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GRN mutations are associated with Lewy body dementia

Paolo Reho, PhD[1], **Shunsuke Koga, MD, PhD**[2], **Zalak Shah, PhD**[1], **Ruth Chia, PhD**[3], **International LBD Genomics Consortium, The American Genome Center**, **Rosa Rademakers, PhD**[2,4], **Clifton L. Dalgard, PhD**[5,6], **Bradley F. Boeve, MD**[7], **Thomas G. Beach, MD, PhD**[8], **Dennis W. Dickson, MD**[2], **Owen A. Ross, PhD**[2,9], **Sonja W. Scholz, MD, PhD**[1,10,*]

[1]Neurodegenerative Diseases Research Unit, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

[2]Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA

[3]Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD, USA

[4]VIB Center for Molecular Neurology, VIB, Antwerp, Belgium

[5]Department of Anatomy, Physiology & Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

[6]The American Genome Center, Collaborative Health Initiative Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

[7]Department of Neurology, Mayo Clinic, Rochester, MN, USA

[8]Banner Sun Health Research Institute, Sun City, AZ, USA

[9]Department of Clinical Genomics, Mayo Clinic, Jacksonville, FL, USA

[10]Department of Neurology, Johns Hopkins University Medical Center, Baltimore, MD, USA

Abstract

Background: Loss-of-function mutations in *GRN* are a cause of familial frontotemporal dementia, and common variants within the gene have been associated with an increased risk of developing Alzheimer's disease and Parkinson's disease. While TDP-43-positive inclusions are characteristic of *GRN*-related neurodegeneration, Lewy body co-pathology has also been observed in many *GRN* mutation carriers.

Methods: We analyzed whole-genome sequence data generated for 2,591 European-ancestry Lewy body dementia (LBD) cases and 4,032 neurologically healthy controls to identify disease-causing mutations in *GRN*.

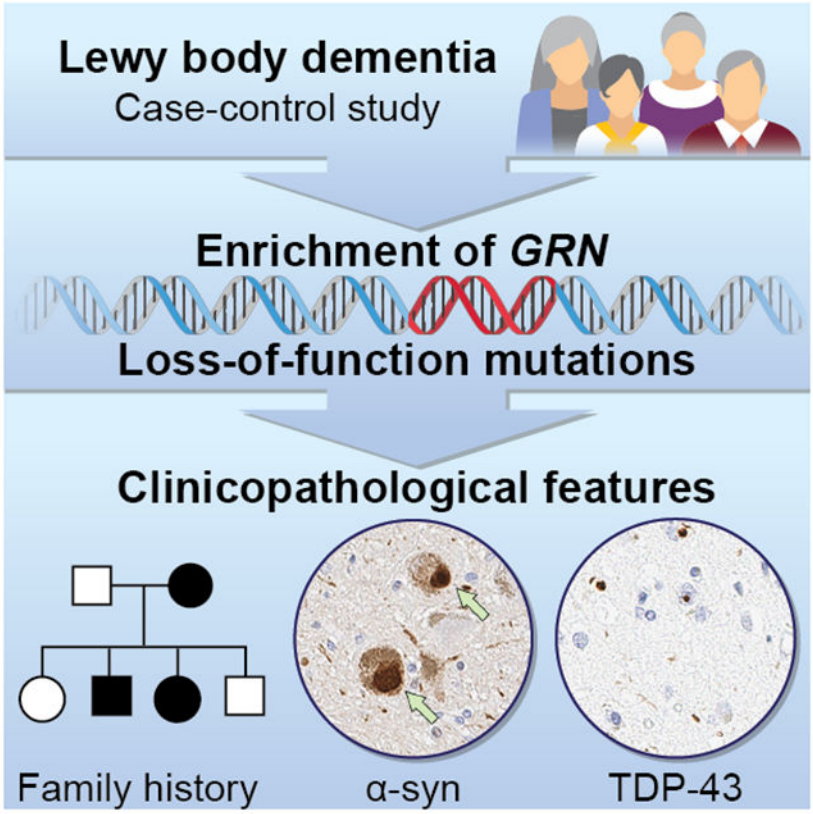
Results: We identified six heterozygous exonic *GRN* mutations in seven study participants (cases: n=6; controls: n=1). Each variant was predicted to be pathogenic or likely pathogenic.

*Correspondence to: Sonja W. Scholz, M.D., Ph.D., Neurodegenerative Diseases Research Unit, National Institutes of Health | NINDS, Bethesda, MD 20892-3707, USA. Tel.: +1 (301) 496-0013 | Fax: +1 (301) 451-7295 | sonja.scholz@nih.gov.

We found significant enrichment of *GRN* loss-of-function mutations in LBD patients compared to controls (SKAT-O *p*-value = 0.0162). Immunohistochemistry in three definite LBD cases demonstrated Lewy body pathology and TDP-43-positive neuronal inclusions.

Conclusions: Our findings suggest that deleterious *GRN* mutations are a rare cause of familial LBD.

Graphical Abstract



Keywords

Lewy body dementia (LBD); frontotemporal lobar degeneration (FTLD); *GRN* mutations; Progranulin; Neurodegeneration

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Progranulin is a broadly expressed and secreted growth factor that plays a role in several biological processes, including neural circuit development, regulation of cell growth, wound healing, lysosomal homeostasis, and neuroinflammation.[1–3] Progranulin is encoded by the *GRN* gene located on chromosome 17q21.31. Heterozygous mutations in this gene are the second most common cause of frontotemporal lobar degeneration (FTLD), accounting for 10% of all FTLD cases.[4, 5] These loss-of-function *GRN* mutations result in haploinsufficiency of progranulin with subsequent lysosomal dysfunction and neurodegeneration.

Recently, genome-wide association studies identified common variants within the *GRN* locus as being associated with other age-related neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.[6–8] Moreover, neuropathological studies in patients carrying pathogenic *GRN* variants found frequent Lewy body co-pathology in addition to the expected TDP-43-positive inclusions, suggesting a potential molecular link between Lewy body diseases and frontotemporal dementia.[9, 10] Interestingly, rare incidental *GRN* mutations have already been reported in Lewy body dementia (LBD) cases, but the relevance of these observations remains unclear.[11, 12] Based on this prior evidence, we postulated that *GRN* mutations might give rise to LBD. The objective of our study was to assess an LBD case-control cohort for pathogenic variants in *GRN* and test whether there is an enrichment of damaging mutations among LBD patients.

METHODS

Subjects

We examined 2,591 LBD cases and 4,032 neurologically healthy controls in which we recently performed whole-genome sequencing.[13] All study participants were of European-ancestry. Patients were diagnosed with pathologically definite (n = 1,789, 69.0%) or clinically probable LBD (n = 802, 31.0%), according to consensus criteria.[14, 15] One hundred and twenty-five (4.8%) of LBD cases had the clinical subtype of Parkinson's disease dementia, and the remainder had the clinical subtype of dementia with Lewy bodies. As expected for this form of dementia, nearly two thirds of LBD cases (63.4%) were males. Control subjects had no history of cognitive decline or neurological deficits on neurological examination. No evidence of significant neurodegenerative disease was detected on histopathological examination, performed on 15% (n = 605) of controls. Further details about the study demographics have been described elsewhere.[13]

Genome sequencing

All cases and controls underwent whole-genome sequencing using PCR-free library preparations followed by 150 base pair, paired-end sequencing on an Illumina HiSeq X Ten platform. The average coverage per genome was 35x. Sequencing, alignment (reference genome build GRCh38), variant calling, and quality control procedures (sample-level and variant-level) followed a standardized workflow described elsewhere.[13]

GRN variant annotation, filtering, and statistical analysis

To identify pathogenic variants in *GRN* (NM_002087), we extracted the *GRN* sequence from the genome data and annotated variants within this gene using Annovar (version 2018-04-16).[16] We next filtered variants by allele frequency, applying a minor allele frequency threshold of <0.01. We examined frameshift mutations, stop-gain mutations, and previously described pathogenic missense variants in the resulting data and reviewed the clinicopathological information of mutation carriers. To test whether loss-of-function mutations in *GRN* are enriched in our LBD cases compared to controls, we performed the Optimized Sequence Kernel Association Test (SKAT-O) with a gene-wide significance threshold of 0.05.[17] This analysis was performed in RVTESTS (vs.2.1.0) using sex, age, and five principal components as covariates.[18]

Data availability

Individual-level genome sequence data for the LBD and resource control genomes are available at dbGaP (https://www.ncbi.nlm.nih.nih.gov/gap/; accession no. phs001963.v1.p1 NIA DementiaSeq) and AMP-PD (https://amp-PD.org).

RESULTS

We examined coding variants in the *GRN* gene in 2,591 LBD cases and 4,032 neurologically healthy controls in which we recently performed whole-genome sequencing.[13] This identified six unique, heterozygous, exonic *GRN* mutations in a total of seven study participants (six LBD cases and one neurologically healthy control, Figure 1A). These mutations included three frameshift mutations (p.Q130Sfs*124, p.T382Sfs*29, p.E498Dfs*11), two stop-gain mutations (p.R493X, p.R535X [identified in a control]), and one missense mutation (p.A9V) located in a highly conserved region of *GRN* (Figure 1B, Table S1). All five frameshift and stop-gain mutations were predicted to be pathogenic and have been previously reported to be disease-causing mutations in patients with frontotemporal dementia or Alzheimer's disease.[4, 5, 9, 19] The p.A9V missense variant (observed in an LBD patient) was classified as 'likely pathogenic' according to the ACMG consensus classification. None of identified *GRN* mutation carriers had a pathogenic variant in the genes *GBA, SNCA, APP, PSEN1, PSEN2* that may otherwise explain the disease in these subjects. Additionally, none of the *GRN* mutation carriers had an *APOE ε4* risk allele (Table S1). Gene burden analysis confirmed a significant enrichment of loss-of-function mutations in *GRN* in LBD cases compared to healthy controls (SKAT-O; p-value = 0.0162). As all pathogenic variant carriers were diagnosed with dementia with Lewy bodies, we performed a subanalysis in dementia with Lewy body cases (excluding 125 patients diagnosed with Parkinson's disease dementia) compared to all controls, which again was found a significant association (SKAT-O; p-value = 0.0147).

The clinicopathological characteristics of all seven *GRN* mutation carriers are summarized in Table S1. The average age at death of LBD cases was 76.8 years (range: 63–83 years), while a neurologically healthy control, carrying the pathogenic p.R535X stop-gain variant, was still alive and cognitively well at age 74. Neuropathological confirmation was available for all LBD cases, all of whom fulfilled pathological consensus criteria for limbic (n = 3) or neocortical (n = 3) dementia with Lewy bodies.[15] Family history information was available for five out of seven mutation carriers, of which two reported a strong family history of dementia. An in-depth review of the histopathology associated with three LBD cases carrying pathogenic frameshift variants revealed widespread α-synuclein-positive Lewy bodies and Lewy neuritis as well as TDP-43-positive neuronal cytoplasmic inclusions and short dystrophic neurites, consistent with FTLD with TDP-43 type A.[20] Figure 2 showcases the immunohistochemical findings of a representative *GRN*-related LBD patient. Notably, this particular case (subject 2 in Table S1, carrying the pathogenic p.Q130Sfs*124 variant) reported a positive clinical history of dementia in several family members (a detailed description of the patient's clinical history and disease course is documented in the Supplementary Note).

DISCUSSION

Mutations in *GRN* are the second most common cause of autosomal dominant FTLD with TDP-43-positive inclusions. Recent evidence has implicated genetic variation at the *GRN* locus in the pathogenesis of a broader spectrum of neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, suggesting pleiotropic effects for this locus.[6–8] Here, we provide genetic and histopathological evidence linking *GRN* mutations and LBD based on a large whole-genome sequence case-control cohort.

First, we identified six LBD cases carrying heterozygous exonic *GRN* mutations, five of which led to a truncated protein and have been already reported as disease-causing.[4, 5, 9, 19] Second, we identified significant gene-wide enrichment of *GRN* loss-of-function mutations in LBD patients, supporting the notion that haploinsufficiency due to damaging mutations can also be a rare cause of familial Lewy body dementia. Third, immunohistochemistry of loss-of-function mutation carriers confirmed the presence of widespread Lewy body disease in addition to TDP-43 pathology in three out of six LBD cases, suggesting a molecular link between synuclein and TDP-43 proteinopathic changes and emphasizing the complexity of neurodegenerative disease related to *GRN* mutations. Taken together, our observations support the hypothesis that *GRN* mutations could be a rare cause of LBD.

Importantly, patient 2, who was found to carry the p.Q130Sfs*124 mutation, had a notable family history of dementia, which was reported as clinically diagnosed Alzheimer's disease affecting his brother, father, and paternal grandfather (Supplementary Note). Although we could not test the other family members, we can assume that the frameshift variant is the disease-causing mutation in this particular family, underlining the role of deleterious *GRN* mutations in familiar LBD.

Our evaluation further identified a missense variant (c.26C>T = p.A9V), located at a highly conserved residue within the signal sequence of the protein (Figure 1) and predicted to be 'likely pathogenic'. Another missense variant at the same amino acid residue (p.A9D = rs63751243) has been previously described as pathogenic and limited evidence suggests that the p.A9V mutation results in cytoplasmic missorting. It is difficult to interpret the clinical relevance of this mutation, as this patient is also homozygous for the protective *TMEM106B* allele.[21–24] Further evidence is needed to draw conclusions about the pathogenicity of this coding variant in LBD.

It is also noteworthy that a stop-gain mutation (p.R535X) was identified in a 74-year neurologically healthy subject who is followed longitudinally (Table S1). Reduced penetrance of pathogenic variants is a well-described phenomenon in late-onset neurodegenerative diseases.[25] Although predicted as pathogenic and previously reported as disease-causing,[9] the variant leads to a truncated protein where 90% of the wild-type sequence is intact, and non-sensitivity to nonsense-mediated decay may explain why the damaging mutation did not cause disease in this particular case. We cannot exclude that the control subject is actually a pre-symptomatic patient who will phenoconvert in the future.

Our study expands our understanding of the genetic architecture underlying LBD. It also adds to the increasing literature of pleiotropic genes as important drivers for multi-

proteinopathic changes in adult neurodegenerative diseases with heterogeneous clinical presentations.[13, 26–29] Although *GRN* mutations are overall rare (0.23% of LBD patients), genetic testing is increasingly available in specialty clinics and allows clinicians to rapidly screen for damaging mutations, particularly in individuals with a family history of dementia. As targeted therapies for *GRN*-related neurodegeneration are already studied in clinical trials (Clinicaltrials.gov: NCT04408625, NCT04747431, NCT04374136, NCT03987295; accessed on 01/21/22), identifying these individuals may open new avenues for research and treatment in LBD, an otherwise fatal form of dementia.

CONCLUSION

In summary, our findings implicate loss-of-function mutations in *GRN* in the pathogenesis of LBD. Molecular screening for damaging *GRN* mutations should be considered in LBD patients to establish a molecular diagnosis, especially in patients with a family history of dementia. Although rare, this information may be clinically relevant, as targeted *GRN* treatment trials are already ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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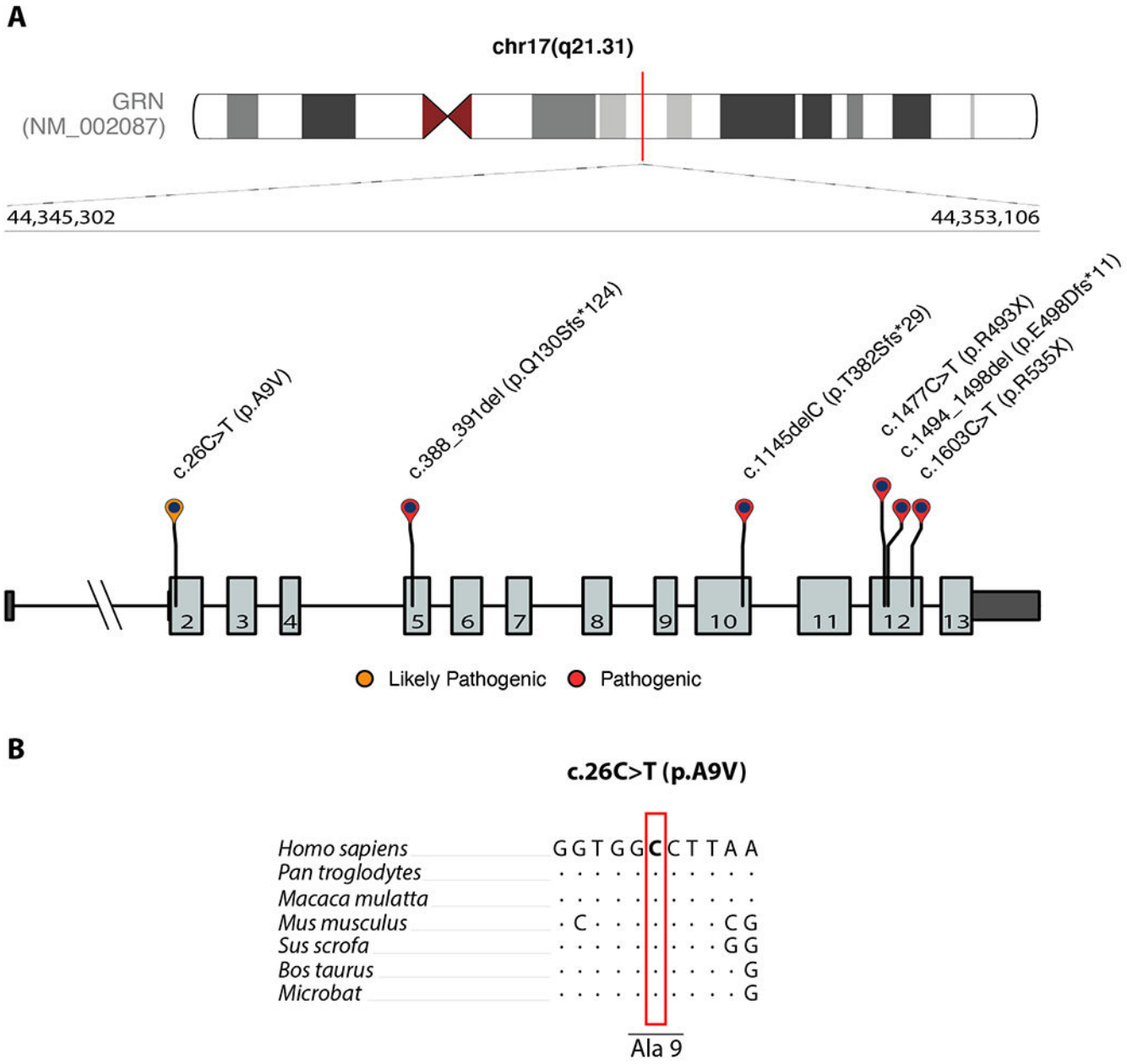

Figure 1. Schematic illustration of *GRN* mutations.

Schematic representation of *GRN* mutations identified in this study. Untranslated regions are illustrated by dark gray boxes, and exons are shown as light gray boxes. The bottom panel shows the nucleotide conservation for the novel coding mutation (c.26C>T = p.A9V) in seven mammals (B). A red box highlights the location of the mutated nucleotide. Dots indicate nucleotides that are identical to the human reference genome sequence.

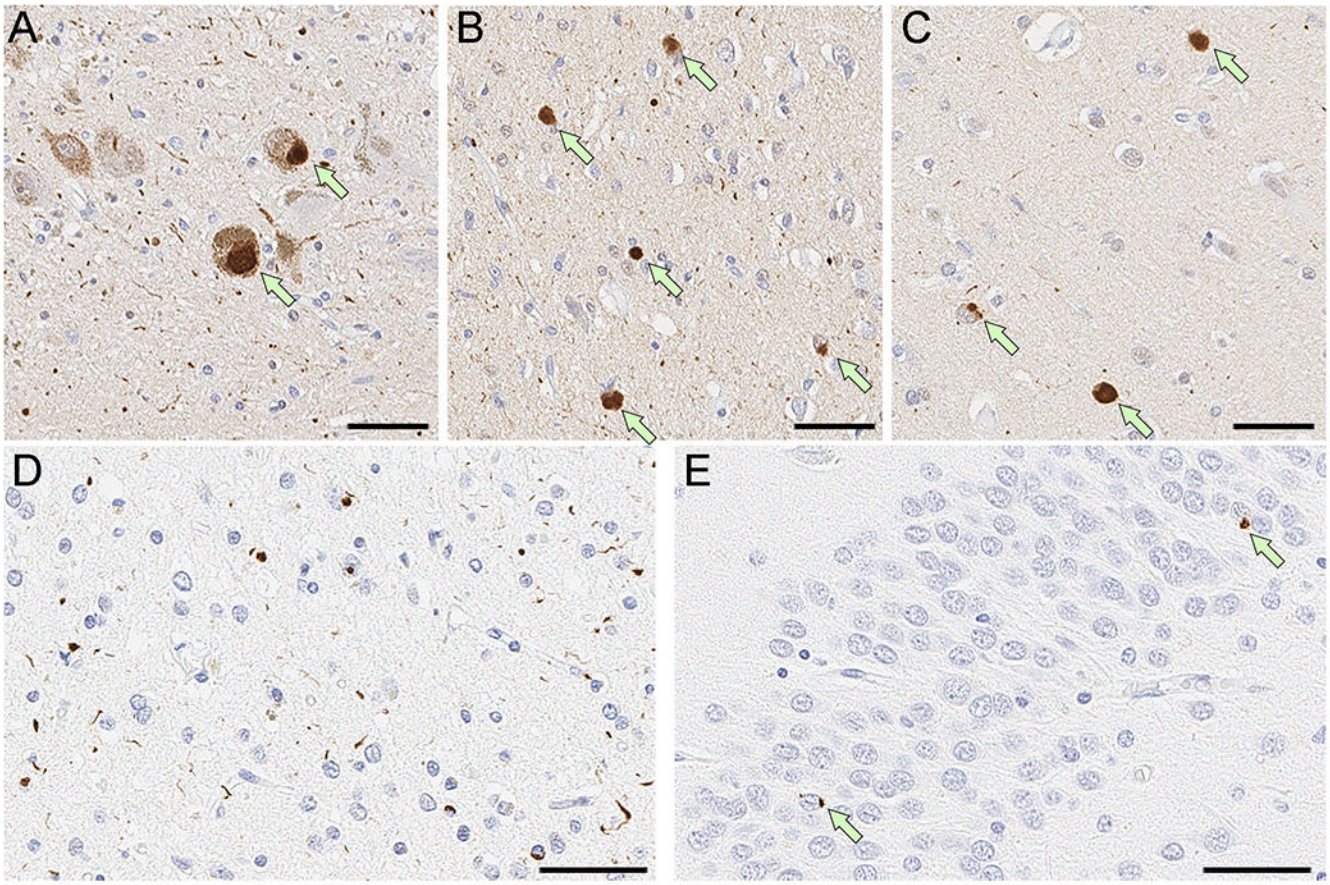

Figure 2. Representative images of α-synuclein and phosphorylated-TDP-43 immunohistochemistry

Shown are the immunohistochemical staining results in a *GRN* mutation carrier (p.Q130Sfs*124) illustrating α-synuclein-positive and TDP-43-positive co-pathologies. Immunohistochemistry for α-synuclein shows Lewy bodies (arrows) in the substantia nigra (A), cingulate gyrus (B), and superior temporal gyrus (C), consistent with diffuse Lewy body disease. Immunohistochemistry for phosphorylated-TDP-43 shows numerous neuronal cytoplasmic inclusions and short dystrophic neurites predominantly in layer II of the middle frontal gyrus (D), consistent with type A pathology. Dentate gyrus has a few compact neuronal cytoplasmic inclusions (arrows in E). Scale bars = 50μm.