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Novel progranulin variants do not disrupt progranulin secretion and cleavage

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Abstract

A subset of frontotemporal dementia cases are neuropathologically defined by tau negative, TDP-43 and ubiquitin positive inclusions in the brain and are associated with mutations in the progranulin gene (*GRN*). Deep sequencing of families exhibiting late onset dementia revealed several novel variants in the *GRN* gene. Due to the small size of these families and limited availability of samples, it was not possible to determine whether the variants segregated with disease. Furthermore, none of these families had autopsy confirmation of diagnosis. We sought to determine if these novel *GRN* variants alter progranulin protein (PGRN) stability, PGRN secretion, and PGRN cleavage in cultured cells. All of the novel *GRN* variants behave like PGRN WT protein, suggesting that these variants represent rare polymorphisms. However, it remains possible that these variants affect other aspects of PGRN function or represent risk factors for dementia when combined with other modifying genes.

Keywords

Late onset Alzheimer's disease; frontotemporal dementia; progranulin; granulin

1. Introduction

Frontotemporal dementia (FTD) is a late onset neurodegenerative disease that is clinically characterized by disturbances in language, behavior, and personality (Cairns, et al., 2007). A subset of FTD cases, termed FTLD-U, are neuropathologically defined by tau negative, TDP-43 and ubiquitin positive inclusions in the brain and are associated with mutations in the progranulin gene (*GRN*) (Baker, et al., 2006, Cruts, et al., 2006).

PGRN is a 68 kDa glycoprotein. Glycosylation of PGRN produces high molecular weight proteins, the most common of which is an 88 kDa protein. PGRN is secreted from the cell as a full-length protein and converted into 6–25 kDa fragments, termed granulins (GRN) via proteolytic processing by elastase and other extracellular proteases. The majority of FTLD-U-associated *GRN* mutations cause premature termination of the coding sequence, resulting

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in nonsense-mediated decay; however, some missense variants have been described that cause FTL-D-U by other mechanisms (Gass, et al., 2012, Mukherjee, et al., 2008, Wang, et al., 2010).

Deep sequencing efforts in 439 clinically diagnosed late onset Alzheimer's disease (LOAD) families, without autopsy confirmation of diagnosis, have identified rare variants in *GRN* in several families (Cruchaga, et al., 2012). Due to the rarity of these variants and the small families and/or incomplete pedigree data, determining pathogenicity of these rare *GRN* variants is challenging. To further investigate the impact of these variants we tested whether they behave like known pathogenic FTL-D-U-associated *GRN* mutations *in vitro*. All novel PGRN variants behaved like PGRN wild-type (WT) protein in the assays studied here. Thus, these rare *GRN* variants are likely to be polymorphisms rather than pathogenic variants but we cannot eliminate the possibility that they are risk factors that modify other PGRN functions or the downstream effects of GRN.

2. Methods

Novel variants were introduced into the pCMVSPORT6 human PGRN WT vector (Wang, et al., 2010) by site-directed mutagenesis using the QuikChangeII Site-Directed Mutagenesis kit (Stratagene). Mutations were confirmed by Sanger sequencing.

PGRN secretion and elastase cleavage were measured as previously described (Wang, et al., 2010) (see Supplemental Information).

3. Results

Recent deep sequencing of the *GRN* gene in clinically diagnosed LOAD families identified several pathogenic mutations and novel variants in *GRN* (Cruchaga, et al., 2012). These novel variants occur in individuals clinically diagnosed with dementia and do not occur in individuals who are cognitively normal; however, some individuals in these families exhibit clinical dementia without carrying the *GRN* variant (Table 1) (Cruchaga, et al., 2012). GRN P85A and GRN G515A were detected among unselected African Americans (EVS, <http://evs.gs.washington.edu/EVS/>, Table 1). These findings make interpretation of the pathogenicity of these novel variants challenging. PolyPhen predicts that 3 of the 4 novel variants are damaging based on the location of the variant, conservation of the residue, and predicted structural disruption with novel amino acid (Table 1) (Cruchaga, et al., 2012). A protein alignment of these novel variants reveals that, with the exception of GRN P85A, these sites are not highly conserved (Suppl. Fig. 1).

To determine how these variants influence PGRN stability and secretion, vectors containing cDNA for PGRN WT or novel variants were transiently transfected in HEK293T cells, and intracellular and extracellular PGRN protein was measured by immunoblotting. Novel variants produced similar intracellular (Fig. 1B) and extracellular (Fig. 1A) PGRN protein levels compared with PGRN WT, while the pathogenic PGRN A9D mutation produced almost no detectable extracellular PGRN (Fig. 1A). We also observed no difference in the PGRN secretion rate between PGRN WT, a previously reported mutation (P248L), and the novel variants (Suppl. Fig. 2) (Shankaran, et al., 2008, Wang, et al., 2010).

Some pathogenic PGRN mutations, such as C521Y, alter cleavage rather than protein stability and secretion (Wang, et al., 2010). To measure the influence of these variants on PGRN cleavage, media from cells transiently expressing PGRN WT and novel variants were treated with elastase. Elastase treatment of PGRN WT produced protein fragments at 55, 37, 30, 26, and 19 kDa (Fig. 1C). Protein fragments at 55 and 37 kDa were absent after 60 minutes of elastase treatment (Fig. 1C). Novel variants failed to alter GRN cleavage in a

similar fashion to the pathogenic GRN C521Y (Fig. 1C), which fails to produce the 55 and 37 kDa protein fragments. The novel *GRN* variants produced protein fragments at 30, 26, and 19 kDa that are more stable than the WT cleavage fragments (Fig. 1C); however, the importance of these fragments in PGRN metabolism and function is unknown.

4. Discussion

A subset of FTD cases are neuropathologically defined by tau negative, TDP-43 and ubiquitin positive inclusions in the brain and are associated with mutations in the *GRN* gene (Baker, et al., 2006, Cruts, et al., 2006). We sought to characterize the pathogenicity of novel *GRN* variants identified in individuals who were clinically diagnosed with dementia. Novel *GRN* variants produced PGRN proteins that exhibit protein stability and secretion similar to PGRN WT. The PGRN proteins produced from the novel *GRN* variants were also cleaved to the same extent as PGRN WT.

The functional role of PGRN and GRN proteins is poorly understood, which makes defining pathogenic variants challenging. Recent evidence suggests that PGRN and GRN proteins function in the CNS inflammatory response (Yin, et al., 2010), microglial activation (Martens, et al., 2012), synaptic transmission (Tapia, et al., 2011), and neurite outgrowth (Gao, et al., 2010). *GRN* mutations with unclear pathogenicity (C139R, P248L, R432C) fail to restore neurite outgrowth in GRN deficient neuronal cultures while a non-pathogenic variant (identified in affected and unaffected individuals) restored neurite outgrowth (Gass, et al., 2012). So, it remains possible that the novel *GRN* variants described here alter these PGRN functions.

Together, these findings illustrate that these rare variants in *GRN* are likely to be polymorphisms rather than pathogenic variants. It remains possible that these rare *GRN* variants represent risk factors for dementia in the presence of additional modifying genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, Snowden J, Adamson J, Sadovnick AD, Rollinson S, Cannon A, Dwosh E, Neary D, Melquist S, Richardson A, Dickson D, Berger Z, Eriksen J, Robinson T, Zehr C, Dickey CA, Crook R, McGowan E, Mann D, Boeve B, Feldman H, Hutton M. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*. 2006; 442(7105):916–9. [PubMed: 16862116]
- Cairns NJ, Bigio EH, Mackenzie IRA, Neumann M, Lee VMY, Hatanpaa KJ, White CL, Schneider JA, Grinberg LT, Halliday G, Duyckaerts C, Lowe JS, Holm IE, Tolnay M, Okamoto K, Yokoo H, Murayama S, Woulfe J, Munoz DG, Dickson DW, Ince PG, Trojanowski JQ, Mann DMA, Degeneration, C.f.F.L. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol*. 2007; 114(1):5–22. [PubMed: 17579875]
- Cruchaga C, Haller G, Chakraverty S, Mayo K, Vallania FL, Mitra RD, Faber K, Williamson J, Bird T, Diaz-Arrastia R, Foroud TM, Boeve BF, Graff-Radford NR, St Jean P, Lawson M, Ehm MG,

- Mayeux R, Goate AM. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. *PLoS One*. 2012; 7(2):e31039.10.1371/journal.pone.0031039 [PubMed: 22312439]
- Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, Rademakers R, Vandenbergh R, Dermaut B, Martin JJ, van Duijn C, Peeters K, Sciot R, Santens P, De Pooter T, Mattheijssens M, Van den Broeck M, Cuijt I, Vennekens K, De Deyn PP, Kumar-Singh S, Van Broeckhoven C. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*. 2006; 442(7105):920–4.10.1038/nature05017 [PubMed: 16862115]
- Gao X, Joselin AP, Wang L, Kar A, Ray P, Bateman A, Goate AM, Wu JY. Progranulin promotes neurite outgrowth and neuronal differentiation by regulating GSK-3 β . *Protein Cell*. 2010; 1(6):552–62. [PubMed: 21204008]
- Gass J, Lee WC, Cook C, Finch N, Stetler C, Jansen-West K, Lewis J, Link CD, Rademakers R, Nykjaer A, Petrucelli L. Progranulin regulates neuronal outgrowth independent of sortilin. *Mol Neurodegener*. 2012; 7:33.10.1186/1750-1326-7-33 [PubMed: 22781549]
- Martens LH, Zhang J, Barmada SJ, Zhou P, Kamiya S, Sun B, Min SW, Gan L, Finkbeiner S, Huang EJ, Farese RV Jr. Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. *J Clin Invest*. 2012;10.1172/JCI63113
- Mukherjee O, Wang J, Gitcho M, Chakraverty S, Taylor-Reinwald L, Shears S, Kauwe JSK, Norton J, Levitch D, Bigio EH, Hatanpaa KJ, White CL, Morris JC, Cairns NJ, Goate A. Molecular characterization of novel progranulin (GRN) mutations in frontotemporal dementia. *Hum Mutat*. 2008; 29(4):512–21. [PubMed: 18183624]
- Shankaran SS, Capell A, Hruscha AT, Fellerer K, Neumann M, Schmid B, Haass C. Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. *J Biol Chem*. 2008; 283(3):1744–53. [PubMed: 17984093]
- Tapia L, Milnerwood A, Guo A, Mills F, Yoshida E, Vasuta C, Mackenzie IR, Raymond L, Cynader M, Jia W, Bamji SX. Progranulin deficiency decreases gross neural connectivity but enhances transmission at individual synapses. *J Neurosci*. 2011; 31(31):11126–32.10.1523/JNEUROSCI.6244-10.2011 [PubMed: 21813674]
- Wang J, Van Damme P, Cruchaga C, Gitcho MA, Vidal JM, Seijo-Martínez M, Wang L, Wu JY, Robberecht W, Goate A. Pathogenic cysteine mutations affect progranulin function and production of mature granulins. *J Neurochem*. 2010; 112(5):1305–15. [PubMed: 20028451]
- Yin F, Banerjee R, Thomas B, Zhou P, Qian L, Jia T, Ma X, Ma Y, Iadecola C, Beal MF, Nathan C, Ding A. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. *J Exp Med*. 2010; 207(1):117–28.10.1084/jem.20091568 [PubMed: 20026663]

Figure 1.

Novel PGRN variants behave like PGRN WT in cultured cells. A–B. HEK293T cells were transiently transfected with vectors containing WT and novel PGRN variants for 48 hours, and media was conditioned for 24 hours. Cell lysates were extracted in non-ionic detergent. A. Media. B. Cell lysate. C. Novel PGRN variants do not alter elastase cleavage. Vectors containing WT and novel PGRN variants were transiently transfected in HEK293T cells for 48 hours, and media was replaced after 24 hours. Media was collected and treated with elastase at 37°C for the time points indicated. Black arrow, PGRN protein cleavage products. Media, cell lysates, and elastase-treated media were analyzed by SDS-PAGE in 4–20% Tris HCl gels in the presence of reducing agent. Immunoblots were probed with a C-terminal PGRN antibody (Invitrogen). Probing immunoblots with an antibody raised against full-length PGRN (R&D) produced similar results (data not shown). Immunoblots are representative of at least four replicate experiments.

Table 1

Novel GRN variants identified in clinically diagnosed LOAD families.

PGRN Variant	Predicted Effect [†]	LOAD Families				LOAD Families* (n=439)	AD Cases# (n=1806)	Control (n=1346)	Unselected*	
		Affected		Unaffected					EA' (n=4300)	AA'' (n=2203)
		Carrier	Non-Carrier	Carrier	Non-Carrier					
P85A	Damaging	3	3	0	1	2	0	0	0	8
D135V	Damaging	1	0	0	0	1	0	0	0	0
M207T	Benign	1	1	0	1	1	0	0	0	0
G515A	Damaging	3	0	0	1	1	0	0	1	131

[†] PolyPhen prediction of functional impact of amino acid change.

^{*} Number of families carrying the variant.

[#] Sporadic AD cases.

[°] Unselected samples from the NHLBI Exome Sequencing Project Exome Variant Server.

[✓] EA, European Americans.

[〃] AA, African Americans.