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Regulation of cathepsin D activity by the FTLD protein progranulin

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Progranulin (PGRN) protein encoded by the granulin (*GRN*) gene has been recently implicated in several neurodegenerative diseases [2,5]. While haploinsufficiency of PGRN leads to frontotemporal lobar degeneration (FTLD) [2,5], the most prevalent form of early onset dementia after Alzheimer's disease (AD), complete loss of PGRN is known to cause neuronal ceroid lipofuscinosis (NCL) [1,13], a group of lysosomal storage diseases. PGRN is a secreted glycoprotein of 7.5 granulin repeats [2,5]. However, within the cell, PGRN is localized to lysosomes through two independent trafficking pathways [8,17]. Furthermore, *GRN* is transcriptionally co-regulated with a number of essential lysosomal genes by the transcriptional factor TFEB [3]. While all this evidence suggests an essential role of PGRN in regulating lysosomal function, how PGRN does so is still unclear.

Cathepsin D (CTSD) is a lysosomal aspartic-type protease involved in many neurodegenerative diseases [14]. Mutations in the cathepsin D gene (*CTSD*) result in NCL in humans [9]. Interestingly, mice deficient in CTSD also develop TDP-43 aggregates (Supplementary Fig. 1) [7], a hallmark of FTLD with *GRN* mutations. FTLD patients with *GRN* mutations exhibit typical pathological features of NCL [7]. These data support that lysosomal dysfunction might serve as a common mechanism for FTLD and NCL and suggest that PGRN and CTSD might function together to regulate lysosomal activities. In support of this hypothesis, granulin motifs are found in cathepsin-like cysteine proteases in plants [11,16]. These lines of evidence led us to postulate that an interaction between PGRN and CTSD may exist.

To test the physical interaction between PGRN and CTSD, FLAG-tagged CTSD was co-transfected with untagged PGRN in HEK293T cells. Cell lysates were immunoprecipitated with anti-FLAG antibodies. PGRN signal is detected in anti-FLAG CTSD immunoprecipitates but not in the controls (Fig. 1a), suggesting a physical interaction between PGRN and CTSD. Since CTSD is known to interact with prosaposin (PSAP) [6,10], which we previously showed to bind to PGRN as well [17], it is possible that the PGRN and CTSD interaction might be bridged by endogenous PSAP in HEK293T cells. To rule out this possibility, we compared the interaction between PGRN and CTSD in control

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N2a cells or N2a cells with PSAP expression depleted using the CRISPR/Cas9 system [17]. PSAP ablation has no effect on PGRN-CTSD binding in the co-immunoprecipitation assay (Fig. 1b), indicating that the interaction between PGRN and CTSD is not mediated by PSAP. Co-immunoprecipitation studies between individual granulins and CTSD suggest that multiple granulin motifs interact with CTSD (Supplemental Fig. 2a and 2b).

With the physical interaction between PGRN and CTSD confirmed, next we wanted to investigate its functional relevance. Since CTSD deficiency results in much more severe lysosomal phenotypes than PGRN deficiency [4], we hypothesized that PGRN might regulate CTSD activities. Therefore, we measured CTSD activities in 2-month-old wild type (WT) and PGRN deficient mice before the appearance of any obvious lysosomal abnormalities or glial activation. Indeed, liver and spleen lysates from PGRN-deficient mice showed significantly lower CTSD activities compared to those from WT mice (Fig. 1c), without any changes in CTSD protein levels or maturation status (Fig. 1d, 1e, Supplementary Fig. 3). Lysates from cerebrum and cerebellum also show a trend of lower CTSD activities in $Grn^{-/-}$ mice (Fig. 1c). Notably, in $Grn^{-/-}$ midbrain, although the protein levels of CTSD are slightly increased, CTSD activities are slightly lower than WT, indicating that the midbrain is one of earliest affected brain regions in $Grn^{-/-}$ mice (Fig. 1c–e). It should be noted that $Grn^{-/-}$ mice do not develop TDP-43 pathology and neurodegeneration as seen in FTLD patients [12]. Thus it is possible that PGRN more strongly regulates CTSD activity in humans than in mice. Indeed, a recent study demonstrated reduced cathepsin D activity in fibroblasts derived from FTLD patients with heterozygous *GRN* mutations [15].

In summary, we demonstrate that PGRN interacts with the lysosomal protease CTSD and maintains its proper activity *in vivo*. CTSD mediated proteolysis is essential to neuronal cell homeostasis through the degradation of aggregates delivered to lysosomes via autophagy or endocytosis [14]. Therefore by regulating CTSD activity, PGRN may modulate protein homeostasis. This could potentially explain the TDP-43 aggregation observed in FTLD with *GRN* mutations. Although the mechanism by which PGRN regulates CTSD activity remains to be determined, our data argues that reduced CTSD activities are a disease mechanism for FTLD with *GRN* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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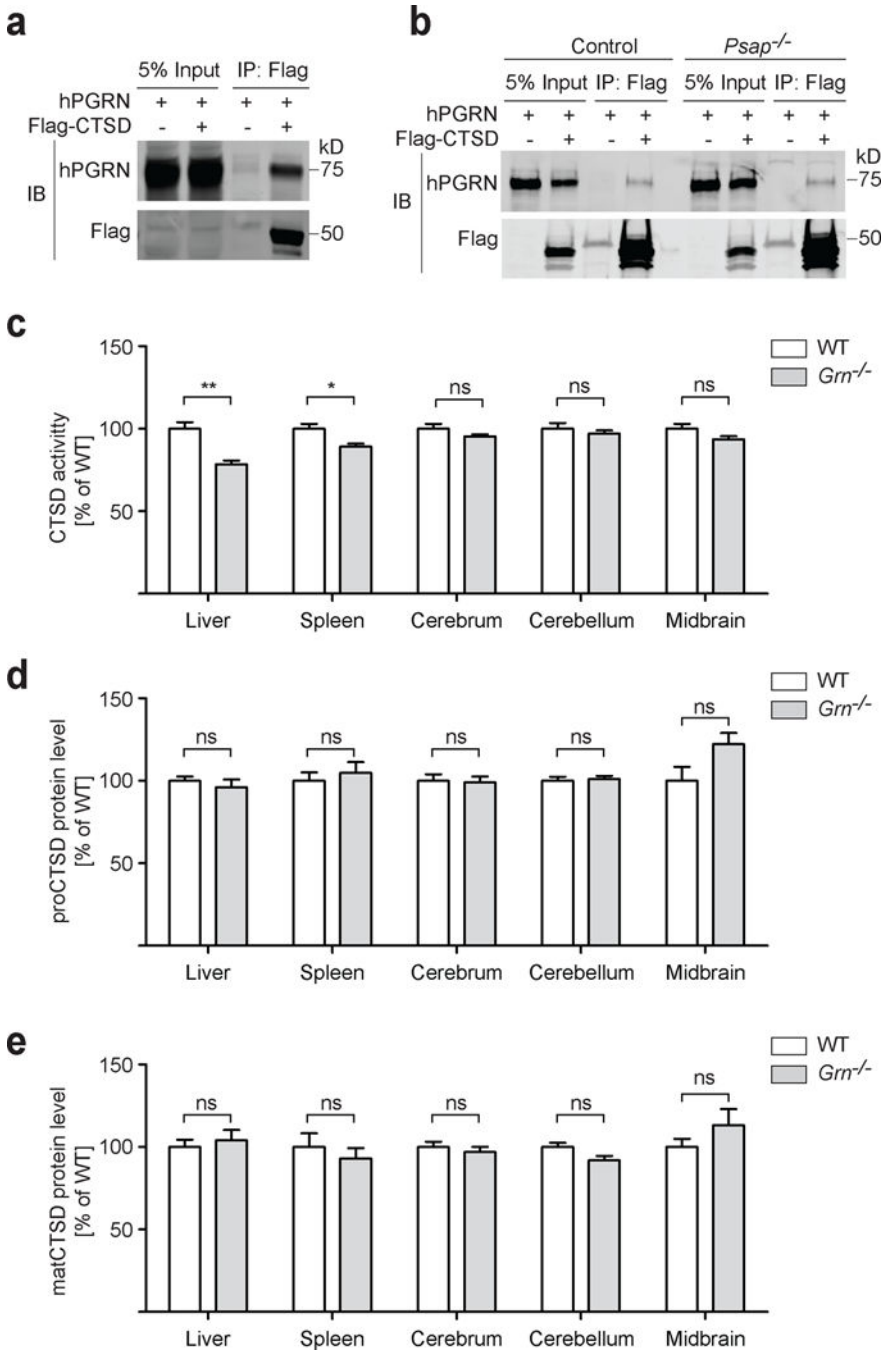

Fig. 1.

PGRN binds to CTSD and regulates its activity. **a** HEK293T cells transfected with indicated constructs were lysed and immunoprecipitated with anti-FLAG antibodies. **b** Control N2a cells or *Psap*$^{-/-}$N2a cells generated using Cas9/CRIPSR were transfected with indicated constructs, lysed and immunoprecipitated with anti-FLAG antibodies. **c**, CTSD activities in tissue lysates of 2 month old WT or *Grn*$^{-/-}$ mice as indicated. **d, e**, The levels of both the pro (d) and mature (e) forms of cathepsin D in the tissue lysates of WT or *Grn*$^{-/-}$ mice are

quantified and normalized to GAPDH. n=5–6, +/− SEM, *p-value <0.05, **p-value <0.01, ns, not significant, Student's t-test.