Progranulin mutations and ALS or ALS-FTD phenotypes

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Key words
amyotrophic lateral sclerosis, amyotrophic lateral sclerosis-frontotemporal dementia, progranulin
ABSTRACT

Objective: Mutations in the progranulin (PGRN) gene were recently described as the cause of ubiquitin positive FTD. Clinical and pathological overlap between ALS and FTD prompted us to screen PGRN in patients with ALS and ALS-FTD. Methods: The PGRN gene was sequenced in 272 cases of sporadic ALS, 40 familial ALS and 49 patients with ALS-FTD. Results: Missense changes were identified in an ALS-FTD patient (p.S120Y) and in a single case of limb-onset sporadic ALS (p.T182M), though the pathogenicity of these variants remains unclear. Conclusion: PGRN mutations are not a common cause of ALS phenotypes.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder predominantly involving motor neurons leading to paralysis and death within 3 to 5 years from symptom onset. The pathogenic mechanism leading to motor neuron degeneration is unknown in the majority of cases. Fronto-temporal dementia (FTD) is a degenerative disorder of the frontal and anterior temporal lobes.1 Clinical, pathological and genetic data suggest that ALS and FTD form a spectrum of disease.2 Approximately 5% of ALS patients have fronto-temporal dementia (ALS-FTD)3 and roughly half of patients with “classical” ALS have subtle frontal and temporal lobe cognitive impairment.4 Many FTD cases similarly develop symptoms of motor neuron involvement during the course of their illness5 and up to one third of FTD patients without overt motor symptoms have loss of anterior horn cells with characteristic ubiquitin inclusions in surviving motor neurons on autopsy.6 Recently, mutations in the progranulin (PGRN) gene have been described as the cause of ubiquitin positive FTD (FTDU).7;8 The overlap between ALS and FTD prompted us to screen 272 cases of sporadic ALS, 40 familial ALS cases and 49 patients diagnosed with ALS-FTD for mutations in this gene.

METHODS

Subjects

Genetic studies were approved by local research ethics committees (NIA IRB protocol #2003-081). Diagnosis of ALS was based on the El Escorial diagnostic criteria.9 Samples used are outlined in table 1. The samples from the Coriell NINDS DNA repository consisted of 45 Caucasian women and 89 Caucasian men with an average age of symptom onset of 54.3 years (range, 26 - 81). Of these, 34 (25.4%) had bulbar-onset disease, 98 (73.1%) had limb-onset weakness and the remaining 2 (1.5%) cases presented with respiratory symptoms. Three sporadic ALS patients were reported to have cognitive changes, but did not have a formal diagnosis of FTD or dementia.

Of the 48 Irish cases, 20 were women and 28 were men. Average age of symptom onset was 58.9 years (range, 29 – 79). Eight (16.7%) patients initially manifested symptoms in bulbar musculature, 38 (79.2%) cases reported limb-onset symptoms and site of onset was unspecified in the remaining two cases (4.1%).
Samples obtained from the brain banks at Johns Hopkins University, Columbia University and the University of Miami consisted of 26 women and 40 men (missing data = 15) with an average of onset of 65.5 years of age (range 27 – 88, MD = 16).

Of the 96 Swedish patients, 49 were males and 47 females. Thirty-one had bulbar-onset MND and 63 had spinal-onset MND. Two presented with cognitive symptoms. The mean age of onset of first symptom was 56.2 years, range 16-85 years. Diagnosis of FTD was based on the consensus Manchester-Lund clinical diagnostic criteria, supported by ancillary investigations. Of the 36 Swedish ALS-FTD cases, 30 patients had ALS plus frontotemporal dementia, four manifested ALS plus language disorders characteristic of the FTD spectrum (i.e. semantic dementia or progressive non-fluent aphasia) and two cases had ante-mortem diagnoses of “pure” FTD with loss of anterior horn cells evident on autopsy (i.e. FTD-MND). Both of these patients had siblings or parents with ALS.

North American control DNA samples were obtained from the Coriell Institute for Medical Research (n = 159, NDPT002, NDPT006, NDPT009) together with 250 additional North American controls ascertained at the Mayo Clinic in Jacksonville, FL and Scottsdale, AZ.

Table 1. Samples in which the PGRN gene was sequenced

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>SALS</th>
<th>FALS</th>
<th>ALS-FTD</th>
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<tbody>
<tr>
<td>North American ALS samples*</td>
<td>134</td>
<td>132</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Johns Hopkins Brain Bank ALS samples</td>
<td>51</td>
<td>46</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Swedish ALS samples</td>
<td>96</td>
<td>30</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>New York Brain Bank at Columbia University ALS samples</td>
<td>23</td>
<td>16</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>University of Miami/NPF Brain Bank ALS samples</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Irish ALS-FTD and ALS samples</td>
<td>48</td>
<td>41</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Italian ALS-FTD sample</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Belgium ALS-FTD sample</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>361</strong></td>
<td><strong>272</strong></td>
<td><strong>40</strong></td>
<td><strong>49</strong></td>
</tr>
</tbody>
</table>

*http://ccr.coriell.org/ninds/catalog/panel/ (sample IDs available upon request)
NPF = National Parkinson Foundation

**PCR amplification and sequencing of PGRN**

DNA was extracted from blood using the Wizard Purification kit (Promega Corp., WI) or from brain tissue using the DNeasy kit (Qiagen, Inc., CA). The 12 coding exons of PGRN and at least 30bp of flanking intronic sequence were PCR amplified using primer pairs listed in appendix 1 and Roche FastStart PCR Master Mix polymerase (Roche Diagnostics Corp., IN). Each product was sequenced using forward and reverse primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry.

**RESULTS**

Sequence analysis of the PGRN gene identified novel heterozygous genetic variants in a single patient diagnosed with ALS-FTD (2.0%, 1 out of 49) and in a single case of limb-onset sporadic ALS (0.4%, 1 out of 272 sporadic cases). Mutations were not present in the 40 familial ALS cases (Appendix 2).
A single nucleotide change, c.578C>A, was identified in an ALS-FTD patient (New York Brain Bank sample T-51) predicting a serine to tyrosine substitution (p.S120Y, RefSeq NM_002087.2). A different nucleotide change, c.764C>T, was identified in a patient diagnosed with sporadic ALS (Coriell ALS repository ND10418) predicting a threonine to methionine substitution at residue 182 (p.T182M). S120Y and T182M were not identified in 818 North American control chromosomes, in public SNP databases, or any of the other 361 ALS/ALS-FTD samples screened as part of this study, nor in 523 previously published FTD samples. A c.1395 A>C base pair change was present in a single Swedish ALS. Though this variant was also not found in 818 control chromosomes, it is unlikely to be pathogenic as it does not alter the amino acid at this codon (i.e. synonymous mutation, p. Pro392Pro). Clinico-pathological details of the patients with the S120Y and T182M PGRN genetic variants are available in supplementary data.

DISCUSSION
We found variants in the PGRN gene in a single case of ALS-FTD and in a single case of limb-onset sporadic ALS, but mutations were not found in the other 271 sporadic ALS, 40 familial ALS or in the 48 additional ALS-FTD samples screened as part of this study. These findings suggest that PGRN mutations are not a common cause of motor neuron degeneration, though the data does not exclude the possibility that PGRN mutations may be relevant in other populations/ethnicities. Furthermore, the possibility of genomic insertion/deletion mutations in ALS patients has not been excluded by this study.

Our findings agree with a previous report that failed to find PGRN mutations in 48 ALS patients (29 sporadic cases and 19 familial cases). To date only one other individual with a PGRN mutation (family UBC-17, individual 60) fulfilled the El Escorial criteria for ALS and it appears that mutations in this gene are most commonly associated with a behavioral FTD syndrome with possible parkinsonism features in the later stages of illness. The paucity of PGRN mutations in ALS patients is surprising given the clinico-pathological evidence indicating that these two ubiquitionopathy syndromes overlap. However, available data indicates that the pathogenic mechanisms of ALS and FTD do not overlap completely and each individual FTD- or ALS-causing gene will display its own phenotype pattern.

The nature of the S120Y and T182M variants suggests that they are not pathogenic. All previously described PGRN mutations are truncating mutations that are thought to lead to nonsense-mediated decay of the mutant mRNA and consequently haploinsufficiency due to loss of functional PGRN protein. The only exception is a c.26C>A point mutation predicting a p.A9D mutation in the signal peptide of the PGRN protein that may lead to incorrect localization of the PGRN peptide within the neuron. Neither the S120Y or the T182M mutations are within the signaling domain or located within any of the tandem repeats of 12 cysteines and these residues are not highly conserved across species. Furthermore, it was not possible to demonstrate segregation of the S120Y mutation with disease as additional DNA samples were not available from this family. However, S120Y and T182M were not found in 818 control chromosomes indicating that they are not common polymorphisms.

In summary, we identified missense nucleotide variants in the PGRN gene in a single case of FTD associated with motor neuron dysfunction (S120Y) and in a single case of limb-onset sporadic ALS (T182M), though the pathogenicity of these variants
remains unclear. We conclude that PGRN mutations are not a common cause of ALS phenotypes.

ACKNOWLEDGEMENTS
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COMPETING INTERESTS
The authors have reported no conflicts of interest.

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REFERENCES


### SUPPLEMENTARY DATA

#### Appendix 1. Primers used to sequence the 12 exons of *PGRN*

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
<th>Product size</th>
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<tr>
<td>Exon 1</td>
<td>AGGTGTTGAGAAGGCTCAGG</td>
<td>CGGTAAAGATGCAGGAGTGG</td>
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<tr>
<td>Exon 2</td>
<td>GTCCCCTTCTGGTGAGTGC</td>
<td>GATCTTTGGAAGCAGGATCG</td>
<td>458</td>
</tr>
<tr>
<td>Exon 3</td>
<td>ACACTGAGCAGGCATCTGG</td>
<td>CTGGAGTCTGGCACTTTTCTCC</td>
<td>512</td>
</tr>
<tr>
<td>Exon 4</td>
<td>AGGGAGGGGACTGGATTGTG</td>
<td>TGCTCTCCCCACCTCCTCAC</td>
<td>466</td>
</tr>
<tr>
<td>Exon 5</td>
<td>GTTATGGTGATGGCTCTTG</td>
<td>CAGCTCACAGCAGGTAGAACC</td>
<td>550</td>
</tr>
<tr>
<td>Exon 6</td>
<td>TGGGACAGCAGTACACACAGG</td>
<td>CTGGCTGAGGAGGACTTAACAGG</td>
<td>448</td>
</tr>
<tr>
<td>Exon 7</td>
<td>GGAGACAGCAGTGCAGGATGTGT</td>
<td>VTGCCCTCCCACCTCCTCAC</td>
<td>498</td>
</tr>
<tr>
<td>Exon 8</td>
<td>CCCCTTCTCTCCTCTTCTAG</td>
<td>CCTCCGCATAGCCCATAG</td>
<td>498</td>
</tr>
<tr>
<td>Exon 9</td>
<td>TGGCTGAGGAGGACTTAACAG</td>
<td>ATCCTCAGACGACACACGC</td>
<td>488</td>
</tr>
<tr>
<td>Exon 10</td>
<td>AGGGAGGGGACTGGATTGTG</td>
<td>GCGAGAAGGGTTGAGGACG</td>
<td>454</td>
</tr>
<tr>
<td>Exon 11</td>
<td>GTTATGGTGATGGCTCTTG</td>
<td>CAGCTCACAGCAGGTAGAACC</td>
<td>550</td>
</tr>
<tr>
<td>Exon 12</td>
<td>AGGGAGGGGACTGGATTGTG</td>
<td>TGCTCTCCCCACCTCCTCAC</td>
<td>512</td>
</tr>
</tbody>
</table>

#### Appendix 2. *PGRN* sequence variants found in both ALS cases and normal control samples (NM_002087.2)

<table>
<thead>
<tr>
<th>Variant</th>
<th>Number of ALS/ALS-FTD cases with variant</th>
<th>Control chromosomes (number /total number screened)</th>
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</thead>
<tbody>
<tr>
<td>R19W (c.274C&gt;T)</td>
<td>1</td>
<td>13/450</td>
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<tr>
<td>D33D (c.318C&gt;T)</td>
<td>1</td>
<td>2/268</td>
</tr>
<tr>
<td>IVS2+7 G&gt;A</td>
<td>11</td>
<td>1/478</td>
</tr>
<tr>
<td>IVS3-47insAGTC</td>
<td>174*</td>
<td>62/231</td>
</tr>
<tr>
<td>V141I (c.640G&gt;A)</td>
<td>1</td>
<td>2/510</td>
</tr>
<tr>
<td>IVS7+7 G&gt;A</td>
<td>40†</td>
<td>28/400</td>
</tr>
<tr>
<td>A324T (c.1189G&gt;A)</td>
<td>3</td>
<td>3/367</td>
</tr>
<tr>
<td>R433W (c.1516C&gt;T)</td>
<td>3</td>
<td>5/184</td>
</tr>
<tr>
<td>P458L (c.1592C&gt;T)</td>
<td>1</td>
<td>25/959</td>
</tr>
<tr>
<td>R556C (c.1885C&gt;T)</td>
<td>1</td>
<td>1/862</td>
</tr>
</tbody>
</table>

*41 homozygous cases, 133 heterozygous cases
† 2 homozygous cases, 38 heterozygous cases
Clinico-pathological description of case T-51
Case T-51 was a Caucasian woman who, at age 56, became obsessed with finding a partner after the death of her husband. At age 58 she repeated stories. Two years later she did not recall that her husband had died of a heart attack. At age 61 she displayed inappropriate sexual behavior, such as approaching strange men on the subway. Her personality changed in that she became dependent, belligerent, obsessive and compulsive. She developed akathesia and mispronounced words. The diagnosis of FTD was established at that time. At age 62 the patient developed left leg weakness, which spread to her arms. Electrophysiological studies confirmed motor neuron dysfunction. SOD1 and MAPT genetic testing were normal. Her father had died of ALS at age 60 and her paternal grandmother had also died of ALS. The patient died at age 63.

On autopsy, there was marked frontal atrophy with milder changes within the rest of the brain (figure 1). All sections were stained with Luxol fast blue, counterstained with hematoxylin and eosin (LHE), and additional immunohistochemistry staining was performed using antibodies directed against ubiquitinated aggregates, phosphorylated tau (AT8), α-synuclein aggregates, β-amyloid and CD68. The pars compacta of the substantia nigra was pale. Histologically, there was marked neuronal loss and reactive gliosis involving the motor cortex (figure 1). Within the pyramidal system there were ubiquitinated aggregates in the neuropil and in scattered neurons. Within neurons, the aggregates were mainly cytoplasmic and rarely nuclear. Scattered atrophic neurons and mild gliosis were also present in the prefrontal cortex. There were scattered, AT8-labeled neurons in the hippocampus, entorhinal cortex, parahippocampal, and occipito-temporalis gyri. Neuritic plaques and amyloid deposits were not found. The changes involving the hypoglossal nucleus, and anterior horn cells were similar to those seen within the motor cortex. Rare motor neurons showed Bunina bodies. Myelin loss was marked within the lateral and ventral corticospinal tracts.

Clinical description of case ND10418
This non-Hispanic, Caucasian right-handed male patient presented at 67 years of age with leg weakness that progressed to involve all four limbs. He met the criteria for probable ALS by El Escorial criteria. He was not demented and there was no known family history of ALS, dementia or Parkinson Disease. He was 69 years of age at the time of sampling without obvious bulbar involvement.
Figure 1. (A) Right hemi-brain from case T-51 carrying S120Y mutation showing marked frontal and anterior temporal lobe atrophy; (B) Luxol fast blue counterstained with hematoxylin and eosin (LHE) stain of hypoglossal nucleus showing a neuron containing a cytoplasmic, eosinophilic inclusion (Bunina body). In addition, neuronal density is decreased and neuropil is traversed by glial fibrillary processes (Original
magnification 630X); (C) LHE stain of motor cortex (Brodmann area 4) showing an atrophic, centrally located Betz cells with scattered, shrunken neurons (400X); (D) Sixth cortical layer of motor cortex (BA4) with neuropil ubiquitinated aggregates (630X); (E) Fifth cortical layer of cingulate gyrus (BA24) showing two neurons with ubiquitinated nuclear inclusions (round compact on the upper right, two punctate on the bottom/center) and comma-shaped cytoplasmic aggregates on the lower left (630X); (F) Fifth cortical layer of superior frontal gyrus (BA 9) showing a neuron with a large, globular ubiquitinated, cytoplasmic inclusion (630X); (G) LHE stain of the cervical cord showing marked myelin loss of the corticospinal tract; (H) LHE stain of cervical corticospinal tract showing marked myelin loss with scattered macrophages (630X).
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