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Association Between Progranulin and β-Amyloid in Dementia With Lewy Bodies

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Recent studies demonstrated that progranulin plays an integral role in the pathogenesis of frontotemporal dementia. To begin to explore the role of progranulin in dementia with Lewy bodies, we investigate its association with pathologic proteins that characterize this disease. We assessed immunoreactivity for progranulin in medial temporal lobe structures of 12 cases of dementia with Lewy bodies. Similar data were collected for β-amyloid burden, and α-synuclein pathology. Blinded investigators used a 0-3–point scale to quantify progranulin burden. Double labeling for progranulin and β-amyloid was also performed. We were able to demonstrate progranulin immunoreactivity throughout the medial temporal lobe in all dementia with Lewy body cases. We identified a significant positive correlation ($r = 0.606$; $P = .037$) between β-amyloid burden and progranulin. There was no significant correlation between α-synuclein pathology or Braak stage and progranulin. Progranulin and β-amyloid colocalized in plaques in dementia with Lewy bodies, suggesting that there is likely a biological association between these 2 aggregated proteins.

Keywords: progranulin; β-amyloid; dementia with Lewy bodies; synucleinopathies; frontotemporal dementia

Introduction

Progranulin (PGRN) is a 68.5 kd glycoprotein that has long been implicated in tumorigenesis, inflammation, and repair, including growth factor signaling pathways. Specifically, PGRN has been directly related to carcinogenesis in breast, ovarian, hepatocellular, and prostate cancers, in addition to gliomas and multiple myelomas.[1] Its role in inflammation and repair is derived from the finding that it is present in activated microglia, and it is overexpressed in several disease states that involve microglial activation, including Alzheimer's disease (AD).[2] In addition, PGRN has often been thought of as a growth factor due to its ability to stimulate growth factor–related signaling pathways, including phosphorylation of Shc, p44/42 mitogen-activated protein kinase, phosphatidylinositol 3-kinase, protein kinase B/AKT, and the p70S6 kinase.[1] Progranulin is expressed in several tissues including cerebellar Purkinje cells and hippocampal pyramidal cells, as well as cortical neurons where it may play a growth factor–like function.[1] Recent discoveries have implicated PGRN in neurodegenerative diseases, particularly in tau-negative frontotemporal dementia with ubiquitin-positive inclusions (FTD-U).[3-7] The gene coding for PGRN has been identified in chromosome 17 near the microtubule-associated protein tau (MAPT) gene, and over 40 abnormalities have been described as underlying the FTD-U phenotype.[2,4,5,7] All of these reduce function of PGRN.

These discoveries imply that PGRN plays an integral role in the pathogenesis of FTD and may also play an important role in other neurodegenerative diseases. To further characterize the function of PGRN, it is important to study how it interacts with other proteins in patients that express PGRN, yet have concurrent abnormal accumulation of other proteins that lead to neurodegeneration. Progranulin has been found in association with β-amyloid (Aβ) plaques and activated microglia in AD (and other inflammatory disease states), but is not found in the inclusions present in patients with PGRN mutations and FTD-U.[3] These

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inclusions have instead been found to contain transactivation response element (TAR)-DNA–binding protein (TDP-43)[8] and in some cases p62 protein.[9] However, the genetic etiology of this disease suggests that there is a relationship between the reduction of PGRN in these patients and the abnormal accumulation of TDP-43.[5] No further studies have been performed at this time to further elucidate the relationship between PGRN and Aβ in AD or any other amyloid diseases.

Dementia with Lewy bodies (DLBs) is the second most common neurodegenerative dementing illness, following AD. Dementia with Lewy body is of particular value in elucidating the pathobiology of neurodegenerative diseases due to the heterogeneous nature of the pathophysiologic processes that are involved in this disease entity. Although DLB is characterized as a synucleinopathy because of the presence of LBs and Lewy neurites (LNs), AD pathology including Aβ plaques and neurofibrillary tangles (NFTs) are found readily in this disease.[10] Therefore, by studying the role of PGRN in DLB, we are able to investigate its relationship with both AD pathology and other major pathological proteins to further elucidate its function.

Methods

Subjects

Twelve medial temporal lobes (MTLs) from cases with a pathological diagnosis of DLB were examined, and 4 aged matched controls. All cases had postmortem delays of less than 12 h. Medial temporal lobe structures were sampled at the level of the lateral geniculate nucleus. Information regarding age at death, gender, and clinical diagnosis was available on 11 of the 12 cases. Of the 11 cases, 7 were men and 4 were women. The age at death ranged from 51 to 87 years with a mean of 74 (Table 1).

Table 1. Individual Case History

Case Number	Gender	Age at Death (years)	Duration of Illness (years)	Clinical Diagnosis
1	Female	69	5	DLB
2	Male	51	N/A	N/A
3	Male	N/A	N/A	Dementia NOS
4	Female	69	9	Parkinsonism
5	Male	69	6	N/A
6	Male	79	5	N/A
7	Male	81	N/A	Dementia NOS
8	Female	83	N/A	N/A
9	Male	70	N/A	N/A
10	Male	87	9	AD/parkinsonism
11	Male	82	20+	Senile dementia
12	Female	70	N/A	DLB

Abbreviations: AD, Alzheimer's disease; DLB, dementia with Lewy body; N/A, not available; NOS, not otherwise specified.

Histological Methods

A formalin-fixed, paraffin-embedded tissue block was sampled at the level of MTL structures (11 at the hippocampus, 1 at the amygdala). Tissue was sectioned at 6μm. The tissue was deparaffinized, hydrated, and then stained for PGRN (R&D Systems, polyclonal, 1:250; Minneapolis, MN), tau (Sigma, 396-s, 1:5; St Louis, MO), α-synuclein (BD Biosciences, 42, 1:300; San Jose, CA), or Aβ (DakoCytomation, 6F/3D, 1:00; Denmark) immunohistochemistry. Incubation times were standardized for each antibody staining procedure (1 h for PGRN, tau, and α-synuclein and overnight for Aβ). To minimize background staining, the tissue samples were immersed for 30 min in a block solution of 3% hydrogen peroxide in 100% methanol. The slides stained for Aβ were immersed in formic acid prior to application of the primary antibody. DakoCytomation link and label system was used, and 3, 3'-diaminobenzidine (DAB) was used as the chromogen. The slides were counterstained using Harris Hematoxylin. Protocols were based on previously published standardized techniques.[11,12]

Quantitative Methods

Quantitative data for assessing PGRN and/or Aβ immunoreactivity in MTL structures were obtained using a 0 to 3 scale, where 0 represented no pathology, and 3 represented the maximal burden of abnormal PGRN immunoreactivity. For the purpose of this study, we referred to the abnormal PGRN immunoreactivity as PGRN plaques. Investigators were blinded regarding clinical data, AD or PGRN pathology, respectively. We chose the same scale to grade PGRN plaques and Aβ plaques to optimize our ability to compare both pathologies accurately.

The presence of neurofibrillary tangles was documented in the transentorhinal layer, proper entorhinal cortex, and isocortical association areas. A Braak stage was assigned to each case to further characterize our findings. Braak stages were determined using immunoreactivity for tau protein in the MTL.[13] Lewy bodies and Lewy neurites were identified in

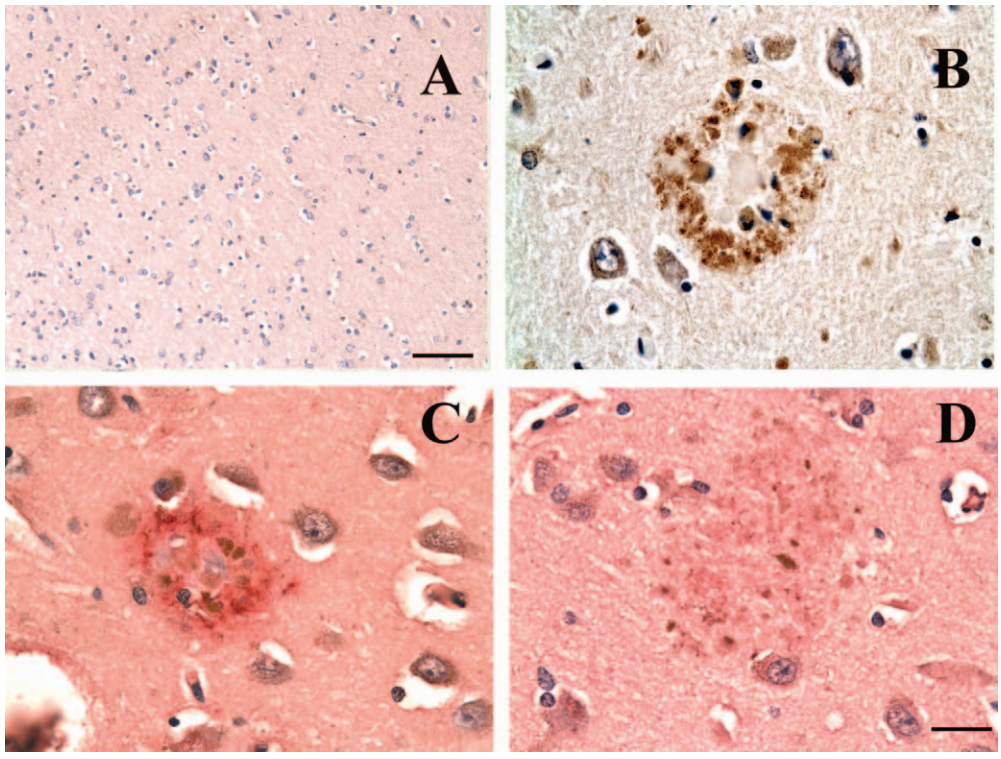

Figure 1. Representative photomicrograph of a control case (A) with single label immunohistochemistry (IHC) for progranulin (PGRN) which does not show any plaques (scale bar = 60 m for A only), a PGRN plaque (B) with single label IHC for PGRN, a PGRN plaque (C) with double labeling for PGRN (brown) and β-amyloid (Aβ; pink), and a PGRN plaque (D) with double labeling for PGRN and Aβ, which did not show any immunostaining for Aβ (scale bar = 20 m for B-D). All photomicrographs are from cases with a pathological diagnosis of dementia with Lewy body (DLB), which were used in the current study.

the entorhinal cortex and the Cornu Ammonis-2 (CA-2) region of the hippocampus proper, respectively, and graded on a 0 to 4 point scale where 0 = no Lewy pathology, 1 = sparse LBs or LNs, 2 = moderate Lewy pathology (LBs or LNs), 3 = severe Lewy pathology, and 4 = very severe Lewy pathology.[14]

Spearman rho correlation coefficients and Mann-Whitney *U* statistics were obtained using SPSS version 14.0.

Results

Abnormal accumulation of proteins immunoreactive for PGRN or PGRN plaques were identified in MTL structures in all 12 DLB cases that were investigated (Figure 1B). We also confirmed the presence of LBs, LNs, and neurofibrillary tangles in all cases. Aβ plaques were present in 11 of 12 cases studied. No significant pathology was identified in the 4 age matched controls that were used (Figure 1A). Double labeling of plaques revealed immunoreactivity for both PGRN and Aβ in most cases (Figure 1C), demonstrating that these 2 entities co-localize within the area of abnormal protein accumulation. In some cases, these plaques demonstrated immunoreactivity for PGRN alone without any immunoreactivity for Aβ (Figure 1D). Double labeling with α-synuclein and PGRN simultaneously failed to show any immunoreactivity within PGRN plaques for α-synuclein.

Mean score for PGRN plaques was 0.78, ranging from 0.5 to 2.0. Mean score for Aβ plaques was 1.78, ranging from 0 to 3 (Table 2). A significant positive correlation (r = 0.606, *P* = .037) was found between Aβ burden and PGRN plaque formation (Figure 2). No significant correlation was found between LBs or LNs and PGRN plaque formation (r = 0.368, *P* = .2 and r = 0.245, *P* = .4 for entorhinal and CA2 regions, respectively). No significant correlations were found between PGRN plaque formation and gender or age at death. No significant correlation was found between Braak stage and PGRN.

Table 2. Pathological Data on Individual Cases

Case Number	PGRN	Amyloid	Braak Stage	LB/LN Entorhinal	LB/LN CA-2
1	1.5	2.5	5	3	1.5
2	2.5	3	6	2.5	1.5
3	0.5	0	1	1	1
4	2	2.5	3	3	3
5	1	2	5	3	1.5
6	1	2.5	4	1.5	2
7	0.5	1.5	4	0.5	0
8	1.5	1.5	4	1.5	1
9	1	1.5	2	3	2.5
10	2	1.5	3	1	1
11	2	2	3	2.5	0.5
12	1	1	1	0	0

Abbreviations: LB/LN, Lewy body/Lewy neurite.; PGRN, progranulin.

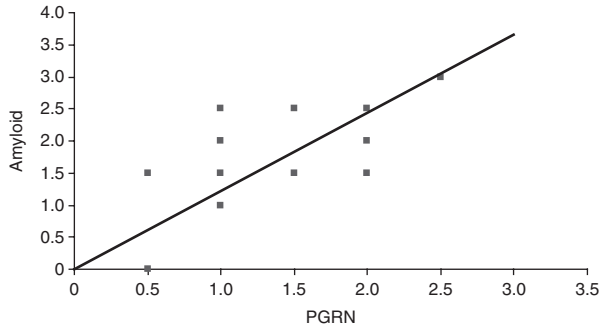

Figure 2. Quantitative data of β-amyloid (Aβ) burden and progranulin (PGRN) burden is represented here, showing the significant correlation between Aβ and PGRN in dementia with Lewy body (DLB).

Discussion

We expand what is known about the biology of PGRN in degenerative diseases by showing that PGRN plaques occur in DLB. To our knowledge, they have not been shown in any synucleinopathy. Abnormal PGRN immunoreactivity occurred in plaque-like structures that often co-localized with Aβ plaques. This co-localization has been previously shown in AD but not in other amyloid diseases.

Progranulin has been shown to be essential to many processes integral to cell functioning. The fact that we have observed the abnormal accumulation of PGRN immunoreactive proteins implies protein dysfunction; however, the mechanisms by which there is abnormal accumulation of PGRN are unclear. We began to characterize protein function by demonstrating PGRNs association with Aβ. Not only does the degree of PGRN immunoexpression correlate independently and significantly with Aβ, but we were able to show that these two proteins co-localize in plaques by double-label immunohistochemistry with antibodies to both proteins simultaneously. Furthermore, the fact that no correlation was found between PGRN plaque burden and α-synuclein pathology, nor did α-synuclein and PGRN co-localize in extracellular plaques found in DLB illustrates further that the process involved in the accumulation of PGRN is related to AD (Aβ) pathology, and not to α-synuclein pathology. The lack of correlation between neuritic (tau) pathology and PGRN burden supports the idea that there is a specific relationship between PGRN and Aβ, excluding other AD-type pathology such as neurofibrillary tangles. It is also important to note that Aβ burden was predominant as compared to PGRN burden in this series of patients.

To date, the only evidence to imply any association between α-synuclein and PGRN was a recent study that reported 2 members of a family with FTD clinical phenotype and a novel PGRN mutation, which exhibited α-synuclein pathology, although tau pathology was also present.[15]

An important finding was the observation that some plaques that were immunoreactive for PGRN did not show immunoreactivity for Aβ, in spite of a positive correlation between these two proteins. This shows that the co-existence of these proteins in these cases is not mandatory, and may imply that there is an evolution of abnormal protein accumulation throughout the disease process where PGRN accumulates early in the disease and Aβ does so later. Alternatively, one protein accumulation may even facilitate the accumulation of the other in early stages of the disease when the immune response is prominent.

Progranulin has been shown to co-localize in Aβ-plaques, dystrophic neurites, and activated microglia in AD.[3] This not only supports the hypothesis that PGRN and Aβ are associated, but also implicates PGRN in the immune response. Further evidence of this concept is supported by the finding that PGRN expression is increased in many diseases that involve microglial activation, including motor neuron disease, lysosomal storage diseases, and Creutzfeldt-Jakob disease.[2,16,17] It remains unclear at this point whether the

immune response is triggered by neurodegeneration and abnormal protein accumulation leading to PGRN overexpression, or it is the immune response itself that is abnormal and leads to neurotoxicity and neurodegeneration.

The relationship between TDP-43 and PGRN has been previously elucidated where the lack of PGRN leads to the abnormal ubiquitination, cleavage, and accumulation of TDP-43 in FTD-U.[2] However, it is not clear whether the overexpression of PGRN contributes to or inhibits the accumulation of AD pathology.

Aβ has been shown to be neurotoxic, although the exact mechanism by which this occurs is not completely clear.[18,19] It is unlikely that PGRN is inherently neurotoxic because it is a growth factor, and neuronal degeneration is associated with reduced PGRN function. Given that FTD PGRN mutations reduce expression, neurodegeneration is more likely related to the loss of growth factor–like properties, rendering the neurons vulnerable to disease processes. Because we do not know whether the accumulating PGRN protein retains its biological activity, it could be argued that a loss of active PGRN could render the individual more susceptible to degenerative diseases.

At this point, it is unclear whether PGRN and Aβ accumulation are triggered by the same process, or whether either of these proteins induces the abnormal accumulation of the other. Increased PGRN in cellular processes may occur as a response to the Aβ deposits. Here, it may function as part of a repair mechanism, as is seen in peripheral nerve injuries.[20] Alternatively, microglial expression of PGRN may be part of an inflammatory cascade triggered by the Aβ plaque. Future work using Western blot analysis to study biochemical characteristics of PGRN including its solubility, aggregation, protein levels in different areas as well as a model systems approach will help us further characterize this protein and understand the relationship between Aβ and PGRN in DLB and other degenerative diseases where amyloid plaques occur.

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