Neurobiology of Aging 34 (2013) 2538–2540

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Brief communication

# Novel progranulin variants do not disrupt progranulin secretion and cleavage

Celeste M. Karch, Amanda T. Jeng, Tara Skorupa, Carlos Cruchaga, Alison M. Goate*

*Department of Psychiatry and Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, MO, USA*

ARTICLE INFO

*Article history:*
Received 18 October 2012
Received in revised form 30 April 2013
Accepted 4 May 2013
Available online 4 June 2013

*Keywords:*
Late-onset Alzheimer's disease
Frontotemporal dementia
Progranulin
Granulin

ABSTRACT

A subset of frontotemporal dementia cases are neuropathologically defined by tau-negative, TAR DNA-binding protein-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 *GRN*. Because of the small size of these families and limited availability of samples, it was not possible to determine whether the variants segregated with the disease. Furthermore, none of these families had autopsy confirmation of diagnosis. We sought to determine if these novel *GRN* variants alter progranulin (PGRN) protein stability, PGRN secretion, and PGRN cleavage in cultured cells. All the novel *GRN* variants behave like PGRN wild-type 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.



## 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, TAR DNA-binding protein-43 (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).

Progranulin (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. Most FTLD-U–associated *GRN* mutations cause premature termination of the coding sequence, resulting in nonsense-mediated decay; however, some missense variants have been described that cause FTLD-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). Because of 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 FTLD-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 *GRN* WT vector (Wang et al., 2010) by site-directed mutagenesis using the QuikChangeII Site-Directed Mutagenesis kit (Strategene). Mutations were confirmed by Sanger sequencing.

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

## 3. Results

Recent deep sequencing of the *GRN* 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 (Exome Variant Server, http://evs.gs.washington.edu/EVS/, Table 1). These

* Corresponding author at: Department of Psychiatry, Washington University School of Medicine, 425 S. Euclid Ave., Campus Box 8134, St. Louis, MO 63110, USA. Tel.: +314 362 8691; fax: +314 747 2983.
*E-mail address:* goatea@psychiatry.wustl.edu (A.M. Goate).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.05.004

**Table 1**
Novel *GRN* variants identified in clinically diagnosed LOAD families

| *GRN* variant | Predicted effect[a] | LOAD families | | | | LOAD families[b] ($n = 439$) | AD cases[c] ($n = 1806$) | Control ($n = 1346$) | Unselected[d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Affected | | Unaffected | | | | | EA ($n = 4300$) | AA ($n = 2203$) |
| | | Carrier | Noncarrier | Carrier | Noncarrier | | | | | |
| 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 |

Key: AA, African Americans; EA, European Americans; *GRN*, progranulin gene; LOAD, late-onset Alzheimer's disease; PGRN, progranulin.
[a] PolyPhen prediction of functional impact of amino acid change.
[b] Number of families carrying the variant.
[c] Sporadic AD cases.
[d] Unselected samples from the NHLBI Exome Sequencing Project Exome Variant Server.

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 (Supplementary Fig. 1).

To determine how these variants influence PGRN stability and secretion, vectors containing *GRN* 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, whereas 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 mutation with unclear pathogenicity (P248L), and the novel variants (Supplementary 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 PGRN 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.

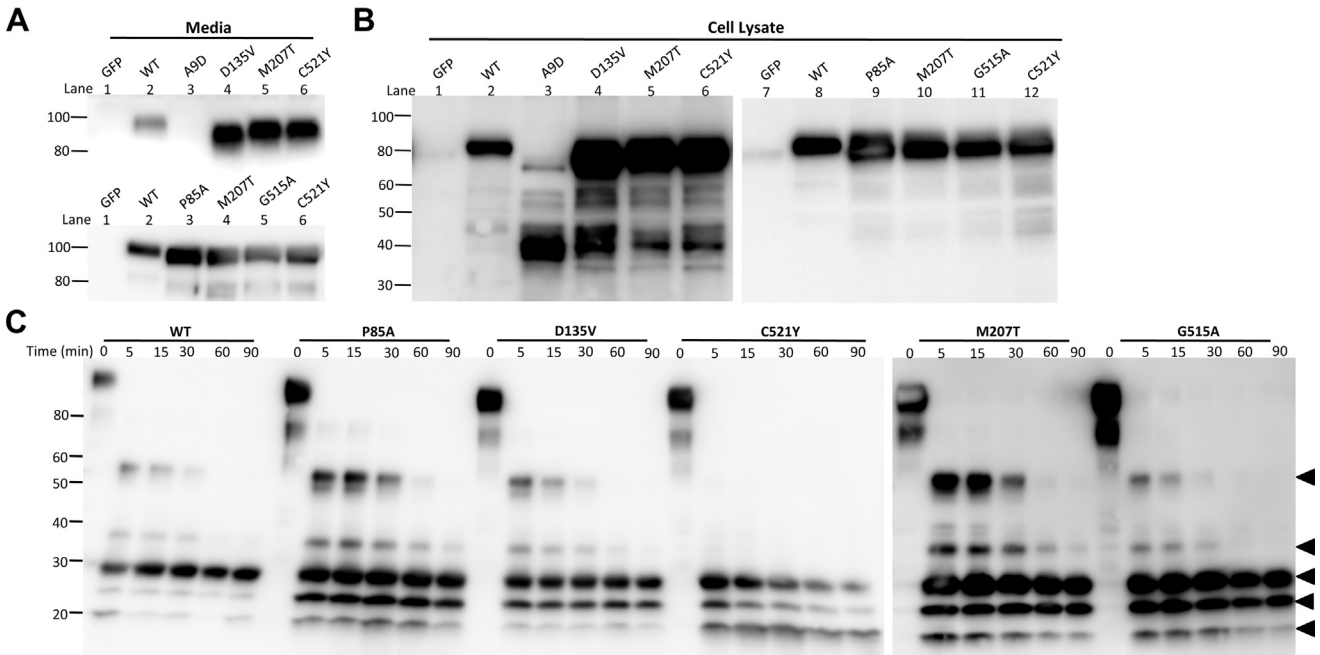


**Fig. 1.** Novel progranulin (PGRN) variants behave like PGRN wild-type (WT) in cultured cells. (A, B) HEK293T cells were transiently transfected with vectors containing WT and novel PGRN variants for 48 hours, and media were conditioned for 24 hours. Cell lysates were extracted in nonionic detergent. (A) Media, (B) cell lysate, and (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 were replaced after 24 hours. Media were collected and treated with elastase at 37 °C for the time points indicated. Black arrows, PGRN protein cleavage products. Media, cell lysates, and elastase-treated media were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis 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 4 replicate experiments.

## 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* (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 the function of PGRN and GRN proteins 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, whereas a nonpathogenic 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.

## Disclosure statement

The authors have no actual or potential conflicts of interest to disclose.

## Acknowledgements

The authors would like to thank National Institute on Aging (NIA)-LOAD investigators (Principal Investigator: Richard Mayeux) who collected the samples used to identify the novel *GRN* variants and the participants and family members, whose participation made this work possible. Funding was provided by U24AG026395 (NIA-LOAD/National Cell Repository for Alzheimer's Disease (NCRAD) Family Study Consortium) and National Institutes of Health-AG035083 (A.M.G.).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2013.05.004.

## References

Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A.D., Rollinson, S., Cannon, A., Dwosh, E., Neary, D., Melquist, S., Richardson, A., Dickson, D., Berger, Z., Eriksen, J., Robinson, T., Zehr, C., Dickey, C.A., Crook, R., Mcgowan, E., Mann, D., Boeve, B., Feldman, H., Hutton, M., 2006. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 442, 916–919.

Cairns, N.J., Bigio, E.H., Mackenzie, I.R.A., Neumann, M., Lee, V.M.-Y., Hatanpaa, K.J., White, C.L., Schneider, J.A., Grinberg, L.T., Halliday, G., Duyckaerts, C., Lowe, J.S., Holm, I.E., Tolnay, M., Okamoto, K., Yokoo, H., Murayama, S., Woulfe, J., Munoz, D.G., Dickson, D.W., Ince, P.G., Trojanowski, J.Q., Mann, D.M.A., Consortium for Frontotemporal Lobar Degeneration, 2007. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol. 114, 5–22.

Cruchaga, C., Haller, G., Chakraverty, S., Mayo, K., Vallania, F.L., Mitra, R.D., Faber, K., Williamson, J., Bird, T., Diaz-Arrastia, R., Foroud, T.M., Boeve, B.F., Graff-Radford, N.R., St Jean, P., Lawson, M., Ehm, M.G., Mayeux, R., Goate, A.M., 2012. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PLoS One 7, e31039.

Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J.J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P.P., Kumar-Singh, S., Van Broeckhoven, C., 2006. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 442, 920–924.

Gao, X., Joselin, A.P., Wang, L., Kar, A., Ray, P., Bateman, A., Goate, A.M., Wu, J.Y., 2010. Progranulin promotes neurite outgrowth and neuronal differentiation by regulating GSK-3β. Protein Cell 1, 552–562.

Gass, J., Lee, W.C., Cook, C., Finch, N., Stetler, C., Jansen-West, K., Lewis, J., Link, C.D., Rademakers, R., Nykjaer, A., Petrucelli, L., 2012. Progranulin regulates neuronal outgrowth independent of sortilin. Mol. Neurodegener. 7, 33.

Martens, L.H., Zhang, J., Barmada, S.J., Zhou, P., Kamiya, S., Sun, B., Min, S.W., Gan, L., Finkbeiner, S., Huang, E.J., Farese Jr., R.V., 2012. Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. J. Clin. Invest 122, 3955–3959.

Mukherjee, O., Wang, J., Gitcho, M., Chakraverty, S., Taylor-Reinwald, L., Shears, S., Kauwe, J.S.K., Norton, J., Levitch, D., Bigio, E.H., Hatanpaa, K.J., White, C.L., Morris, J.C., Cairns, N.J., Goate, A., 2008. Molecular characterization of novel progranulin (GRN) mutations in frontotemporal dementia. Hum. Mutat. 29, 512–521.

Shankaran, S.S., Capell, A., Hruscha, A.T., Fellerer, K., Neumann, M., Schmid, B., Haass, C., 2008. Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. J. Biol. Chem. 283, 1744–1753.

Tapia, L., Milnerwood, A., Guo, A., Mills, F., Yoshida, E., Vasuta, C., Mackenzie, I.R., Raymond, L., Cynader, M., Jia, W., Bamji, S.X., 2011. Progranulin deficiency decreases gross neural connectivity but enhances transmission at individual synapses. J. Neurosci. 31, 11126–11132.

Wang, J., Van Damme, P., Cruchaga, C., Gitcho, M.A., Vidal, J.M., Seijo-Martínez, M., Wang, L., Wu, J.Y., Robberecht, W., Goate, A., 2010. Pathogenic cysteine mutations affect progranulin function and production of mature granulins. J. Neurochem. 112, 1305–1315.

Yin, F., Banerjee, R., Thomas, B., Zhou, P., Qian, L., Jia, T., Ma, X., Ma, Y., Iadecola, C., Beal, M.F., Nathan, C., Ding, A., 2010. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. J. Exp. Med. 207, 117–128.