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The lysosomal function of progranulin, a guardian against neurodegeneration

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Abstract

Progranulin (PGRN), encoded by the *GRN* gene in humans, is a secreted growth factor implicated in a multitude of processes ranging from regulation of inflammation to wound healing and tumorigenesis. The clinical importance of PGRN became especially evident in 2006, when heterozygous mutations in the *GRN* gene, resulting in haploinsuffiency, were found to be one of the main causes of frontotemporal lobar degeneration (FTLD). FTLD is a clinically heterogenous disease that results in the progressive atrophy of the frontal and temporal lobes of the brain. Despite significant research, the exact function of PGRN and its mechanistic relationship to FTLD remain unclear. However, growing evidence suggests a role for PGRN in the lysosome – most striking being that homozygous *GRN* mutation leads to neuronal ceroid lipofuscinosis (NCL), a lysosomal storage disease (LSD). Since this discovery, several links between PGRN and the lysosome have been established, including the existence of two independent lysosomal trafficking pathways, intralysosomal processing of PGRN into discrete functional peptides, and direct and indirect regulation of lysosomal hydrolases. Here we summarize cellular functions of PGRN, roles of PGRN in the nervous system and its link to multiple neurodegenerative diseases, with a particular focus dedicated to recent lysosome-related mechanistic developments.

Introduction

GRN mutation causes frontotemporal lobar degeneration and neuronal ceroid lipofuscinosis

The clinical importance of progranulin (PGRN; also known as acrogranin; granulin-epithelin precursor (GEP); GP88; PC cell-derived growth factor (PCDGF); and proepithelin (PEPI)) became evident in 2006, when heterozygous mutations in the *GRN* gene, resulting in haploinsuffiency, were found to cause frontotemporal lobar degeneration (FTLD) [8, 28, 36, 72, 118, 81]. FTLD is a clinically heterogenous, incurable neurodegenerative disease resulting in frontotemporal dementia (FTD), most often presenting with drastic alterations in behavior and personality, including social disinhibition as well as gradual decline in language capabilities [84]. Although the exact mechanism is unknown, the disease is characterized by progressive atrophy of the frontal and temporal lobes of the brain. FTLD is the second leading cause of early-onset dementia after Alzheimer's disease (AD), and is the

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third most common cause of cortical dementia [102, 84]. FTD can clinically be divided into distinct subtypes based upon earliest primary signs of disease. Behavioral-variant FTD is associated with sometimes severe alterations in personality and behavior, while primary progressive aphasia (PPA), which can be subdivided into semantic dementia (SD) and progressive nonfluent aphasia (PNFA), is associated with marked dysfunction in language processing and production. Interestingly, there is also overlap between the development of FTLD and the motor neuron disease, amyotrophic lateral sclerosis (ALS), with many FTLD patients developing motor neuron disease and many ALS patients showing cognitive decline [69]. In addition to classification by clinical presentation, FTLD can also be grouped by common histopathological features. Chief findings include inclusion bodies positive for tau protein (FTLD-Tau), ubiquitinated TAR DNA-binding protein 43 (TDP-43) (FTLD-TDP), or fused in sarcoma protein (FTLD-FUS) [73]. Although not all cases have a clear genetic component, there are several gene mutations known to result in FTLD, with major contributors including *GRN*, microtubule-associated protein tau (*MAPT*) and chromosome 9 open reading frame 72 (*C9orf72*) [99]. Since it was first associated with FTLD, more than 60 disease-causing *GRN* mutations have been identified [38, 99]. Accounting for ~20–25% of familial FTLD cases and ~10% of all FTLD cases, with the majority producing truncated transcripts that are degraded by nonsense-mediated mRNA decay, *GRN* mutation is primarily associated with the FTLD-TDP subtype and shows minimal connection with ALS [111].

Perhaps the most important finding leading to a hypothetical lysosomal function of PGRN was the 2012 discovery of a pair of siblings with adult-onset neuronal ceroid lipofuscinosis (NCL), with exome sequencing pointing to a homozygous variant in *GRN* resulting in a premature stop [117]. Like FTLD, NCLs are a family of neurodegenerative diseases that are symptomatically and pathologically diverse [88, 78, 62]. Clinical presentations encompass cognitive and motor deterioration, epilepsy, and retinopathy, with pathology including lysosomal accumulation of lipofuscin, an autofluorescent lipid and protein aggregate. NCLs were previously classified by age of onset, but with progressing molecular technologies, are now classified by genetic cause, of which 13 specific genes *(CLN1-13)*, including *GRN (CLN11)*, have been identified [88, 27]. Since the first cases of *GRN*-related NCL came to prominence, a second family has been identified wherein both parents developed FTLD and were found to be *GRN* mutant carriers, while their daughter who received two copies of the mutant allele developed adult-onset NCL, confirming the dosage effects of PGRN in disease manifestation [3]. More importantly, NCL-related phenotypes are reported in FTLD patients with *GRN* mutation [142, 41, 134], supporting the theory that lysosomal dysfunction might serve as a common mechanism of these two diseases.

GRN mutation is associated with AD

In addition to *GRN* mutation directly causing FTLD and NCL, PGRN has been linked to the pathophysiology of AD, although the exact nature of the relationship is less well-defined. AD is the most common senile dementia, characterized by the accumulation of insoluble amyloid-β (Aβ) plaques, neurofibrillary tangles of hyperphosphorylated tau, and gradual memory impairment. A number of groups have found that the T-allele of a *GRN* single nucleotide polymorphism (SNP), rs5848, in the 3′-untranslated region (3′-UTR), is

associated with an increased risk of AD, which has been further supported by meta-analytic studies [68, 114, 57, 146]. The risk of AD development is inversely proportional to serum PGRN levels, as AD patients carrying homozygous T alleles have lower serum PGRN levels [51], possibly due to translational inhibition consequent to increased miRNA binding [101].

In AD mouse models, PGRN expression is dramatically upregulated in microglia [94] and in axons [42] around Aβ plaques. However, conflicting results have been obtained regarding the effect of PGRN in AD. PGRN has been demonstrated to attenuate pathological phenotypes, including Aβ plaque burden, neuroinflammatory markers, and memory impairment in AD mouse models in some studies [76, 137]. Other studies, however, have demonstrated that some AD phenotypes are *improved* by PGRN deficiency [49, 122]. The root of this discrepancy is currently unclear, but it has been proposed to be due to variations in mouse strains used or age of animals assessed. Despite this, it is clear from human studies that PGRN, while likely not causative in cases of AD, may be an important marker and modulator of disease susceptibility.

PGRN is a conserved, multifunctional pro-protein with unique structure

Structurally, PGRN is a 593 amino acid, 88 kDa protein that is highly glycosylated (~65 kDa without glycosylation) with 4 confirmed, and 1 putative, N-linked glycosylation sites [119]. It is comprised of seven full and one half conserved granulin domains connected by short linker regions [130]. The general structure of the granulin domains is unique, with each full domain consisting of approximately 55 residues, with 2 (granulin G) or 4 (other granulins) double cysteine motifs and 4 single cysteine motifs. Structural studies of human granulin A and a granulin derived from carp revealed stacks of β-hairpins held together tightly by six disulfide bonds [50, 131, 140]. Despite this bonding, there appears to be great variability in the rigidity and flexibility of each mammalian granulin [130]. Notably, PGRN can be proteolytically processed to release individual granulin peptides, which are evidenced to possess functions independent of, and sometimes in contrast to, the full-length precursor. These granulin peptides were, in fact, discovered a few years before PGRN, having been isolated from epidermal carcinoma, rat kidney, and inflammatory exudates [15, 10, 115]. PGRN/granulin homologs are found across taxa, ranging from plants to invertebrates, such as sponges, worms, and insects, to vertebrates, including fish and mammals [92]. Transcriptionally, the *GRN* gene is expressed to varying degrees across a wide range of tissues and cell types, including a variety of neurons, immune cells, and epithelial cells [29]. While the exact function of PGRN remains elusive, it has been found to be involved in numerous normal physiologic and pathologic processes.

PGRN is a growth factor involved in inflammation, wound healing, and tumorigenesis

When granulin peptides were first discovered in 1990, their primary role was attributed to the modulation of cell growth [44]. Originally termed “epithelins”, the first two peptides to be isolated were shown to have opposing effects on the *in vitro* growth of a murine keratinocyte cell line [115, 98]. As with its derivative peptides, full-length PGRN can function as a growth factor. Recombinant PGRN supplied in the culture media of two epithelial cell lines showed stimulation of proliferation [29]. Similarly, PGRN has been demonstrated to stimulate endothelial cell migration and vessel growth *in vitro* and *in vivo*

[128, 46]. Multiple studies have shown that PGRN is able to modulate MAPK/ERK, PI3K/Akt, and FAK signaling pathways, despite the lack of a previously identified PGRN signaling receptor [155, 70, 45]. A recent study, however, indicates that PGRN binds to the receptor tyrosine kinase, EPH receptor A2 (EphA2) and, through this binding, is able to activate MAPK and Akt signaling pathways (Table 1) (Fig. 1) [85].

Owing at least in part to its mitogenic and angiogenic effects, PGRN expression has been linked to several cancers, generally with direct correlation between increasing PGRN expression and cancer severity [1, 32, 112, 126, 127]. PGRN may also be a useful biomarker in a number of cancers, such as ovarian epithelial cancer and malignant lymphoma, where elevated serum PGRN levels can be indicative of disease and prognostic of lower survival [151, 6].

In addition to the roles PGRN plays in cell proliferation, wound healing, and tumorigenesis, it is a well-established modulator of immune function [53, 129, 21, 124, 59]. In *C. elegans*, a PGRN homolog was reported to slow clearance of apoptotic cells [58]. Microglia, the resident immune cells in the brain, especially those which have become reactive following insult or trauma, produce and secrete especially high levels of PGRN [52, 162, 77, 96, 83], and PGRN has been shown to regulate microglial activation, migration, phagocytosis, and synapse pruning [71, 135, 97, 74, 153]. Interestingly, PGRN and granulin peptides have been found to possess opposing inflammatory functions, with PGRN generally being anti-inflammatory and granulin peptides pro-inflammatory [164, 63]. In fact, studies in *C. elegans* have suggested that granulin peptides might be toxic [107], although this has yet to be reported in mammalian systems. Because of this stark contrast, extracellular processing of PGRN into granulin peptides may be tightly regulated, and PGRN can be cleaved by multiple proteases, including neutrophil elastase, a disintegrin and metalloproteinase with thrombospondin motifs 7 (ADAMTS-7), proteinase 3, matrix metalloproteinase (MMP)-9, MMP-12, and MMP-14 (Fig. 1) [60, 144, 164, 121, 19]. Thus, one role of PGRN in neurodegenerative diseases is regulating microglia-mediated inflammatory responses. There is some evidence that PGRN may bind the tumor necrosis factor (TNF) receptors, with competitive inhibition of TNF-α providing a mechanism of action (Fig. 1) [125]. However, there has been dispute about the nature of this relationship and whether it is physiologically real [25].

Considering the relationship between PGRN and neurodegeneration and its described functions as a growth and survival factor, it is not surprising that PGRN and at least one granulin peptide can function as neurotrophic factors, promoting neuron survival and neurite outgrowth *in vitro* and *in vivo,* both in mammalian systems and in Zebrafish [135, 106, 26, 35, 141, 64, 37, 30, 29]. A proteomic screen and gene ontology enrichment analysis has revealed an association between PGRN and Notch receptor signaling, with reported binding between PGRN and Notch1, 2, 3, and 4 (Fig. 1) [5]. However, it is not known whether this interaction mediates the neurotrophic effects of PGRN.

While loss of a direct neurotrophic or inflammation regulatory effect of PGRN could contribute to neurodegeneration in FTLD, the genetic link between PGRN and NCL strongly supports a lysosomal function of PGRN, as all the NCL genes discovered so far play a direct

or indirect role in regulating lysosomal function [27]. With this understanding, there has been a new focus on identifying this potential function.

PGRN and the Lysosome

Lysosomal dysfunction in models of PGRN deficiency

Several groups have utilized mouse and cellular models of PGRN deficiency to try to recapitulate the disease processes of *GRN*-related FTLD and NCL. Although pathology in *Grn*$^{-/-}$ mouse models tends to be more moderate than in human cases, a number of phenotypes have been reported, including exaggerated inflammatory responses, microgliosis, astrogliosis, and behavioral dysfunction such as OCD-like and disinhibition-like behavior [153, 74, 154, 71, 104]. In addition to these broad observations, signs of lysosomal dysfunction have also been reported. Aged *Grn*$^{-/-}$ mice develop lipofuscin deposits and enlarged lysosomes, signatures of NCL (Fig. 2a, 2b) [143, 2, 95]. As occurs with FTLD with *GRN* mutations (FTLD-*GRN*), at least one group has observed phosphorylated TDP-43 accumulation in *Grn*$^{-/-}$ mice [154]. Additional links to the endolysosomal system include diffuse or granular cytosolic ubiquitin deposits [143, 2, 154] and aggregation of the autophagy-related protein, ubiquitin-binding protein p62 [123]. Reduced autophagic flux and autophagy-dependent clearance have also been reported in PGRN-deficient mice [22]. Human FTLD-*GRN* patient-derived primary fibroblasts showed decreased lysosomal protease activity, and lymphoblasts contained NCL-like storage material [142]. Similarly, FTLD-*GRN* patient induced pluripotent stem cell (iPSC)-derived cortical neurons have been shown to develop NCL- and FTLD-like pathologies, such as enlarged vesicles, lipofuscin accumulation, and a specific lysosomal cathepsin deficiency [134]. These models provide strong evidence for lysosomal dysfunction in cases of PGRN deficiency. Importantly, there is pathologic overlap between FTLD-*GRN* patients and cells derived from them and cases of NCL, suggesting a mechanistic link between these two diseases.

GRN transcriptomics and lipidomics

Transcriptomic analyses have also provided novel insights into the relationship between PGRN and the lysosome. Transcription factor EB (TFEB) is known to regulate the expression of most lysosomal genes [108]. In deleterious situations where nutrients are scarce or lysosomal function is diminished, TFEB translocates to the nucleus and stimulates lysosomal biogenesis. Studies have found that the *GRN* gene is under the transcriptional control of TFEB, supporting a lysosomal association [12, 108].

Multiple transcriptomic studies have also confirmed a critical lysosomal role of PGRN and a transcriptional upregulation of many lysosomal genes in response to PGRN deficiency in mice [33, 122, 61, 123, 71]. A microglia-specific transcriptomic study revealed an age-dependent upregulation of many lysosomal genes and genes related to innate immunity, including *Cd68*, triggering receptor expressed on myeloid cells 2 (*Trem2*), and complement genes *C1qa*, *C1qb*, *C1qc* and *C3* in response to PGRN deficiency [71]. Lysosomal dysfunction has been previously linked to dysregulation of innate immunity [82], although the mechanistic connection between these two processes still needs to be worked out.

Using a model of primary human neural progenitor cells with shRNA *GRN* knockdown, upregulation of genes related to apoptotic regulation and Wnt signaling were identified with gene ontology analysis [105]. Additionally, enrichment in ontology categories related to ubiquitination was observed, with upregulation of associated genes including ubiquitin-conjugating and -ligating enzymes. As ubiquitin-positive TDP-43 inclusions are a hallmark of FTLD-*GRN* and lysosomal degradation is one of the primary mechanisms of processing ubiquitinated proteins, this is further evidence of an association between loss of PGRN and lysosomal dysfunction.

Based upon previous findings demonstrating altered lysosome homeostasis in association with *GRN* loss, one group hypothesized that PGRN might help regulate lysosomal lipid metabolism [33]. Lipidomic analysis demonstrated dosage-dependent differences in brain lipids in both humans and mice with PGRN insufficiency, mainly found to be related to triacylglyceride, diacylglyceride, phosphatidylethanolamine, and phosphatidylserine subspecies. Transcriptomic analysis of $Grn^{+/-}$ and $Grn^{-/-}$ mouse brains showed a number of differentially expressed transcripts involved in lipid metabolism, including lipid hydrolases that could potentially explain lipodomic changes observed.

Lysosomal trafficking of PGRN

Among the earliest findings linking PGRN to the lysosome was the discovery that PGRN is lysosomally localized (Fig. 2c, 2d) [52, 83]. The endocytic receptor, sortilin, was identified as a high affinity binding partner of PGRN and shown to mediate its lysosomal trafficking (Table 1) [52]. This sortilin-mediated sorting is likely to occur both at the trans-Golgi network (TGN) in the biosynthetic pathway, and at plasma membrane in the endocytic pathway (Fig. 1). In the brain, PGRN is expressed by microglia, neurons, astrocytes, endothelial cells, and oligodendrocyte precursor cells, with microglia having the highest expression levels [156]. Thus, the extracellular pool of PGRN is likely derived from several sources. Ablation of sortilin in mice leads to a 5-fold increase in the levels of serum PGRN [52]. Sortilin is a VPS10 family protein highly expressed in neurons, which is also known to bind the nerve growth factor precursor (proNGF) and neurotensin [89, 75, 31, 24]. The binding of PGRN to sortilin is dependent on the final 3 amino acids, QLL, and more specifically, the terminal carboxylate of PGRN [157], and loss of these completely abolishes the interaction. Consistent with the role of sortilin as a PGRN trafficking receptor, sortilin is not required for the neurotrophic function of PGRN in cell culture [37, 30]. However, a recent study demonstrated that the PGRN-sortilin interaction strengthens and maintains developing climbing fiber inputs, counteracting synapse elimination in the developing cerebellum [133]. It remains to be determined whether this is dependent on sortilin-mediated PGRN endocytosis or on active signaling at the plasma membrane.

In sortilin knockout mice, PGRN is still successfully delivered to neuronal lysosomes, suggesting the essential existence of an alternative trafficking pathway [52, 161]. Interestingly, this was shown to be mediated not by direct binding to another transmembrane trafficking receptor, but through an indirect mechanism whereby PGRN binds to the soluble lysosomal protein, prosaposin (PSAP) (Table 1). When PSAP binds to either of its own trafficking receptors, the cation-independent mannose-6-phosphate receptor (CI-M6PR) or

the low-density lipoprotein receptor-related protein 1 (LRP1), it carries PGRN along with it to the lysosome. Like sortilin, PSAP is able to traffic PGRN from the secretory pathway as well as from the extracellular space (Fig. 1). The importance of this was underscored when loss of just one functional *Psap* allele was sufficient to significantly increase plasma PGRN levels in mice [161]. Additionally, the reciprocal trafficking, wherein PGRN transports PSAP to the lysosome via sortilin, has also been shown to occur [162].

PSAP is a proprotein composed of four homologous saposin domains (A, B, C, and D), and is known to be proteolytically processed in the lysosome into individual saposin peptides [90]. These peptides are responsible for activating different lysosomal sphingolipid-degrading enzymes and loss of PSAP function has been linked to lysosomal storage diseases (LSDs), including Gaucher disease (GD) and metachromatic leukodystrophy. The interaction between PGRN and PSAP is mediated by the linker region connecting saposins B and C and multiple granulin domains, with granulin D and E showing the most substantial binding [160]. More recently, the reciprocal relationship has been shown to be occur, where PGRN is able to deliver PSAP to the lysosome via sortilin (Fig. 1) [162].

The discoveries of independent PGRN trafficking pathways correlate nicely with studies searching for genetic determinants of plasma PGRN levels. Genome-wide association studies (GWAS) linked plasma PGRN levels to SNPs in both sortilin and PSAP, further confirming the importance of these two routes of trafficking [20, 86]. However, this does not preclude the possibility that additional pathways exist. In theory, PGRN might be carried to the lysosome by binding to other soluble lysosomal proteins, like PSAP, or by interacting with additional lysosomal trafficking receptors.

PGRN loss reduces lysosomal saposins

Ensuing research on the PGRN-PSAP relationship indicates that one of the mechanisms of pathology in cases of PGRN deficiency may be a reduction in neuronal lysosomal saposin levels due to a loss of indirect PSAP lysosomal trafficking via sortilin, which is highly expressed in neurons (Fig. 1) [162]. In support of the importance of this trafficking pathway, immunofluorescence imaging of brain sections from either *Grn*$^{-/-}$ or *Sort*$^{-/-}$ mice showed a significant reduction in neuronal PSAP, and brain sections from FTLD-*GRN* patients showed a significant reduction in neuronal PSAP, saposin A, saposin B, and saposin C in the orbitofrontal cortex compared to healthy control or AD patient brain. Interestingly, saposin D, a known component of lipofuscin, accumulates in enlarged lysosomes of FTLD-*GRN* brain samples, which might reflect different biochemical properties and degradation routes of saposin peptides in the lysosome. Importantly, this reduction in saposin levels was specific to FTLD-*GRN,* but not FTLD-Tau patients, further implicating PGRN in this effect. Furthermore, loss of PSAP in mice was found to cause pathology and behavioral phenotypes reminiscent of FTLD. Therefore, by regulating PSAP trafficking, PGRN determines neuronal saposin levels and loss of neuronal saposins is likely to contribute to FTLD-like phenotypes.

Progranulin is lysosomally processed into granulin peptides

With accumulating evidence that, in addition to being highly secreted, PGRN is localized to the lysosome and an understanding that it can be proteolytically processed into granulin peptides in the extracellular space, a natural corollary was that PGRN could be processed within the lysosome in a manner similar that of its binding partner, PSAP. An important implication of this is that, once liberated, granulin peptides could potentially each be involved in unique interactions, regulating different aspects of lysosomal physiology like the saposin peptides derived from PSAP. In fact, recent studies suggest that this may be the case (Fig. 1).

Several laboratories have independently found that PGRN can be processed intracellularly in a lysosome-dependent manner [159, 48]. Granulin peptides were readily detected in lysates from several immortalized cell lines, and extracellular PGRN was efficiently endocytosed and processed into granulin peptides. While PGRN was detected in both the cell lysate and conditioned media collected from these cells, granulin peptides were almost undetectable in the media, indicating that, at least in these conditions, the peptides are not secreted nor produced extracellularly [48, 159]. This is also an assurance that the peptides detected internally were not endocytosed from the media.

Chemical inhibition of lysosomes was found to increase full-length PGRN, while decreasing granulin peptides, indicating that proper lysosome functioning is necessary for peptide generation. Perturbation of PGRN lysosomal trafficking also led to reduced production of granulin peptides. Additionally, cathepsin L (CTSL) was identified as a PGRN protease capable of efficiently cleaving PGRN into distinct peptides [159, 48, 67]. Liquid chromatography-mass spectrometry was used to identify the CTSL cleavage sites within PGRN, which were found to reside within its linker regions and were mostly distinct from those of neutrophil elastase [67].

These data are significant because, while it has been postulated that granulin peptides may exist in the lysosome, cumulatively, this is the first clear evidence of their lysosomal occurrence. These results support the hypothesis that granulin peptides may be functional units within the lysosome and relevant to the disease mechanisms of FTLD and NCL. Importantly, haploinsufficiency of full-length PGRN in FTLD leads to haploinsuffiency of granulin peptides [48]. While the functions of granulin peptides remain to be fully elucidated, at the time of this writing, two different lysosomal enzymes have been shown to be positively regulated by PGRN or granulin peptides: cathepsin D (CTSD) and glucocerebrosidase (GBA).

PGRN regulates CTSD activity

The first evidence of a conceivable association between PGRN and lysosomal proteases comes from studies in *Arabidopsis* and several other plant species. Certain plant vacuolar cysteine proteases, such as the *Arabidopsis* protein, Responsive-to-Desiccation-21 (RD21), contain a granulin domain that is homologous to those found in mammalian PGRN [148, 43, 132]. The function of this domain is still unclear, but multiple studies have suggested that it

is involved in the regulation, either positive or negative, of the maturation and enzymatic functioning of these proteases [148, 150, 149].

The existence of vacuolar combined granulin-proteases intimates the possibility of the existence of an analogous relationship in mammalian cells. Recently, three groups independently identified a functional relationship between PGRN, as well as select granulin peptides, and the lysosomal enzyme, CTSD [158, 11, 134]. CTSD is an aspartyl protease responsible for degrading proteins in the lysosome. Mutations in CTSD have been associated with AD and, as occurs with PGRN deficiency, CTSD loss in humans results in NCL [139, 113] and CTSD-deficient mice develop phosphorylated TDP-43 aggregates [41].

A correlation between *Grn*-deficiency and CTSD expression was previously established when $Grn^{-/-}$ mice were found to have an increase in both the immature and mature forms of CTSD, which rise up to 10× compared to WT mice by 20–24 months of age [41]. In a model of facial crush injury, CTSD was the most highly transcriptionally upregulated gene in the facial motor nucleus of $Grn^{-/-}$ mice [11]. Likewise, human FTLD-*GRN* patient iPSC-derived cortical neurons showed an increase in mature CTSD protein by day 35 post-differentiation [134].

While the upregulation of CTSD expression could be due to broader effects of PGRN loss, a novel interaction between PGRN and CTSD was established via overexpression and co-immunoprecipitation [158], as well as by pulldown of recombinant PGRN or granulin E added to mouse brain lysate [11]. Perhaps the most profound new findings are that CTSD activity is reduced in instances of PGRN deficiency and that PGRN and granulin E specifically modulate the activity of this protease. CTSD activity was significantly decreased across a range of tissue lysates from $Grn^{-/-}$ mice by as early as 2 months of age [158], and heterozygous *GRN* mutant iPSC-derived cortical neurons showed a significant decrease in CTSD activity, despite the above-mentioned increase in mature CTSD protein levels. Remarkably, the reduction in CTSD activity in brain lysates of aged $Grn^{-/-}$ mice, normalized to mature CTSD, could be rescued by the addition of recombinant PGRN [11]. A direct augmentation of CTSD activity was demonstrated when recombinant PGRN or granulin E increased the activity of recombinant CTSD in a dose-dependent manner [11, 134]. Additionally, recombinant PGRN was found to increase the stability of CTSD at temperatures exceeding 37°C [11].

These new discoveries make CTSD a protein of great interest with respect to the role of PGRN in the development of FTLD and NCL. With the observed direct binding, moderation of CTSD stability and activity, and overlap in disease and pathology, it is reasonable that CTSD deficiency subsequent to PGRN loss may be at least a partial mechanism of these two diseases. This also spurs the notion that PGRN may directly modulate the activity of other lysosomal enzymes, especially proteases similar to CTSD.

PGRN is a co-chaperone for GBA

A second lysosomal enzyme that has become of interest to PGRN-related pathologies is GBA, a β-glucosidase that cleaves glucocerebroside into glucose and ceramide [13]. Genetic mutation, leading to GBA deficiency, causes GD, the most common LSD [14, 13]. GBA

mutation is also a recognized risk factor for Parkinson's disease (PD), with 5–10% of patients carrying a mutant gene [109].

An association between PGRN and GD was reported in 2016, when a significant decrease in serum PGRN levels was found in GD patients [55]. Likewise, under induced chronic inflammatory conditions, *Grn*$^{-/-}$ mice showed GD phenotypes, including hepatosplenomegaly, glycolipid accumulation in bone marrow, and appearance of Gaucher-like cells in multiple tissues, which could be rescued by supplementation with human GBA analogue [55]. GBA was found to be mislocalized and aggregated in the cytoplasm in *Grn*$^{-/-}$ mice under these conditions. Upon further investigation, PGRN, primarily through the C-terminal granulin E region, was shown to mediate an interaction between heat shock protein 70 (HSP70) and GBA, as well as the GBA trafficking receptor, lysosome membrane protein 2 (LIMP-2) [54]. However, GBA protein levels and enzymatic activity were not affected in *Grn*$^{-/-}$ mouse tissue lysate [55].

These studies identify PGRN as a functional co-chaperone of HSP70, GBA, and LIMP-2 and suggest that in cases of PGRN deficiency, loss of HSP70 chaperone function may result in mislocalization and loss of function of GBA. As with CTSD deficiency due to PGRN loss, GBA deficiency may be a mechanism of PGRN-related FTLD and NCL. These results also open new avenues of research, as it is possible that PGRN, in conjunction with HSP70 or possibly other HSPs, acts as a co-chaperone for other lysosomal enzymes.

It is worth noting that PSAP is known to interact with CTSD [47, 40, 66], and saposin peptides with GBA [80, 79]. As PGRN can regulate CTSD activity and CTSD is the major contributor to PSAP processing to active saposins, it stands to reason that changes in PGRN levels may potentially alter PSAP processing. Likewise, GBA, an enzyme that is specifically activated by saposin C (and to a lesser extent, saposin A), could be impacted by this. Because of the complexity of these associations, more work is required to determine how PGRN loss affects these proteins and what the downstream consequences are.

TMEM106B is a risk factor for FTLD with *GRN* mutations

In 2010, a GWAS to detect gene loci imparting susceptibility to FTLD-TDP identified a then uncharacterized transmembrane protein, TMEM106B, which showed risk vulnerability specific to *GRN* mutation carriers [136]. There are some indications that the pathologic connection to TMEM106B is related to increased levels of the protein. First, elevated levels of TMEM106B have been found in the post-mortem brains of FTLD-TDP patients [23]. Second, Nicholson, et al. identified a coding variant, p.T185S, in linkage disequilibrium with a protective minor allele of the gene [87, 34, 138]. They found that upon overexpression, the S185 variant levels were approximately 40% those of the T185 variant. Inhibiting protein synthesis or lysosomal degradation in cell culture demonstrated that this was due to differential rates of degradation. These results suggest that the protective nature of this variant may be due to its more rapid degradation and subsequent lower levels.

While the function of the protein and its association with FTLD remain undetermined, early characterization found it to be a glycosylated, type II transmembrane protein primarily localized to endosomes and lysosomes [16, 65, 23]. With the association between elevated

TMEM106B levels and disease, significant effort has gone into examining the effects of TMEM106B overexpression. The most obvious phenotype associated with TMEM106B overexpression is lysosomal enlargement, which has been observed in multiple cell lines [16, 120, 23, 17]. Overexpression also results in a decreased capacity to degrade endocytic cargo [16], which could be due to inhibition of lysosomal acidification [23]. Likewise, it has been shown to increase intracellular PGRN levels [23, 16], which was recently demonstrated to likely be due to inhibition of processing into granulin peptides [48].

One study has identified an interaction between TMEM106B and microtubule-associated protein 6 (MAP6), and indicated a role for TMEM106B in dendritic trafficking of lysosomes [110]. An interaction between TMEM106B and the FTLD-related protein, charged multivesicular body protein 2B (CHMP2B), a component of endosomal sorting complexes required for transport-III (ESCRT-III), has also been reported [56]. Recently, Klein, et al. generated a *Tmem106b* knockout mouse model and found that loss of the protein resulted in a downregulation of several lysosomal enzymes and vacuolar-ATPase AP1 and V0 domain subunits [61]. Similar to overexpression models, *Tmem106b*$^{-/-}$ primary cortical neurons showed a decrease in lysosomal acidification and, likely related to this, the authors found that TMEM106B interacts with the V-ATPase AP1 subunit in HEK293T cells.

Loss of TMEM106B was also found to ameliorate many of the pathological phenotypes associated with *Grn*$^{-/-}$ mice [61]. In double knockout animals, TMEM106B loss corrected retinal degeneration and rescued hyperactivity in *Grn*$^{-/-}$ mice. However, some detriments associated with PGRN deficiency, including lipofuscin accumulation and CD68 upregulation in microglia were not corrected [71, 122]. The authors argue that PGRN and TMEM106B regulate lysosomal biology in opposite directions.

To determine the *in vivo* effect of TMEM106B overexpression, a transgenic mouse line incorporating human TMEM106B under the neuron-specific CAMKII alpha promoter was created [163]. However, TMEM106B levels were found to be tightly regulated and were not elevated in the transgenic line compared to WT mice. Because TMEM106B is a risk factor for *GRN* mutant carriers, the authors wanted to see if PGRN regulates TMEM106B homeostasis. *Grn*$^{-/-}$ mice developed elevated levels of TMEM106B protein in the cortex with aging, which is further increased by the expression of the transgene. In the *Grn*$^{-/-}$ background, expression of the TMEM106B transgene was associated with greater lipofuscin accumulation and a higher percentage of neurons containing enlarged lysosomes, indicating greater lysosomal dysfunction. These results nicely recapitulate the genetic interaction between *GRN* and *TMEM106B* seen in human FTLD cases.

Intriguingly, a recent analysis identified a dominant D252N mutation in TMEM106B as a cause of hypomyelinating leukodystrophy [116, 152]. This disease is characterized by an intractable deficit in myelination leading to clinical signs, such as nystagmus, ataxia, and spasticity. It is not clear at the moment how TMEM106B mutation causes this disease, or whether this relates in any way to its relationship with PGRN and FTLD. In addition to this new disease association, a recent study identified *TMEM106B* and *GRN* as two main determinants of differential aging in the cerebral cortex with genome-wide significance [103].

It is still uncertain what the exact function of TMEM106B is or how it alters FTLD susceptibility. However, a great deal has been learned about the protein since it rose to prominence eight years ago. It is now understood that TMEM106B is an important regulator of lysosomal morphology and function, potentially through moderation of acidification, and that an increase in TMEM106B levels produces grossly enlarged, poorly functioning lysosomes. This, alone or in combination with a reduction in PGRN processing, could potentially exacerbate lysosomal defects associated with PGRN haploinsufficiency in FTLD.

Conclusions

The mechanistic link between PGRN and neurodegeneration has been elusive since its deficiency was first found to result in FTLD over a decade ago. Since that time, however, steady progress has been made in dissecting its function. Commonalities between PGRN-related FTLD and NCL pathology have been identified, PGRN has been shown to be trafficked to and processed in the lysosome, and direct mechanisms of lysosomal dysfunction due to PGRN loss have been established. Taken together, these results strongly support a necessary lysosomal function of PGRN that likely contributes to neurodegeneration. However, it remains to be shown that findings such as regulation of CTSD and GBA function by PGRN directly influence FTLD and NCL pathology. More so, these leaps in our understanding also raise new questions. Does PGRN modulate the activity of other lysosomal enzymes? Do other granulin peptides possess lysosomal functionality? Is there a greater significance to the overlap between binding partners and downstream functions of PGRN and PSAP? Notably, in addition to *GRN*, many other FTLD-associated genes, including valosin-containing protein (*VCP)/p97*, *CHMP2B*, sequestosome 1 (*SQSTM1)*, TANK-binding kinase 1 (*TBK1),* and optineurin (*OPTN),* are involved in endolysosome trafficking and the autophagy-lysosome pathway [100]. Therefore, lysosomal dysfunction may serve as a common mechanism of FTLD. As research pushes forward and we better understand the biological role of PGRN, we move closer to determining the mechanisms of related neurodegenerative diseases and developing new therapeutics to treat such conditions.

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List of Abbreviations in Main Text

3′-UTR	3′-untranslated region
Aβ	Amyloid-β
AD	Alzheimer’s disease
ADAMTS-7	A disintegrin and metalloproteinase with thrombospondin motifs 7
ALS	Amyotrophic lateral sclerosis
C9orf72	Chromosome 9 open reading frame 72

CHMP2B	Charged multivesicular body protein 2B
CI-M6PR	Cation-independent mannose-6-phosphate receptor
CLN11	Ceroid-lipofuscinosis, neuronal 11
CPPIC	Cysteine protease of protease-inhibitor complex
CTSD	Cathepsin D
CTSL	Cathepsin L
EphA2	EPH receptor A2
ESCRT-III	Endosomal sorting complexes required for transport-III
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FTLD-FUS	FTLD with fused in sarcoma protein-positive inclusions
FTLD-*GRN*	FTLD with *GRN* mutation
FTLD-TAU	FTLD with tau-positive inclusions
FTLD-TDP	FTLD with TAR DNA-binding protein 43-positive inclusions
GBA	Glucocerebrosidase
GD	Gaucher disease
GEP	Granulin-epithelin precursor
GWAS	Genome-wide association study
HSP70	Heat shock protein 70
iPSC	Induced pluripotent stem cell
LIMP-2	Lysosome membrane protein 2
LRP1	Low-density lipoprotein receptor-related protein 1
LSD	Lysosomal storage disease
MAP6	Microtubule-associated protein 6
MAPT	Microtubule-associated protein tau
NCL	Neuronal ceroid lipofuscinosis
OPTN	Optineurin
OVA	Ovalbumin
PCDGF	PC cell-derived growth factor

PD	Parkinson's disease
PEPI	Proepithelin
PGRN	Progranulin
PNFA	Progressive nonfluent aphasia
PPA	Primary progressive aphasia
proNGF	Nerve growth factor precursor
PSAP	Prosaposin
RD21	Responsive-to-dessication-21
SD	Semantic dementia
SQSTM1	Sequestosome 1
SNP	Single nucleotide polymorphism
TBK1	TANK-binding kinase 1
TDP-43	TAR DNA-binding protein 43
TFEB	Transcription factor EB
TGN	Trans-Golgi network
TNF	Tumor necrosis factor
Trem2	Triggering receptor expressed on myeloid cells 2
VCP	Valosin-containing protein

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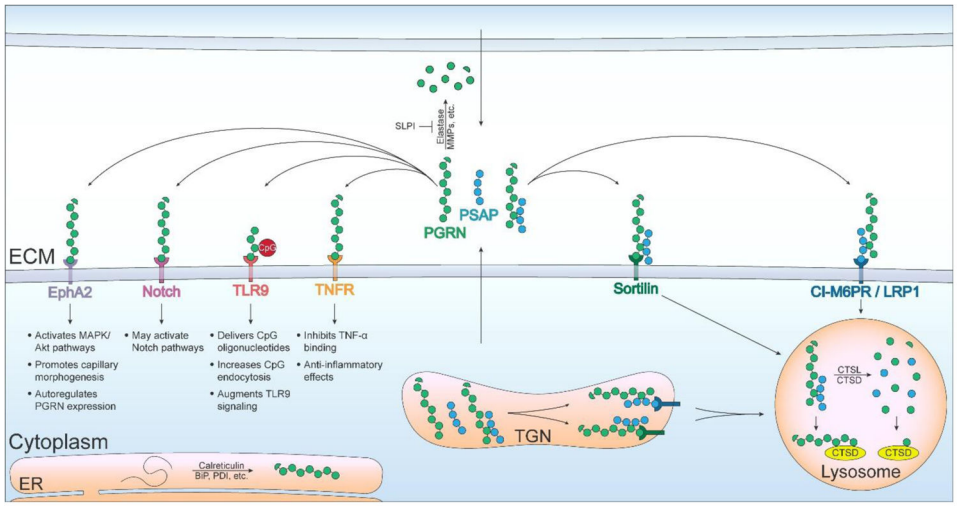

Figure 1. PGRN interactions, trafficking, and proteolysis

Several PGRN receptors have been identified, including EphA2, Notch, TLR9, and TNFR. Chaperones, such as BiP and PDI ensure proper folding of PGRN in the ER. Mature PGRN can be delivered to the lysosome from the TGN or extracellular space by two independent pathways. 1) PGRN binds directly to the trafficking receptor, sortilin, which delivers it to the lysosome. 2) PGRN binds to the soluble lysosomal protein, PSAP, which then binds to either of its own trafficking receptors, the CI-M6PR or LRP1, and carries PGRN with it to the lysosome. The complementary relationship also exists, wherein PGRN transports PSAP to the lysosome via sortilin. Upon reaching the lysosome, PGRN can be cleaved by CTSL into individual granulin peptides and PSAP cleaved by CTSD into individual saposins. PGRN and Grn E can then bind to CTSD and augment its activity.

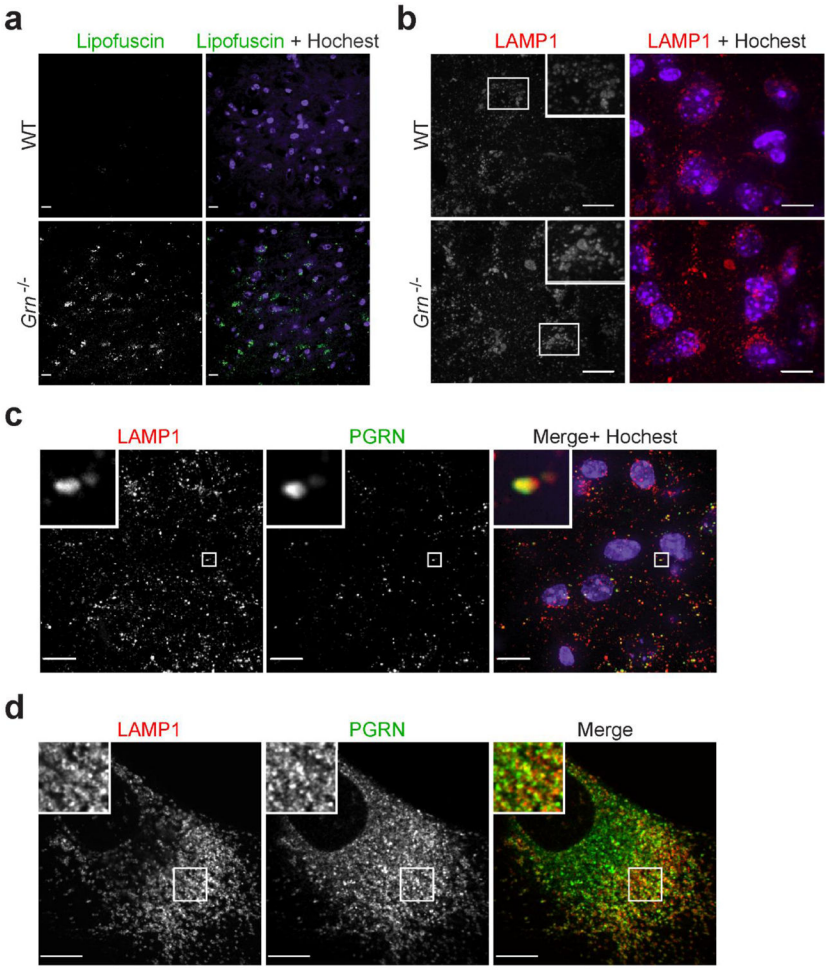

Figure 2. PGRN is a lysosome-resident protein important for lysosome function

a) Increased lipofuscin accumulation in *Grn*$^{-/-}$ mice. Brain sections from 4-month-old WT and *Grn*$^{-/-}$ mice were imaged at 488 nm to detect auto-fluorescence (green). Hoechst 33342 was used as a nuclear marker. Scale bar = 10 μm.

b) Lysosome enlargement in *Grn*$^{-/-}$ mice. Brain sections from 10-month-old WT and *Grn*$^{-/-}$ mice were immunostained with rat anti-mouse LAMP1 antibodies. Hoescht 33342 was used as a nuclear marker. Scale bar = 10 μm.

c) PGRN co-localizes with the lysosome marker, LAMP1, in mouse brain. A brain section from a 1-month-old WT mouse was immunostained with sheep anti-mouse PGRN and rat anti-mouse LAMP1 antibodies. Hoechst 33342 was used as a nuclear marker. Scale bar = 10 μm.

d) PGRN co-localizes with the lysosome marker, LAMP1 in human fibroblasts. Primary human fibroblasts were immunostained with goat anti-human PGRN and rabbit anti-human LAMP1 antibodies. Scale bar = 10 μm

Table 1

Known PGRN Protein Interactors

Class	Protein	Description	Functional Significance	Ref.
ECM/Serum Proteins	COMP	Cartilage-related protein	Enhances PGRN proliferative effect on chondrocytes	[147]
	HDL/apo A-I	Lipoprotein/HDL protein	May limit PGRN processing to suppress inflammation	[91]
	Perlecan	Proteoglycan	Diminishes mitogenic effects of PGRN	[39]
Secreted Proteins	ADAMTS-7	Protease, digests COMP	Processes PGRN into granulin peptides	[7]
	MMP-9[a]/12/14	Endopeptidase	Processes PGRN into granulin peptides	[145, 121, 18]
	NE	Serine protease	Processes PGRN into granulin peptides	[164]
	PR3	Serine protease	Processes PGRN into granulin peptides	[60]
	SLPI	Inhibitor of NE	Sequesters PGRN, prevents processing by NE	[164]
Membrane Proteins	Dlk1b	EGF-like homeotic protein	Unknown function	[9]
	EphA2	Receptor tyrosine kinase	Upregulates PGRN, activates MAPK/Akt	[85]
	Notch 1–4	Growth signaling receptors	Promotes peripheral nerve regeneration	[5]
	Sortilin	Endocytic/signaling receptor	Traffics PGRN to the lysosome	[52]
	TLR9	Immune receptor	PGRN products aid CpG oligonucleotide delivery	[93]
	TNFR1/2	Inflammatory signaling	PGRN may competitively inhibit TNFR	[125]
Lysosomal Proteins	CTSD	Aspartyl protease	PGRN stabilizes and activates	[134, 11, 158]
	CTSL	Cysteine protease	Processes PGRN into granulin peptides	[159, 48, 67]
	GBA	Glucocerebrosidase	PGRN acts as co-chaperone with Hsp70	[55, 54]
	PSAP	Precursor to saposins	Lysosomal trafficking and level regulation	[161, 162, 160]
ER Proteins	BiP	Chaperone	Likely aids in PGRN folding and secretion	[4]
	Calreticulin	Chaperone	Likely aids in PGRN folding and secretion	[4]
	ERp5/57/72	Disulfide bond regulation	Likely aids in PGRN folding and secretion	[4]
	GRP94	HSP/Chaperone	Likely aids in PGRN folding and secretion	[4]
	Hsp70	HSP/Chaperone	PGRN/Hsp70 act as co-chaperones for GBA/ LIMP-2	[54, 4]
	PDI	Disulfide bond regulation	Likely aids in PGRN folding and secretion	[4]

[a] PGRN in media was increased in MMP-9 knockdown cells, but binding and cleavage were not shown,

[b] yeast two-hybrid

Abbreviations: ADAMTS-7, a disintegrin and metalloproteinase with thrombospondin motifs 7; BiP, binding immunoglobulin protein; COMP, cartilage oligomeric matrix protein; CTSD, cathepsin D; CTSL, cathepsin L; Dlk1; delta-like protein 1; EGF, epidermal growth factor; EphA2, ephrin type-A receptor 2; ERp, endoplasmic reticulum protein; GBA, glucocerebrosidase; GRP94, heat shock protein Hsp90 family protein; HDL/apo A-, high-density lipoprotein/apolipoprotein A1; Hsp70, heat shock protein 70; PSAP, prosaposin; MMP, matrix metalloproteinase; NE, neutrophil elastase; PDI, protein disulfide isomerase; PR3, proteinase 3; SLPI, secretory leukocyte protease inhibitor; TLR9, toll-like receptor 9; TNFR, tumor necrosis factor receptor.