal loss in 2C of the SN; NFT in hippocampus (maximum, 10/mm²)</td>
</tr>
<tr>
<td>DR25.1 (DR25 III-17)/F</td>
<td>FTD</td>
<td>69</td>
<td>Spontaneous speech, As behavior, hyperorality</td>
<td>Aspraxia, verbal apraxia, As behavior</td>
<td>Cortical and subcortical atrophy, maximal frontally, L&gt;R; PWML (MRI)</td>
<td>Bilateral frontal, parietal, and temporal HP, L&gt;R; right cerebellar HP (PET)</td>
<td>Frontotemporal atrophy with FTDU</td>
</tr>
<tr>
<td>DR25.5 (DR25 III-18)/M</td>
<td>FTD</td>
<td>70</td>
<td>Aspraxia, apathy, As behavior, As personality</td>
<td>Aspraxia, verbal apraxia, As behavior, As personality</td>
<td>Cortical and subcortical atrophy; PWML, lacunar infarctions in the basal ganglia bilaterally (CT)</td>
<td>Corticosubcortical atrophy; PWML, lacunar infarctions in the basal ganglia bilaterally (CT)</td>
<td>Relative frontoparietal HP extending into both anterior temporal lobes; preserved sensorimotor cortex activity (SPECT)</td>
</tr>
<tr>
<td>DR25.14/F</td>
<td>AD</td>
<td>76</td>
<td>Memory, verbal expression, apathy</td>
<td>CSF biomarker profile typical for AD</td>
<td>Corticosubcortical atrophy; PWML, lacunar infarctions in the basal ganglia bilaterally (CT)</td>
<td>Cortical and subcortical atrophy, maximal frontally, L&gt;R; PWML (MRI)</td>
<td>Relative frontoparietal HP extending into both anterior temporal lobes; preserved sensorimotor cortex activity (SPECT)</td>
</tr>
</tbody>
</table>

(continued)

memory and activities of daily living, her language impairment was characterized by aphasia with reduced fluency, excessive phonologic paraphasia of spoken and written language, verbal apraxia, and perseverations. One year after disease onset, the patient started displaying behavioral changes such as disinhibited laughter and stereotypic involuntary movements of the tongue and jaw.

**PATHOLOGIC CHARACTERISTICS**

The AD patients DR25.14 and DR142.1 died without autopsy (Table 3). The PD patient DR205.1 died at the age of 61 years, and gross examination of autopsied brain samples showed severe cortical atrophy, particularly of the frontal lobe. The caudate nucleus was also atrophied, and the substantia nigra and locus coeruleus were severely depigmented. Histochemical and immunohistochemical data confirmed severe neuronal loss and showed gliosis in all neocortical regions analyzed, with many surviving neurons containing lipofuscin. Anti-UBI immunoreactivity visualized a huge burden of threadlike inclusions in layers II and III, deeper cortical layers (Figure 4A), and white matter (Figure 4B). These inclusions stained positive with UBI and SNCA and negative with AT8 antibodies. In the neocortical regions and basal ganglia, UBI-positive and tau- and SNCA-negative inclusions were observed. Lenticular, cat-eye types of inclusions were especially abundant in the basal ganglia (Figure 4C), overlapping with infragrey Lewy body inclusions observed in the brainstem, caudate nucleus, and occasionally cortical regions.
**Table 3. Overview of Clinical and Pathologic Characteristics of Mutation Carriers (cont)**

<table>
<thead>
<tr>
<th>Patient# / Sex</th>
<th>Clinical Diagnosis</th>
<th>Age at Onset, y</th>
<th>Age at Death/Current Age, y</th>
<th>Presenting Impairments</th>
<th>Additional Features</th>
<th>Structural Neuroimaging (CT/MRI)</th>
<th>Functional Neuroimaging (SPECT/PET)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR26.1 (DR26 II-1)/M</td>
<td>PNFA</td>
<td>65</td>
<td>68†</td>
<td>Progressive apraxia of speech</td>
<td>Global subcortical and cortical atrophy, maximal frontally and temporally, L&gt;R (MRI)</td>
<td>Relative frontal, temporal, and parietal HP, L&gt;R; relative HP of basal ganglia and lentiform nucleus, R cerebellar HP (SPECT)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>DR27.1 (DR27 III-4)/F</td>
<td>FTLD</td>
<td>58</td>
<td>64†</td>
<td>↓Behavior (decorum, aggression)</td>
<td>Corticosubcortical atrophy, maximal frontotemporally R&gt;L; PWML (MRI)</td>
<td>Bilateral frontal, temporal, and parietal HP, R&gt;L; R HP at parietooccipital transition; L cerebellar HP (PET)</td>
<td>Frontotemporal atrophy with FTLDU</td>
<td></td>
</tr>
<tr>
<td>DR28.1 (DR28 III-3)/M</td>
<td>PNFA</td>
<td>57</td>
<td>62†</td>
<td>PNFA</td>
<td>Parkinsonism (tremor; rigidity)</td>
<td>Relative frontal, temporal, and parietal HP, L&gt;R (SPECT)</td>
<td>Frontotemporal and parietal lobe atrophy with FTLD; mild neuronal loss in ZC of the SN; SP (maximum, 40/mm²) with rare NFT</td>
<td></td>
</tr>
<tr>
<td>DR31.1 (DR31 II-1)/M</td>
<td>PNFA</td>
<td>66</td>
<td>70†</td>
<td>PNFA</td>
<td>Global cortical and minor subcortical temporal atrophy L&gt;R (MRI)</td>
<td>Marked relative bilateral frontal, and temporal HP, L&gt;R; diastasis of frontal cortical activity (SPECT)</td>
<td>Frontotemporal atrophy with FTLDU; neuronal loss in ZC of the SN; rare perivascular plaque deposits; NFT (maximum, 7/mm²)</td>
<td></td>
</tr>
<tr>
<td>DR119.1/F</td>
<td>PNFA</td>
<td>45</td>
<td>47</td>
<td>↓Word finding, ↓verbal expression, ↓memory</td>
<td>Relative frontal, temporal, and parietal HP, L&gt;R; diastasis of frontal cortical activity (SPECT)</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>DR142.1/F</td>
<td>AD</td>
<td>66</td>
<td>76†</td>
<td>Spontaneous speech, loss of decorum</td>
<td>Relative frontal, temporal, and parietal HP, L&gt;R; diastasis of frontal cortical activity; preserved sensorimotor cortex activity (SPECT)</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>DR205.1/M</td>
<td>PD and frontal dysfunction</td>
<td>56</td>
<td>61†</td>
<td>Resting tremor, rigidity, bradykinesia</td>
<td>Mutism, echolalia, loss of judgment, disinhibition</td>
<td>NA</td>
<td>FTLDU: frontal atrophy &gt; temporal; concurrent PD; SP (maximum, 120/mm²) with rare NFT</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Aβ1-42, amyloid-β peptide; AD, Alzheimer disease; CSF, cerebrospinal fluid; CT, computed tomography; FTLD, frontotemporal lobar degeneration; FTLDU, ubiquitin-positive frontotemporal lobar degeneration; HP, hypoperfusion; L, left; MRI, magnetic resonance imaging; NA, not available; NFT, neurofibrillary tangle; PD, Parkinson disease; PET, positron emission tomography; PNFA, progressive nonfluent aphasia; PPA, primary progressive aphasia; PWML, periventricular white matter lesions; R, right; SN, substantia nigra; SP, senile plaques; SPECT, single-photon emission computed tomography; ZN, zona compacta; downward arrow, decreased; upward triangle, change in.

*# Patients with identifiers between brackets represent individuals for whom detailed clinical and/or pathologic data were described previously.2,20*

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(Figure 4D). The UBI-positive inclusions (neuronal intranuclear inclusions and neuronal cytoplasmic inclusions) contained TDP-43 (Figure 4E). The patient was diagnosed as having mixed pathologic features of diffuse Lewy body disease and FTLDU. Numerous vessel-related Aβ-positive dense-core plaques were also observed in the cortical and hippocampal regions (Figure 4F).

**DR8 FOUNDER FAMILY**

The current DR8 founder pedigree consists of 10 branches that extend throughout 7 generations and comprise up to 250 relatives, 237 of whom had genealogic data (Figure 2). Mean age at onset in 39 patients was 64.4 years, ranging from 43 to 78 years, with most (25/39 = 64%) showing their first symptoms between 60 and 75 years (Figure 3). Some mutation carriers (9/34 = 26%) were still cognitively healthy at an age older than 60 years. Thirty-five percent of patients are male. Of 16 patients, detailed medical information was available that showed prominent language impairment (progressive nonfluent aphasia and reduced spontaneous speech) and behavioral and personality changes, of which apathy was most frequently noted (n = 6; Table 3). In 3 of 13 patients with FTLD (Table 3), impairment of memory was an early symptom. Parkinsonism was observed in 1 patient with a diagnosis of progressive nonfluent aphasia, which was attributed to the use of antipsychotic medication. However, for several siblings parkinsonian symptoms were reported by members of the family.

Pathologic diagnosis of FTLDU was obtained in 8 patients (S. K., et al, unpublished data, 2007), with concurrent PD in patient DR205.1 and AD (Braak stage A-II) in patient DR2.3. In 4 patients, mild to marked neuro-
nal loss of the zona compacta of the substantia nigra was observed, with melanin in the astrocytic cytoplasm in 3 patients. Rare Lewy bodies were also present in 1 other patient (DR8.1; Table 3). In 3 brains (patients DR25.1, DR28.1 and DR31.1; Table 3), rare amyloid deposits or neurofibrillary tangles were observed, which were limited to hippocampal areas in patients DR31.1 and DR25.1.

Extensive mutation analysis of PGRN in patients with well-diagnosed AD (n = 666) and PD (n = 255) identified 1 patient with AD with a novel nonsense mutation, p.Arg535X, and 2 patients with AD and 1 with PD who carried the null mutation IVS0 + 5G>C, which we previously described in the extended Belgian FTLD-U-17 founder family.2 So far, 37 of 44 PGRN mutations (http://www.molgen.ua.ac.be/FTDMutations) predicted the formation of a PTC and subsequent loss of the mutant transcript by NMD. In contrast, our data indicated that p.Arg535X was not sensitive to NMD. This finding can be explained by the location of the mutation in the penultimate exon 11 of PGRN at 42 nucleotides from the exon 11/12 boundary. When a PTC is located within less than 50 to 55 nucleo-
tides from the last exon-exon junction, the NMD surveillance complex cannot be formed and the mutated transcript escapes degradation. Thus, in the case of p.Arg535X, the formation of a truncated protein missing 59 C-terminal amino acids can be expected. Since the patient is still alive, we were unable to test for the presence or absence of the C-truncated PGRN in the brain, leaving it undecided whether this mutation exerts a pathologic function via haploinsufficiency or an aberrant gain of function. Of interest is that the mutation is located in the last GRN domain (GRN E) and might influence its highly conserved cysteine fold, potentially leading to degradation. However, although PGRN null mutations are frequently found in patients with FTLD (5%-10%), in AD and PD PGRN null mutations are clearly not a frequent cause of disease (0.45% in AD and 0.39% in PD). Interestingly, we identified 7 patient-specific missense mutations that were present in 11 patients with AD or PD. We recently provided evidence in favor of a pathogenic nature for several missense mutations; however, because missense mutations are also present in control individuals, further functional assessment will be necessary.

Both the patients with AD and PD who carried the IVS0 + 5G>C mutation had their conditions diagnosed with internationally accepted clinical diagnostic criteria. The 2 patients with AD presented with marked deficits of recent memory and modest behavioral disturbances of frontal lobe origin, as was reflected by a relatively low MFS of 4. In hindsight, both patients with AD displayed a reduced spontaneous speech and/or apathy, a clinical manifestation reminiscent of the affected relatives of the DR8 founder family. However, these symptoms occurred on a background of typical AD symptoms, such as loss of episodic memory and disorientation. Although both patients’ conditions could not be neuropathologically confirmed, the CSF biomarker profile of patient DR25.14 was typical for AD, with low CSF Aβ1-42 levels. Also, all autopsy diagnoses in the DR8 founder family were consistent with FTLDU. Mixed FTLDU and AD pathologic features were described for patients from the FTLDU family HDDD1 and were also observed in another patient from the DR8 founder family who underwent autopsy (patient DR2.3; clinically diagnosed as having FTLD; age at death, 72 years). Pathologic examination of this patient’s brain revealed early AD pathologic features (Braak A-II) in addition to typical FTLDU-17 abnormalities.

Patient DR205.1 presented with typical PD, although frontal lobe features were apparent. Cognitive impairment is often observed in patients with PD, with an average prevalence of 40% in cross-sectional studies and a cumulative prevalence approaching 80%. It predominantly involves frontal lobe features, including decreased verbal fluency, apathy, and difficulties in impulse control and interpreting social clues. Postmortem examination of the patient’s brain showed concurrent diffuse Lewy body abnormalities and severe FTLDU pathologic features. Interestingly, vessel-related amyloid-β pathologic features were present as well.

Several explanations exist for the high clinical heterogeneity observed in 1V50 + 5G>C carriers in founder family DR8. Because PGRN seems to be important in the maintenance of neuronal cells, neuronal degeneration due to loss of PGRN might be fully responsible for the observed clinical heterogeneity (eg, because of neuronal damage in the basal ganglia); however, the presence of pathologic correlates of PD and AD argues against this. Alternately, in addition to FTLDU, a separate disease
Figure 4. Brain immunohistochemical analysis of patient DR205.1. A, Ubiquitin (UBI)-positive neuronal cytoplasmic inclusions, neuronal intranuclear inclusions, and threadlike inclusions in layers II and III and in deeper cortical layers (original magnification ×4). B, Neuritic pathologic features in the white matter (original magnification ×10). C, A severe degree of abnormality was observed in the basal ganglia. Arrowheads point to 2 UBI-positive neuronal intranuclear inclusions in the same section (original magnification ×40). D, Infrequent Lewy bodies were also observed in the cortex and the basal ganglia, as shown in the latter region (arrowhead) (α-synuclein [SNCA] staining, original magnification ×40). E, Rabbit TAR DNA-binding protein 43 antisera (TDP-43) staining was localized in both the neuronal cytoplasmic and neuronal intranuclear inclusions (arrowheads), whereas normal nuclear TDP-43 staining was observed in unaffected neurons (original magnification ×40). F, Amyloid-β (Aβ)-stained dense-core plaques were also observed in cortical and hippocampal regions, as shown in the temporal cortical region (original magnification ×20).
mechanism or a modifying factor might be present because of age-related or genetic factors, giving rise to different diseases and, in the case of the patients described herein, to different clinical phenotypes. Incomplete penetrance and the wide range of ages at onset observed in the DR8 founder family argue in favor of the existence of modifying factors. Our observations suggest that etiologic heterogeneity exists in these patients and that the different pathomechanisms drive each other in an exacerbated phenotype. Loss of neuroprotection through partial loss of PGRN might lower a threshold for PD or AD pathologic features to arise, and in turn, incipient PD or AD pathologic features might exacerbate those of FTLDU. Relatively severe FTLDU abnormalities in the basal ganglia and the vessel-related amyloid Aβ pathologic features in patient DR205.1 point in this direction. Moreover, we previously observed signs of clinical heterogeneity in the Dutch FTLDU-17 family 1083 with patients with both FTLD and AD segregating a PGRN null mutation, p.Gln125X. Family 1083 was originally identified as an early-onset AD family through its AD proband. Extensive clinical follow-up studies of family 1083 showed that overall clinical diagnoses were more consistent with clinical FTLD, although in some patients a diagnosis of AD could not be excluded, and in 1 patient, parkinsonism coexisted.

In conclusion, null mutations in PGRN are not a major cause of AD or PD. Furthermore, we provided evidence of intrafamilial clinical heterogeneity of PGRN null mutations, since carriers of the same mutation (eg, IVS0 + 5G>C) presented clinically with AD, PD, or FTLD. Whether the clinical manifestation of AD or PD can be explained by a wide phenotypic spectrum of PGRN mutations or the co-occurrence of FTLD and AD or PD due to other, age-related factors remains to be elucidated. Diagnostic testing of PGRN should, however, be considered in patients with familial clinical AD or PD for whom mutations in other relevant genes have been excluded.

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Announcement

Trial Registration Required. In concert with the International Committee of Medical Journal Editors (ICMJE), Archives of Neurology will require, as a condition of consideration for publication, registration of all trials in a public trials registry (such as http://ClinicalTrials.gov). Trials must be registered at or before the onset of patient enrollment. This policy applies to any clinical trial starting enrollment after July 1, 2005. For trials that began enrollment before this date, registration will be required by September 13, 2005, before considering the trial for publication. The trial registration number should be supplied at the time of submission.

For details about this new policy, and for information on how the ICMJE defines a clinical trial, see the editorial by DeAngelis et al in the January issue of Archives of Dermatology (2005;141:76-77). Also see the Instructions to Authors on our Web site: www.archneurol.com.