Microglial Upregulation of Progranulin as a Marker of Motor Neuron Degeneration

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Abstract

Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are overlapping neurodegenerative disorders. Mutations in the growth factor progranulin (PGRN) gene cause FTLD, sometimes in conjunction with ALS; such mutations are also observed in some ALS patients. Most PGRN mutations underlying FTLD are null mutations that result in reduced PGRN levels. We investigated PGRN expression in human ALS and in mouse models of motor neuron degeneration. Progranulin plasma or CSF levels in newly diagnosed ALS patients did not differ from those in healthy or disease controls (PGRN mutation-negative FTLD and Alzheimer disease patients). In the mutant SOD1<sup>G93A</sup> mouse model of ALS, spinal cord PGRN levels were normal in presymptomatic animals but increased during the degenerative process. This increase in PGRN correlated with enhanced expression of PGRN in microglia. In CSF, PGRN levels were normal in presymptomatic and early symptomatic animals, but with disease progression, a raise in PGRN was detectable. These data indicate that upregulation of PGRN is a marker of the microglial response that occurs with progression in motor neuron diseases.

Key Words: Amyotrophic lateral sclerosis, Frontotemporal lobar degeneration, Microglia, Progranulin, Tau.

INTRODUCTION

Progranulin (PGRN) is a multifunctional growth factor that contains a signal peptide and 7.5 granulin domains that are highly conserved throughout evolution (1–3). The protein is widely expressed; it is secreted and is then proteolytically cleaved into granulins (GRN A-F and paragranulin) by elastase in the extracellular matrix (4, 5). Progranulin has been implicated in development, tumor growth, inflammation, and wound healing (6, 7).

Although no PGRN receptor has been identified to date, PGRN stimulates the mitogen-activated protein kinase and the phosphatidylinositol 3-kinase pathways (8). The full-length precursor and some of the GRN peptides act as autocrine growth factors for different cell lines (9), but some of the GRN peptides inhibit proliferation in other cell lines (3). Therefore, the PGRN gene encodes a family of proteins (i.e., the full-length precursor, 7 GRN peptides, paragranulin, and several intermediates), which likely exert different actions on different targets and receptors (6).

Although PGRN has been studied most extensively in tumor growth, inflammation, and wound healing, dominant mutations in the PGRN gene were recently identified as a cause of familial frontotemporal lobar degeneration (FTLD), (10, 11) sometimes in conjunction with amyotrophic lateral sclerosis (ALS) (12, 13), a neurodegenerative disorder linked to FTLD (14–17). Most disease-causing mutations are loss-of-function mutations that result in reduced PGRN messenger RNA (mRNA) and/or protein levels in all tissues studied to date (10, 11, 18–23). Therefore, haploinsufficiency with shortage of PGRN is thought to be the disease-causing mechanism. In line with this hypothesis, we recently observed a survival-promoting effect of PGRN on cultured cortical and motor neurons (19).

Missense mutations, some of unproven pathogenic nature, have also been observed in patients with FTLD (24–26),

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Alzheimer disease (AD) (27), and in some patients with ALS (28–30). In addition, PGRN has been found to be a genetic modifier of the course of sporadic ALS (29).

Apart from reductions in PGRN observed in PGRN mutations carriers, upregulation of PGRN seems to be part of the inflammatory response after acute (31–33) or chronic insults (10, 34–40) in the CNS.

Whether changes in PGRN expression levels also occur in ALS is currently unknown. In this study, the expression of PGRN was studied in ALS patients and in mouse models of motor neuron degeneration.

**MATERIALS AND METHODS**

**Patient Samples**

All human plasma and cerebrospinal fluid (CSF) samples were obtained with informed consent and approved by the local ethical committee. Patient samples were collected at the neuromuscular and memory clinic of the University Hospital Leuven (Belgium) and of the Radboud University Nijmegen Medical Center (the Netherlands). The diagnoses of ALS, FTLD, or AD were made based on published criteria (41–43). For most patients, there was no postmortem study to confirm the diagnosis. Patient demographic data for the CSF study are given in Table 1. Patient data for the study of plasma PGRN levels are shown in Table 2. Patients were sampled during the diagnostic early phase of the disease. For most patients, the lumbar puncture was planned at the first visit. Cerebrospinal fluid control samples were taken after informed consent from patients who underwent a lumbar puncture for diagnostic reasons and who later were found to have normal CSF results on routine testing. Healthy spouses were used as controls for plasma samples. Platelet-poor plasma samples were obtained as described (44). Cerebrospinal fluid samples were obtained by lumbar puncture, collected in polypropylene tubes, centrifuged, and stored at −80°C until use. Progranulin mutations were excluded by sequencing all exons, exon-intron boundaries, and the 5′ UTR, as previously described (11).

**Human Progranulin Enzyme-Linked Immunosorbent Assay**

Progranulin levels were measured by enzyme-linked immunosorbent assay (ELISA), as previously described (19, 22).

In brief, 96-well plates were coated with monoclonal anti-human PGRN antibody (R&D Systems, Minneapolis, MN), blocked with 1% BSA, before addition of samples and standard, that is recombinant human PGRN (R&D Systems). Bound PGRN was detected using biotinylated polyclonal anti-human PGRN antibody (R&D Systems), ABC complex and orthophenylenediamine. Absorbance was measured at 490 nm on a VictorX3 plate reader (Perkin Elmer, Waltham, MA). The combination of antibodies used in this sandwich ELISA recognizes only the full-length PGRN (19). Progranulin was detectable in plasma and CSF, with average values comparable to other reports (20, 21).

**Animal Models With Motor Neuron Degeneration**

All experiments on rodents were approved by the local ethical committee of the K.U. Leuven, Belgium. Mutant SOD1G93A mice [B6SJL-TgN(SOD1-G93A)1Gur/J; stock number 002726] were obtained from Jackson Laboratory (Bar Harbor, ME). Homozygous transgenic mice with a deletion in the hypoxia-response element in the vascular endothelial growth factor (VEGF) gene (VEGFΔ0/Δ0) (45), and transgenic mice overexpressing human mutant tauP301L driven by the mouse Thy1 gene promoter (46) were previously generated. Heterozygous mutant SOD1G93A mice display an aggressive form of motor neuron degeneration (47) with onset of signs at around 80 days and a progressive disease course with death at around 140 to 150 days. Homozygous VEGFΔ0/Δ0 mice

### Table 2. Demographic Data of Amyotrophic Lateral Sclerosis Patients and Controls Used in Plasma Progranulin Measurements

<table>
<thead>
<tr>
<th></th>
<th>ALS Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>135</td>
<td>51</td>
</tr>
<tr>
<td>Age, mean ± SD, yr</td>
<td>69 ± 13</td>
<td>58 ± 13</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>73 (54)</td>
<td>28 (55)</td>
</tr>
<tr>
<td>Diagnostic delay, mean ± SD, mo</td>
<td>22 ± 13</td>
<td>57 ± 13</td>
</tr>
<tr>
<td>Disease duration at sampling, mean ± SD, mo</td>
<td>14 ± 17</td>
<td>12 ± 15</td>
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</tbody>
</table>

Progranulin levels were measured in plasma samples from controls and ALS patients. ALS, amyotrophic lateral sclerosis; NA, not applicable.

### Table 1. Reduced CSF Progranulin Levels Are Restricted to Patients With PGRN Null Mutations

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Patients, n</th>
<th>Age, Mean ± SD</th>
<th>Men, n (%)</th>
<th>Diagnostic Delay, Mean ± SD, mo</th>
<th>Disease Duration at LP, Mean ± SD, mo</th>
<th>PGRN Level, CSF ± SEM, ng/mL</th>
<th>p (vs Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31</td>
<td>53.5 ± 18.4</td>
<td>13 (42)</td>
<td>NA</td>
<td>NA</td>
<td>6.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>ALS</td>
<td>29</td>
<td>60.2 ± 12.4</td>
<td>17 (59)</td>
<td>12.2 ± 9.0</td>
<td>13.9 ± 8.9</td>
<td>7.0 ± 0.5</td>
<td>0.99</td>
</tr>
<tr>
<td>FTLD no PGRN mutation</td>
<td>24</td>
<td>61.1 ± 12.2</td>
<td>17 (71)</td>
<td>36.2 ± 22.1</td>
<td>35.7 ± 22.1</td>
<td>6.9 ± 0.6</td>
<td>0.98</td>
</tr>
<tr>
<td>FTLD PGRN mutation</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.2 ± 1.0</td>
<td>0.04</td>
</tr>
<tr>
<td>FTLD-ALS</td>
<td>4</td>
<td>63.4 ± 8.5</td>
<td>16 (47)</td>
<td>29.5 ± 22.1</td>
<td>30.0 ± 22.8</td>
<td>6.0 ± 0.5</td>
<td>0.69</td>
</tr>
<tr>
<td>AD</td>
<td>34</td>
<td>63.4 ± 8.5</td>
<td>16 (47)</td>
<td>29.5 ± 22.1</td>
<td>30.0 ± 22.8</td>
<td>6.0 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Progranulin (PGRN) levels were measured in CSF from controls and patients with amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD) without PGRN mutations, FTLD-ALS patients, and Alzheimer disease (AD) patients. For comparison, data on samples from FTLD patients with PGRN null mutation (19) were added. Analysis of variance with post hoc comparison with control was used to calculate significance.

LP, lumbar puncture; n, number; NA, not applicable.
develop a slowly progressive motor neuron degeneration phenotype that usually starts at around 5 months, and survival is usually beyond 1 year (45). In mutant TauP301L mice, onset of disease is around 8 to 9 months. In homozygous mice, disease is characterized by an aggressive motor phenotype with motor neuron loss in spinal cord and extensive brainstem tau pathology that results in premature death before the age of 12 months (46, 48).

Isolation of Microglia From Spinal Cord

Microglial cells were isolated from adult spinal cord, as previously described (49). Briefly, mice were transcardially perfused with ice-cold PBS, and spinal cords were dissected. The cords were mechanically homogenized and filtered through a 70-μm cell strainer to obtain a single cell suspension. Cells were then loaded on a Percoll gradient (40%-80%) (Percoll; GE Healthcare, Uppsala, Sweden). After centrifugation, mononuclear cells from the interface were isolated and stained with a phycocerythrin-coupled antibody against the myeloid cell marker CD11b (Becton Dickinson, Franklin Lake, NJ). CD11b+ cells were then gated and sorted using a flow cytometer FACS ARIA (Becton Dickinson).

RNA Extraction and Complementary DNA Preparation

RNA from total spinal cord lysates or freshly isolated spinal microglia was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and further purified using the RNeasy cleanup protocol with on-column DNase digestion (Qiagen, Valencia, CA). One microgram of RNA was transcribed to complementary DNA using the QuantiTect reverse transcription kit (Qiagen), according to the manufacturer’s instructions.

Quantitative Polymerase Chain Reaction

For real-time polymerase chain reaction (PCR) of mouse progranulin, the forward primer was 5′ TTG GGT ACT GGA GTG AAG TGG AT 3′, the reverse primer was 5′ GCC CAG ACG TGT CAT TCC CT 3′, and the probe was FAM-CCT AAC TGA GCT CCC-MGB. Thermal cycling was performed on a 7300 Sequence Detection System (Applied Biosystems, Carlsbad, CA). Each reaction was performed in duplicate. All samples were normalized to the level of β-actin RNA.

Immunohistochemistry

All mice were anesthetized using 10% pentobarbital sodium in PBS. After transcardiac perfusion with ice-cold PBS followed by fixation with 4% paraformaldehyde, spinal cords were dissected and postfixed for 2 hours in 4% paraformaldehyde. Free-floating spinal cord sections (20 μm) were cut on a cryostat (Leica, Wetzlar, Germany) and stained with a phycoerythrin-coupled antibody against the human PGRN antibody (R&D Systems) and a mouse monoclonal CD68 (a microglial cell/macrophage marker) and progranulin; Alexa Fluor 555 and Alexa Fluor 488 were used to visualize 4′,6-diamidino-2-phenylindole (Sigma) as chromogen were used to develop the stain.

Measurement of PGRN Protein Levels by ELISA in Mice

Progranulin protein levels in CSF and spinal cord lysates from mice were measured using a mouse progranulin ELISA kit (AdipoGen, Seoul, Korea). Cerebrospinal fluid samples were obtained by suboccipital puncture in mice anesthetized using intraperitoneal pentobarbital sodium. Spinal cord samples were lysed in RIPA buffer (50 mmol/L Tris, 150 mmol/L NaCl, 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate, and a Complete protease inhibitor cocktail tablet; Roche, Basel, Switzerland); PGRN content was measured according to the manufacturer’s manual. The protein concentration was measured using the BCA protein assay (Pierce, Rockford, IL), and values were normalized to the protein content in the sample.

Statistics

Data are shown as mean ± SEM. Student t-tests or Mann-Whitney U test were used to calculate significance. When more
than 2 groups were compared, a one-way analysis of variance with Fisher least significant difference post hoc test was used.

RESULTS

Progranulin Protein in Human CSF and Plasma

Samples of ALS, FTLD, AD, and control CSF were obtained in the diagnostic phase, when the patients were first presented. Mutations in the PGRN gene were excluded. Patient characteristics are shown in Table 1. The measured levels did not depend on age or sex of the patients or on the duration of sample storage before analysis (data not shown). No differences were noted between ALS patients and controls (Table 1). Patients with the combination of ALS and FTLD, with FTLD alone, or with AD had normal CSF PGRN levels (Table 1). No correlation between the disease duration at the time of lumbar puncture and PGRN levels was observed.

The PGRN plasma levels in ALS patients (n = 91) were not different from those in controls (n = 56, p = 0.4). The mean was 261 ± 25 and 231 ± 24 ng/mL in ALS patients and controls, respectively. In 1 patient without a family history of ALS, an R110Q mutation was found. The plasma level in this patient was within the reference range (200 ng/mL). Of the 91 patients, 7 patients carried the GA instead of GG genotype at rs9897526, which was previously shown to reduce the age of onset of ALS by 7.7 years and to reduce survival (29). The mean PGRN plasma levels were slightly lower in GA carriers (266 ± 27 for GG and 202 ± 36 ng/mL for GA, p = 0.5), but these differences were not significant. Altogether, these results suggest that reductions in PGRN levels are restricted to patients carrying PGRN loss-of-function mutations.

Spinal PGRN Levels Increase With Motor Neuron Degeneration

Progranulin mRNA levels were measured by quantitative PCR in spinal cord lysates from presymptomatic (50 days) and late symptomatic (130 days) SOD1G93A mice. We also included young (50 days) and aged (1 year) nontransgenic animals. There was a slight increase in PGRN mRNA levels with aging in nontransgenic animals, but significance was not reached (Fig. 1A). Presymptomatic animals had normal PGRN mRNA levels, whereas in late symptomatic mutant SOD1G93A mice, there was a distinct upregulation of PGRN mRNA levels (Fig. 1A), that is, a 261% increase in PGRN transcripts compared with presymptomatic animals.

Full-length PGRN protein levels were measured by ELISA in spinal cord lysates from mutant SOD1G93A mice and controls. Similar PGRN levels were observed in young and old nontransgenic animals and presymptomatic mutant SOD1G93A mice (Fig. 1B). In contrast, PGRN levels were significantly higher in the diseased mutant SOD1G93A spinal cord (Fig. 1B). Compared with presymptomatic animals, there was a 33% increase in PGRN protein levels. In comparison to changes in mRNA levels, the increase in full-length PGRN protein level was smaller, possibly because a considerable amount of PGRN is secreted and cleaved into smaller fragments.

Microglial Upregulation of PGRN

In the healthy mouse spinal cord, PGRN expression was observed in neurons in the ventral and dorsal horn, as shown by NeuN double staining (Figure, Supplemental Digital Content 1, http://links.lww.com/NEN/A187). Large caliber SMI-32 (neurofilament heavy) + motor neurons were also PGRN+ (Figs. 2A–C). In presymptomatic mutant SOD1G93A mice, (before the massive expansion of the CD11b+ microglial...
FIGURE 2. Progranulin (PGRN) expression in the mouse spinal cord. (A–C) PGRN+ motor neurons in the ventral horn of the spinal cord. Progranulin staining (A), SMI32 staining (B), and overlay (C) of ventral part of spinal cord from nontransgenic mouse; inset shows detail of motor neuron. (D–L) Progranulin staining of the ventral part of the spinal cord in presymptomatic, early, and late symptomatic mutant SOD1(G93A) mice (in red, D, G, J), CD11b staining (in green, E, H, K), and overlay (F, I, L); 2 motor neurons are marked by white arrows in D. (D–L) There is a gradual increase in the number of PGRN+ microglia in the spinal cord of mutant SOD1(G93A) mice. (M–O) At the end stage, surviving cells with motor neuron morphology (CD11b-negative, white arrows) remained PGRN+ in mutant SOD1(G93A) mice. Scale bar = 50 μm.
population), large motor neurons were also PGRN⁺; resting CD11b⁺ microglia did not express strong PGRN staining (Figs. 2D–F). However, in symptomatic stages, numerous intensely PGRN⁺ microglia were observed in the ventral horn of the spinal cord (Figs. 2G–L), suggesting that the microglial response is responsible for the upregulation of PGRN. In the diseased spinal cord, PGRN did not only colocalize with the microglial marker CD11b (Figure, Supplemental Digital Content 2, parts A–C, http://links.lww.com/NEN/A188), but also with CD11c, a dendritic cell marker expressed by mature microglia (Figure, Supplemental Digital Content 2, parts D–F, http://links.lww.com/NEN/A188). The surviving motor neurons in spinal cords of symptomatic mice remained PGRN⁺ (Figs. 2M–O). Progranulin immunoreactivity was not seen in

**FIGURE 3.** Microglial upregulation of progranulin (PRGN). (A) Microglial cells were isolated from adult spinal cord of SOD1G93A mice by loading the cell suspension on a 40% to 80% Percoll gradient. Viable mononuclear cells were gated (P1) and cells immunoreactive for CD11b-phycoerythrin (PE) were collected for further analysis (49). (B) Progranulin mRNA expression measured by quantitative PCR in freshly isolated spinal microglia from presymptomatic and symptomatic mutant SOD1G93A mice (*, p < 0.05).

**FIGURE 4.** Progranulin (PGRN) mRNA and protein levels increase with motor neuron degeneration in VEGF⁻/⁻ and mutant tauP301L mice. (A) Spinal cord PGRN mRNA levels measured by quantitative PCR and normalized to β-actin levels in presymptomatic and symptomatic VEGF⁻/⁻ and mutant TauP301L mice. (B) Spinal cord PGRN protein levels measured by ELISA normalized to protein content.
reactive astrocytes or in NG2+ glial precursor cells (Figure, Supplemental Digital Content 3, http://links.lww.com/NEN/A189).

To confirm the microglial upregulation of PGRN with disease progression, we performed quantitative PCR on freshly isolated spinal cord microglial cells from presymptomatic and late symptomatic mutant SOD1G93A mice. Microglial cells isolated from diseased mice had a much higher PGRN expression (Fig. 3).

We also analyzed 2 less frequently studied animal models in which motor neuron degeneration occurs, i.e. homozygous transgenic mice with a deletion in the hypoxia-response element in VEGFΔ6 (45), and homozygous transgenic mice overexpressing human mutant tauT301E driven by the mouse Thy1 gene promoter (46). When these mice developed signs of motor neuron degeneration, a similar increase in PGRN levels was observed in the spinal cord at the mRNA and protein levels (Fig. 4). Immunostaining confirmed that the motor neuron loss was also characterized by PGRN staining in microglia (results not shown).

In control human spinal cords, PGRN immunoreactivity was seen in several cell types, including large motor neurons in the ventral horn (Fig. 5A). Compared with control spinal cord without microgliosis (Figs. 5B, C), the spinal cord of ALS patients showed a pronounced microglial response, consisting of CD68+ microglia. These microglia were intensely PGRN+ (Figs. 5D–F), as described in a recent study (50). Surviving cells with neuronal morphology in the ventral horn of the spinal cord remained PGRN+ (Figs. 5G–I).

Because PGRN is a secreted protein, we also assessed PGRN levels in the CSF of mutant SOD1G93A mice. Small volumes of CSF (~15 μL) were obtained by suboccipital puncture in anesthetized mice, and the samples were analyzed by ELISA. In presymptomatic mutant SOD1G93A mice, PGRN levels were equal to nontransgenic controls. In late...
symptomatic mice, there were elevated PGRN CSF levels (Fig. 6). Interestingly, early symptomatic mice (around the age of 80 days, when muscle denervation is already present [51, 52] and when the microglial response has already been initiated [53]) did not yet display the elevated PGRN levels in CSF. These data are in accordance with the human findings in CSF and suggest that microglial PGRN is secreted and accumulates in CSF with disease progression.

**DISCUSSION**

Interest in the role of progranulin in the CNS was raised by the discovery of disease-causing mutations in the progranulin gene in patients with FTLD (10, 11). Most mutations result in reduced PGRN levels, and shortage of PGRN is thought to underlie neuronal death. In addition, missense mutations were also observed in some sporadic FTLD patients (25, 26), in AD patients (27), and in some patients with ALS (28–30). Progranulin has been identified as a genetic modifier of the phenotype of motor neuron degeneration (29), but mutations segregating with the disease have not been observed in classic ALS families to date. How the sequence variants found in individual patients contribute to the disease pathogenesis is unclear at present.

We found that PGRN levels are normal in plasma and CSF from newly diagnosed ALS patients, but expression increases with disease progression in the spinal cord of transgenic animal models experiencing motor neuron degeneration, mainly due to an elevated expression in microglia. This increase was detectable in CSF, suggesting that PGRN may become a disease state marker of motor neuron degeneration.

It has been shown by several groups that FTLD patients with null mutations have reduced PGRN protein levels in their blood and CSF (18–22). Given the potential pathogenic role of PGRN in ALS, we measured PGRN levels in plasma and CSF from ALS patients. The normal PGRN values in ALS patients, as well as in FTLD patients and AD patients without PGRN mutations, suggest that shortage of PGRN is restricted to neurodegeneration caused by PGRN null mutations. Interestingly, the SNP rs9897526 associated with more severe disease in ALS patients (29) resulted in slightly lower PGRN plasma levels. Significance was not reached because of the small sample size of patients with the at-risk genotype in this study. Larger studies are required to study the effect of the rs9897526 SNP on PGRN protein levels.

In some patients with FTLD or AD and missense mutations of unknown pathogenic nature, PGRN blood levels are slightly reduced, whereas other mutations do not affect the PGRN levels (18, 21, 22). Here we report normal PGRN plasma levels in an ALS patient with an R110Q mutation. Recently, another missense mutation (C521Y) with proven segregation in a family with progressive nonfluent aphasia was found to leave PGRN plasma levels untouched (54). How missense mutations with normal protein expression can cause disease is currently unknown. Shortage of PGRN cannot be excluded at present because changes in protein processing or function may still result in loss of function.

In mouse models of motor neuron degeneration, we observed upregulation of PGRN in the spinal cord, mainly because of the increased expression in microglial cells. Strongly PGRN+ microglia were also observed in the ventral horn of the spinal cord in ALS patients. Upregulation of PGRN transcripts after acute or chronic damage to the CNS has been observed in previous mRNA expression studies. Progranulin mRNA was upregulated in mouse brain lysates after Sindbis infection (32), in microglial cells isolated from Creutzfeldt-Jakob disease inoculated mouse brains (36), in mouse brain from a transgenic model of mucopolysaccharidosis I and IIIB (37), in amyloid plaques (39), and in human lumbar spinal cord from ALS patients (38).

The strong expression of PGRN in microglia has been noted not only in ALS (50) but also around neuritic plaques in AD patients (10, 39). Interestingly, also in patients with FTLD caused by loss-of-function mutations in the PGRN gene, intense PGRN staining was observed in activated microglial cells (34, 35). Microglial upregulation occurs despite reduced blood, CSF, and brain levels of PGRN in these patients (18–22), suggesting that the healthy allele can compensate for the diseased allele carrying a null mutation in activated microglial cells. Uprogulation of progranulin in microglial cells seems to be a common response to injury in the CNS. Progranulin produced by microglia and released in the extracellular milieu may be one of the factors derived from microglia that can protect neurons from degeneration and may be important in microglial proliferation. Further research is required to unravel the functions PGRN in microglia and to clarify the effects of secreted PGRN by microglial cells on the microenvironment.

In the adult CNS, PGRN expression is limited to some neuronal populations, such as cortical and hippocampal pyramidal neurons and Purkinje cells (55). Here, we confirm that motor neurons in the spinal cord also express PGRN (56). Although downregulation of PGRN in spinal motor neurons has been observed after axotomy (57), motor neurons in the
diseased spinal cord in mouse models of motor neuron degeneration or ALS patients remained PGRN+. The functions of PGRN in motor neurons require further elucidation.

Despite advances in the search for biomarkers in ALS, markers that are sensitive to presymptomatic diagnosis or to the disease progression of motor neuron degeneration are still lacking (58). In view of microglial upregulation of PGRN (i.e., a secreted protein associated with disease progression), we evaluated the potential of PGRN as a diagnostic biomarker. No differences in PGRN serum or CSF levels were found between ALS patients or controls, indicating that PGRN ELISA on CSF is not a useful diagnostic biomarker for ALS. Normal CSF levels of PGRN were found in early symptomatic mutant SOD1G93A mice, but there was a distinct increase in CSF PGRN with disease progression. The CSF samples from ALS patients in our study obtained during the early diagnostic phase of the disease were not different from controls. In view of the variable disease course of ALS patients, however, serial CSF sampling in ALS patients with disease progression into later disease stages would be necessary to confirm a disease state–dependent upregulation of PGRN in ALS. Cerebrospinal fluid PGRN levels may reflect the degree of microgliosis and thus might be an independent disease state marker. Owing to improved symptomatic care of ALS patients, the survival of patients in advanced stages of the disease is improving, without affecting the decline in functional measures in the earlier stages of the disease (59).

Therefore, markers that reflect the biologic disease state in more advanced stages would be of additional value as a surrogate marker of disease severity in clinical studies.

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