

SHORT REPORT

Progranulin mutations and amyotrophic lateral sclerosis or amyotrophic lateral sclerosis–frontotemporal dementia phenotypes

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Objective: Mutations in the progranulin (*PGRN*) gene were recently described as the cause of ubiquitin positive frontotemporal dementia (FTD). Clinical and pathological overlap between amyotrophic lateral sclerosis (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 cases of familial ALS and in 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), although the pathogenicity of these variants remains unclear.

Conclusion: *PGRN* mutations are not a common cause of ALS phenotypes.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder predominantly involving motor neurons leading to paralysis and death within 3–5 years from symptom onset. The pathogenic mechanism leading to motor neuron degeneration is unknown in the majority of cases. Frontotemporal 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 FTD (ALS–FTD)³ and approximately 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.^{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 the site of onset was unspecified in the remaining 2 (4.1%) cases.

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 onset of 65.5 years of age (range 27–88, missing data = 16).

Of the 96 Swedish patients, 49 were males and 47 females. Thirty-one had bulbar onset motor neuron disease and 63 had spinal onset motor neuron disease. Two presented with cognitive symptoms. The mean age of onset of first symptom was 56.2 years (range 16–85). Diagnosis of FTD was based on the consensus Manchester–Lund clinical diagnostic criteria,¹⁰ supported by ancillary investigations. Of the 36 Swedish ALS–FTD cases, 30 patients had ALS with FTD, four manifested ALS with language disorders characteristic of the FTD spectrum (ie, 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 (ie, FTD–motor neuron disease). 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, Florida and Scottsdale, Arizona.

PCR amplification and sequencing of *PGRN*

DNA was extracted from blood using the Wizard purification kit (Promega Corp., Wisconsin, USA) or from brain tissue using the DNeasy kit (Qiagen, Inc., California, USA). The 12 coding exons of *PGRN* and at least 30 bp of flanking intronic sequence were PCR amplified using primer pairs listed in appendix 1 (see <http://www.jnnp.com/supplemental>) and Roche FastStart PCR Master Mix polymerase (Roche Diagnostics Corp., Indiana, USA). Each product was sequenced using forward and reverse primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry.

Abbreviations: ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia

Table 1 Samples in which the *PGRN* gene was sequenced

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

ALS, amyotrophic lateral sclerosis; FALS, familial amyotrophic lateral sclerosis; FTD, frontotemporal dementia; NPF, National Parkinson Foundation; SALS, sporadic amyotrophic lateral sclerosis.
*<http://ccr.coriell.org/ninds/catalog/panel/> (sample IDs available on request).

RESULTS

Sequence analysis of the *PGRN* gene identified novel heterozygous genetic variants in a single patient diagnosed with ALS-FTD (2.0%, 1 of 49) and in a single case of limb onset sporadic ALS (0.4%, 1 of 272 sporadic cases). Mutations were not present in the 40 familial ALS cases (see appendix 2 at <http://www.jnnp.com/supplemental>).

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 databases of single nucleotide polymorphisms or in any of the other 361 ALS/ALS-FTD samples screened as part of this study, or in 523 previously published FTD samples.^{7-8,11} A c.1395 A>C base pair change was present in a single Swedish ALS case. Although 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 (ie, synonymous mutation, p.Pro392Pro). Autopsy of sample T-51 revealed prominent frontal atrophy with marked neuronal loss and reactive gliosis involving the motor cortex, hypoglossal nucleus and anterior horn cells (see supplementary fig 1 at <http://www.jnnp.com/supplemental>). Rare motor neurons showed Bunina bodies. Myelin loss was marked within the lateral and ventral corticospinal tracts.

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, although the data do 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).¹¹ 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 behavioural FTD syndrome with possible parkinsonism features in the later stages of illness.^{7-8,11} The paucity of *PGRN* mutations in ALS patients is surprising given the clinicopathological evidence indicating that these two ubiquitinationopathy syndromes overlap. However, available data indicate 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.^{7-8,11} 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 localisation of the *PGRN* peptide within the neuron.^{11,12} Neither the S120Y nor the T182M mutations are within the signalling 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), although the pathogenicity of these variants remains unclear. We conclude that *PGRN* mutations are not a common cause of ALS phenotypes.

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Appendices 1 and 2, and supplementary data can be viewed on the *J Neurol Neurosurg Psychiatry* website at <http://www.jnnp.com/supplemental>.

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SUPPLEMENTARY DATA

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

	Sequence	Product size
Exon 1 Forward	AGGTGTTGAGAAGGCTCAGG	485
Exon 1 Reverse	CGGTAAAGATGCAGGAGTGG	
Exon 2 Forward	GTCCCCTTCTGGTGAGTGC	458
Exon 2 Reverse	GATCTTTGGAAGCAGGATCG	
Exon 3 Forward	ACACTGAGCAGGCATCTGG	512
Exon 3 Reverse	CTGGAGTCTTGCACTTTCTCC	
Exon 4 Forward	AGGGAGGGACTGGATTGTG	550
Exon 4 Reverse	TGCTCTCCCACCTCCTCAC	
Exon 5 Forward	GTTATGGTCGATGGCTCCTG	466
Exon 5 Reverse	CAGCTCACAGCAGGTAGAACC	
Exon 6 Forward	TGCCCAGAGGACTAACAGG	448
Exon 6 Reverse	CAGGCTCAGTAGCACACAGG	
Exon 7 Forward	CTGGCTGATGCAGGGTTC	452
Exon 7 Reverse	CTGTGAGGGAAGCAGAGAGG	
Exon 8 Forward	CCCCTTTCCTCCCTTTTAGG	388
Exon 8 Reverse	CGGGACAGCAGTGTATGTGG	
Exon 9 Forward	GCTGCTGCCCTTTTACCC	498
Exon 9 Reverse	CCGCCTCTCCTGCTTACAG	
Exon 10 Forward	CCTCCGCATAGCCCATAG	498
Exon 10 Reverse	ATCCTCGCAGCACACAGC	
Exon 11 Forward	AGACATCGGCTGTGACCAG	488
Exon 11 Reverse	GCGAGAGGGTTGGACGAG	
Exon 12 Forward	ACCTGCTGCCGAGACAAC	454
Exon 12 Reverse	CCACAACCCCTTCTGAC	

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

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

*41 homozygous cases, 133 heterozygous cases

† 2 homozygous cases, 38 heterozygous cases

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.

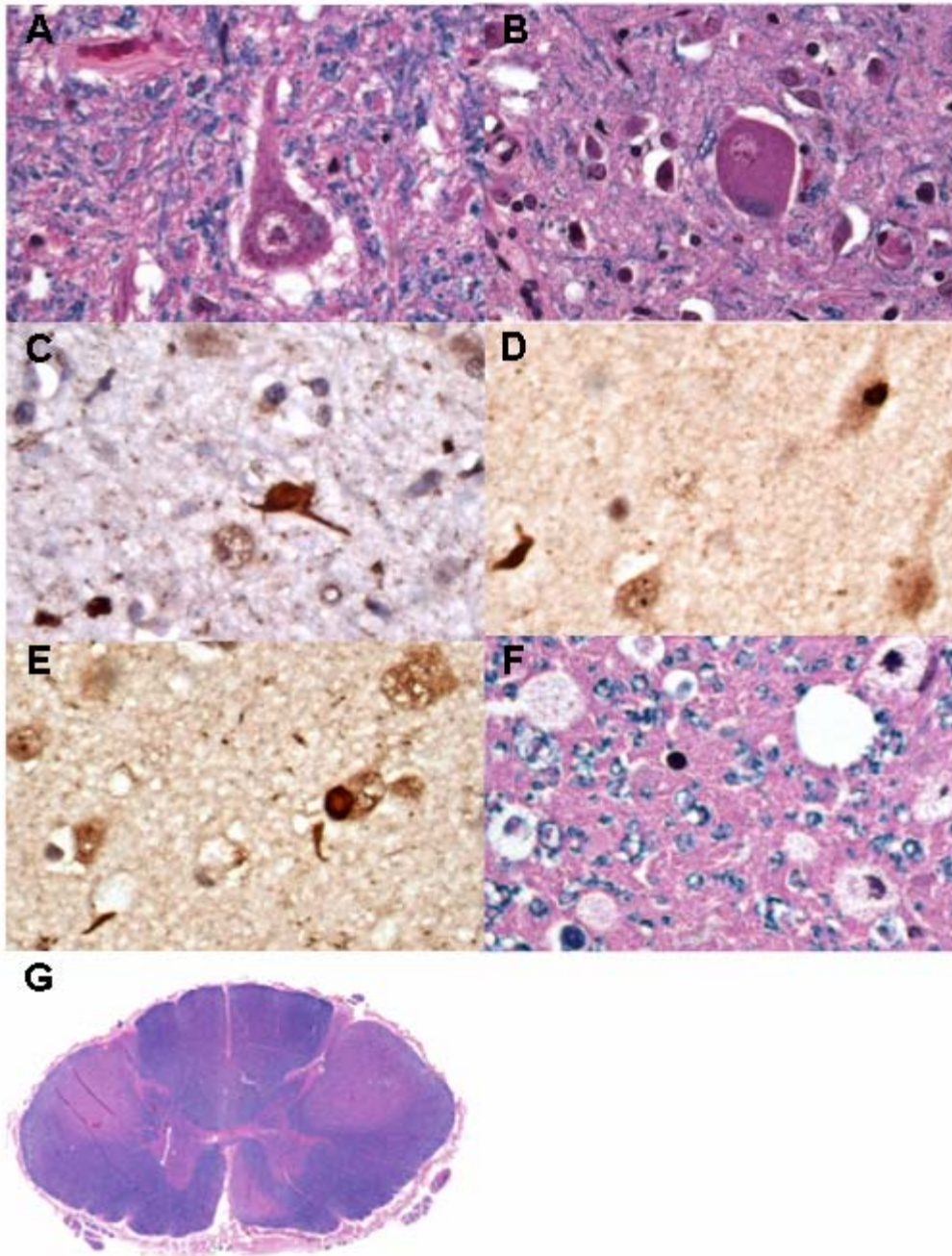


Figure 1. Histology of case T-51 carrying S120Y mutation. (A) Luxol fast blue counterstained with hematoxylin and eosin (LHE) stain of hypoglossal nucleus showing a neuron containing a cytoplasmic, eosinophilic inclusion (Bunina body). In addition, neuronal density is decreased and neuropil is traversed by glial fibrillary processes (Original magnification 630X); (B) LHE stain of motor cortex (Brodmann area 4) showing

an atrophic, centrally located Betz cells with scattered, shrunken neurons (400X); (C) Sixth cortical layer of motor cortex (BA4) with neuropil ubiquitinated aggregates (630X); (D) 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); (E) Fifth cortical layer of superior frontal gyrus (BA 9) showing a neuron with a large, globular ubiquitinated, cytoplasmic inclusion (630X); (F) LHE stain of the cervical cord showing marked myelin loss of the corticospinal tract; (G) LHE stain of cervical corticospinal tract showing marked myelin loss with scattered macrophages (630X).