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Mov Disord. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Mov Disord. 2017 January ; 32(1): 115–123. doi:10.1002/mds.26815.

Study of *LRRK2* variation in tauopathy: progressive supranuclear palsy and corticobasal degeneration

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Abstract

BACKGROUND—Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2)* are the most common genetic cause of Parkinson's disease (PD). Unexpectedly, tau pathology has been reported in a subset of *LRRK2* mutation carriers.

METHODS—To estimate the frequency of pathogenic *LRRK2* mutations, and to evaluate the association of common *LRRK2* variants with risk of primary tauopathies, we studied 1039 progressive supranuclear palsy (PSP) and 145 corticobasal degeneration patients from the Mayo Clinic Florida brain bank and 1790 controls ascertained at Mayo Clinic. Sanger sequencing of *LRRK2* exons 30, 31, 35 and 41 was performed in all patients and genotyping of all 17 known exonic variants with minor allele frequency >0.5% was performed in patients and controls.

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Financial disclosure/Conflict of interest related to research covered in this article:
Authors report no disclosures relevant to the article.

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RESULTS—*LRRK2* mutational screening identified two known pathogenic mutations (p.G2019S and p.R1441C), each in one PSP patient, the novel p.A1413T mutation in a PSP patient and the rare p.R1707K mutation in a corticobasal degeneration patient. Both p.A1413T and p.R1707K mutations were predicted damaging by at least 2 of 3 prediction programs and affect evolutionary conserved sites of LRRK2. Association analysis using common *LRRK2* variants only showed nominal association of the p.L153L variant with PSP.

CONCLUSIONS—Our study confirms the presence of pathogenic and potentially pathogenic *LRRK2* mutations in pathologically confirmed primary tauopathies, albeit with low frequency. In contrast to PD, common *LRRK2* variants do not appear to play a major role in determining PSP and corticobasal degeneration risk.

Keywords

LRRK2; tauopathy; PSP; CBD - mutation

INTRODUCTION

Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are primary neurodegenerative tauopathies characterized by neuronal loss and gliosis associated with abnormal aggregation of hyperphosphorylated tau. A tau isoform that has four ≈31 amino acid repeats in the microtubule-binding domain (4R tau) tends to preferentially accumulate within lesions of both PSP and CBD. Despite having overlapping features, PSP and CBD have distinctive neuropathological and biochemical profiles. In PSP, neurofibrillary tangles (NFT), neuropil threads (NTs) and tau-positive tufted astrocytes (TAs) are predominantly found in basal ganglia and brainstem, whereas in CBD pretangles, NTs and astrocytic plaques are found in gray and white matter of cortex, basal ganglia, diencephalon and rostral brainstem.[1] The classical clinical presentation of PSP is called PSP with Richardson's syndrome (PSP-RS) and consists of an akinetic-rigid syndrome, supranuclear gaze palsy, cognitive impairment and falls. However, at least eight additional clinical types have been reported.[2] CBD presents clinically with diverse motor, sensory, behavioral and cognitive symptoms.[3]

Mutations in leucine-rich repeat kinase 2 (*LRRK2)* are the most common genetic cause of Parkinson's disease (PD). Rare and common *LRRK2* variants have been associated with both familial and sporadic PD with clear pathogenic evidence for at least five variants: p.R1441C, p.R1441G, p.Y1699C, p.G2019S, and p.I2020T. Although predominantly associated with Lewy body (LB) pathology and clinical typical Parkinsonism, *LRRK2* mutations can present with other non-PD manifestations including significant tau-positive aggregates and NFT.[4] The search for *LRRK2* mutations in other non-PD populations has resulted in the identification of mutations in clinical PSP,[5] and corticobasal syndrome[6] but also in negative reports in clinical [7-9] and in pathology-confirmed PSP and CBD. [10-13] Most studies, however, analyzed small patient cohorts and, with a few exceptions, they limited the analysis to a few known *LRRK2* variants. In this study, we aimed to determine the frequency of known and novel pathogenic *LRRK2* mutations in a large series of autopsy-confirmed PSP and CBD cases, and to evaluate the association of common *LRRK2* variants with the risk of both diseases.

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METHODS

Study subjects

1039 PSP and 145 CBD autopsy-confirmed cases and 1790 clinical controls were included in this study. PSP and CBD brains were submitted to the Mayo Clinic brain bank for neurodegenerative disorders between 1998 and 2014. Neuropathologic diagnosis was rendered by a single neuropathologist (DWD) and followed published criteria for PSP[14] and CBD[15] Neuropathology studies included immunohistochemistry for phospho-tau and semi-quantitative analysis of neuronal and glial tau pathology in 20 brain regions of each case, thioflavin-S fluorescent microscopy, and the measurement of Braak NFT stage and Thal amyloid phase.[16] Clinical and demographic information was collected from available medical records. Summary of the characteristics of the PSP, CBD and control populations are displayed in Supplementary Table 1. Controls were neurologically normal volunteers evaluated in neurology clinics at Mayo Clinic in Jacksonville, FL. Study subjects were recruited through protocols approved by the Mayo Clinic IRB.

Genetic analysis

Genomic DNA was extracted from brain tissue in PSP and CBD cases and from peripheral blood in the control population. For the mutational screening, *LRRK2* exons 30, 31, 35 and 41 and flanking intronic sequences were sequenced bidirectionally in all cases as previously described. [17] The DNA mutation annotation was based on the *LRRK2* cDNA sequence of NM_198578.3. Phylogenetic and molecular evolutionary analyses were conducted using MEGA 7.09 running the MUSCLE program. For common variant association, all 17 *LRRK2* exonic variants that were previously observed with a minor allele frequency of 0.5% or greater by the Genetic Epidemiology of Parkinson's Disease Consortium (GEO-PD)[18] were genotyped in cases and controls. Twelve of them (p.L153L, p.N551K, p.I723V, p.L953L, p.R1398H, p.K1423K, p.R1514Q, p.P1542S, p.N2081D, p.E2108E, p.G2385G and p.M2397T) were genotyped using Sequenom MassARRAY iPLEX Gold chemistry. The remaining 5 common variants were genotyped by Sanger sequencing of exon 34 (p.G1624G, p.K1637K, p.M1646T and p.S1647T) and 37 (p.G1819G). The rs76904798 variant that was recently identified as a risk factor for PD in a large meta-analysis of GWAS[19] was genotyped by TaqMan genotyping assay. All genotype call rates were >99%, and there was no evidence of a departure from Hardy-Weinberg equilibrium in controls for any variant (all P>0.05). Linkage-disequilibrium between variants in controls is shown Supplementary tables 2a & 2b.

Statistical analysis

Associations of each common *LRRK2* variant with risk of PSP and CBD were assessed (i.e. each disease separately vs. controls) using logistic regression models adjusting for age (age at death in PSP and CBD patients and age at last follow-up in controls) and gender. Definite and potentially pathogenic *LRRK2* mutation carriers, related individuals as well as patients with an ethnicity different than Caucasian were excluded for this analysis which resulted in a total of 998 PSP and 135 CBD autopsy-confirmed cases (supplementary table 1). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated, and each *LRRK2* variant was examined under a dominant model (i.e. presence vs. absence of the minor allele) due to

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the small number of homozygotes of the minor allele for many of the variants. The association of the p.N551K-R1398H-K1423K haplotype with risk of PSP and CBD was evaluated using a logistic regression framework with adjustments for age and gender; ORs and 95% CIs were obtained in comparison to the common C-G-G haplotype.[20] Associations of a haplotype involving all 18 *LRRK2* variants with risk of PSP and CBD were examined using score tests for association with adjustments for age and gender.[21] Haplotypes occurring in less than 1% of subjects were excluded from all haplotype analysis. In order to adjust for multiple testing in single-variant analysis, we utilized a single-step minP permutation correction separately when evaluating associations of *LRRK2* variants with PSP and CBD; after this adjustment, p-values ≤ 0.0036 (PSP vs. controls) and ≤ 0.0038 (CBD vs. controls) were considered as statistically significant. All statistical analysis was performed using R Statistical Software version 3.0.2.

RESULTS

LRRK2 mutation screening

Sequencing of *LRRK2* exons 30, 31, 35, and 41 in our PSP and CBD cases resulted in the identification of four definite or potentially pathogenic mutations. Three PSP cases were identified carrying *LRRK2* mutations: one p.G2019S, one novel p.A1413T and one p.R1441C mutation. The latter individual is the proband of Family D that has been previously reported.[22] The p.A1413T mutation was identified in a Hispanic PSP patient and results from a c.4237 G>A heterozygous change in exon 30. This mutation has not been reported before in dbSNP, EVS database (http://evs.gs.washington.edu/EVS/ April 2016) or ExAC (http://exac.broadinstitute.org April 2016) which includes exome data of 5,789 individuals from a Latino population. This variant was predicted to be “disease causing” by Mutation Taster and “possibly damaging” by Polyphen-2 programs and is located in a conserved site that is part of the LRRK2 Roc domain (Figure 1A and 1B).

In the CBD cohort, one case was found to carry a heterozygous c.5120 G>A change in exon 35 that results in a p.R1707K change (Figure 1A). This variant is reported in dbSNP (rs200769426) as identified by *LRRK2* deep sequencing in one individual from a total of 14,002 participants.[23] The carrier of this variant is a 19 year-old female affected with irritable bowel disease-constipation (IBS) who is native from England (McCarthy L, GSK, personal communication). This variant is predicted to be “damaging” with SIFT, Mutation Taster and Polyphen-2 programs and is located in an evolutionarily conserved site in the LRRK2 C-terminal of ROC (COR) domain (Figure 1C).

We also genotyped *LRRK2* p.R1628P (rs33949390), since although this variant is observed rarely in Caucasians,[18] it has been shown to be a PD risk factor in Asian populations.[24] The p.R1628P variant was not observed in controls, but was seen in two PSP cases (0.2% of patients) and one CBD case (0.7% of patients) all of them of Caucasian ethnicity.

LRRK2 common SNP association

An evaluation of associations of common *LRRK2* variants with risk of PSP and CBD is provided in Table 1. Though the size of our PSP cohort provided enough statistical power to

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detect significant associations, no *LRRK2* variants were found associated with risk of PSP after adjustment for multiple testing; however, there was a nominally significant association with the p.L153L variant (OR: 1.22, 95% CI: 1.04 – 1.44, P=0.014). A similar non-significant trend was noted for CBD (OR: 1.42, 95% CI: 0.97 – 2.08, P=0.065) but we were underpowered to detect associations in this cohort because of its size. There were no other nominal associations with risk of PSP or CBD for any of the other *LRRK2* variants that were examined (all $P \geq 0.10$). Of particular interest, the p.M1646T PD risk factor was not associated with PSP (OR: 1.03, 95% CI: 0.63 – 1.68, P=0.90) or CBD (OR: 1.12, 95% CI: 0.39 – 3.19, P=0.84). A detailed summary of allele and genotype frequencies for common variants is provided in Supplementary tables 3a-3c.

The p.N551K-R1398H-K1423K haplotype that is protective for PD[18, 25] was not found to be associated with risk of PSP (OR: 0.99, 95% CI: 0.80 – 1.23, P=0.92) or CBD (OR: 1.16, 95% CI: 0.73 – 1.85, P=0.52). When examining a general haplotype that included all 18 common *LRRK2* variants, there was no association with PSP (P=0.69); however, there was a significant association with CBD (P=0.011). As displayed in Table 2, there was one haplotype (haplotype 1) that was less common in CBD patients than controls (3.2% vs. 6.0%, P=0.024), and two haplotypes (haplotypes 17 and 18) that were more common in CBD patients compared to controls (Haplotype 17: 2.3% vs. 1.1%, P=0.033; Haplotype 18: 4.0% vs. 1.8%, P=0.016).

Clinicopathological characteristics of the LRRK2 p.G2019S PSP carrier

This patient (patient #4 in supplementary tables 4a-c) was an 80-year-old white Jewish woman who presented with progressive gait difficulties and falls at the age of 73. In the early stage of the disease the patient developed dysarthria, dysphagia, intermittent left hand tremor and vertical gaze palsy and retrocollis. Three years later the patient became wheelchair-bound and was found to have pseudobulbar palsy and mild memory impairment (27/30 on Mini-Mental State Examination (MMSE)). There was hand-as-object apraxia on the left, bradydiadochokinesia, spasticity, and ataxia of her upper limbs. Under the suspicion of probable PSP, the PSP Rating Scale was conducted and the patient scored 51/100. Her Frontal Assessment Battery score was 13/18. MRI showed mild generalized atrophy. On autopsy, her brain weight was 1080 grams. There was mild atrophy over the frontal convexity with mild enlargement of the frontal horn of the lateral ventricle (Figure 2A). The subthalamic nucleus (STN) was slightly atrophic and the cerebellar hilus, substantia nigra (SN) and the locus coeruleus were remarkable for decreased pigmentation. Microscopically, there was moderate neuronal loss and gliosis with NFT, coiled bodies, and TAs in the STN. The globus pallidus (GP) had mild gliosis. There was myelinated fiber loss and gliosis in the superior cerebellar peduncle (SCP), and the dentate nucleus (DN) was mildly atrophic with severe neuronal loss and grumose degeneration. There was moderate neuronal loss and gliosis with globose NFT, but no LBs in the SN. The raphe nuclei, locus ceruleus and pontine and medullary reticular formation had many pretangles and threads. On thioflavin-S staining, many senile plaques (SP) and sparse NFT were detected. The neuropathologic diagnosis was PSP with early stage AD.

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Clinicopathological characteristics of the LRRK2 p.A1413T PSP carrier

This patient (patient # 5 in supplementary tables 4a-c) was a 79-year-old Filipino man who presented at the age of 72 years with progressing memory problems and gait difficulties with severe postural instability and falls as well as apraxia of eyelid opening. On neurological examination one year before his death the patient showed rigidity of his limbs and axial rigidity with retrocollis, vertical supranuclear palsy, hypomimia, spastic dysarthria, severe bradykinesia, but no tremor. The patient was wheelchair bound and had urinary incontinency. MRI revealed brainstem atrophy. Trials of levodopa (up to 600mg/d), donepezil, onabotulinumtoxinA, quetiapine, risperidone had no durable benefit. On autopsy his brain weighed 1100 g. There was a mild atrophy over the frontal convexity, remarkable STN and SN atrophy (Figure 2B). There were also decreased pigmentation of the SN and locus coeruleus, and mild atrophy of the DN with minimal discoloration of the cerebellar hilus. There was extensive neuronal and glial pathology, including pretangles, NTs, flame-shaped and globoid NFT, TAs (Figure 2C), grains (Figure 2D), coiled bodies and a few ballooned neurons; thus, the clinical diagnosis of PSP could be confirmed by the neuropathology examination. The SN showed moderate to marked neuronal loss with globose NFT, but no LBs (Figure 2E-F). The basal ganglia had no SP, but NFT and TAs. The GP had gliosis, and the STN had mild neuronal loss, marked gliosis and many NFT (Figure 2G-H). On thioflavin-S staining, no SP and only rare NFT were noted.

Clinicopathological characteristics of the LRRK2 p.R1707K CBD carrier

This patient was an 84-year-old right-handed woman with a six year history of progressive cognitive decline. MMSE showed a four point decline in scores per year, reaching 18/30 when last tested four years before she died. Around that time a clinical examination revealed apathy. Except for Babinski's sign on the left, no further neurological abnormalities were recorded. Her family history was negative for neurodegenerative disorders, but notable for stroke in her sister. The patient's tentative diagnosis was mixed multi-infarct and AD-type dementia. On autopsy her brain weighed 1000 grams and showed diffuse cortical atrophy, most prominent in the superior frontal gyrus with marked enlargement of the frontal horn of the lateral ventricles (Figure 3A, *asterisk*). There was mild atrophy of the anterior corpus callosum, amygdala, hippocampus and midbrain, but the basal ganglia, thalamus and STN were unremarkable. The SN showed marked depigmentation and there was no atrophy of the SCP and no atrophy or discoloration of the DN. Tau immunohistochemistry showed many cortical pretangles and threads in gray and white matter, as well as numerous astrocytic plaques (Figure 3B). There was thinning of cortical ribbon with spongiosis, gliosis and ballooned neurons particularly in parietal, limbic and superior frontal cortices (Figure 3C-D). The hippocampus and amygdala had no significant neuronal loss, but many pretangles and threads. Ballooned neurons were present in the amygdala. The basal ganglia had many pretangles, threads and astrocytic plaques in the corpus stratum. The SN had marked neuronal loss with NFT ("corticobasal bodies") (Figure 3 E-F). There was much less severe tau pathology in lower brainstem and nearly completely absent in the cerebellum. NFT and amyloid deposition on Thioflavin-S staining corresponded with Braak NFT stage II and Thal amyloid phase 3. The findings were compatible with CBD with additional AD-type pathology.

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DISCUSSION

Mutations in *LRRK2* are a common genetic factor for both familial and sporadic PD but studies have suggested that *LRRK2* mutations may also be involved in PSP and related tauopathies such as CBD. In this study, we performed a systematic genetic analysis of *LRRK2* mutations and genetic association studies in the largest cohort of autopsy-confirmed PSP and CBD studied to date. In line with earlier reports, we identified three *LRRK2* mutations in PSP patients, and we also report the first CBD case with a likely pathogenic *LRRK2* mutation. Therefore, while these mutation frequencies are low, our findings do confirm that *LRRK2* mutations can lead to the development of primary tauopathies and emphasize *LRRK2* should be considered the second most common causal gene for this group of diseases after *MAPT*. Moreover, since our study only focused on *LRRK2* coding exons in which pathogenic mutations had previously been reported, we cannot rule out the presence of additional mutations in other *LRRK2* regions.

We have previously defined two main types of clinicopathological presentations in *LRRK2* mutation carriers: the most common defined as the the typical PD type, and a second type presenting with “pure nigral degeneration” with no LB pathology. [26] A third type of clinicopathological manifestation of *LRRK2* mutations has been suggested by Spanaki et al. to classify a transitional form between PD and PSP observed in a carrier of the p.R1441H mutation,[27] and the p.R1441C mutation carrier from Family D (this patient correspond to the mutation carrier reported in this study and to patient #1 in supplementary tables 4a-c). [22] Later on, Ruffmann et al. further defined this third type of *LRRK2* mutation carriers by describing the similarities between the p.R1441C mutation carrier from Family D and two additional p.G2019S carriers (patients #2 and #3 in supplementary tables 4a-c) manifesting with PSP-like 4R tauopathy pathology with mainly subcortical involvement. [28] The parkinsonian presentation and milder severity of PSP pathology in these three cases are consistent with their classification as PSP-P. [29] Moreover, the suspected specificity of *LRRK2* mutations for PSP-P may explain the failure to detect *LRRK2* mutations in clinical PSP cohorts, which would be mostly patients with PSP-RS.[9] Since to date, these three cases were the only *LRRK2* mutation carriers presenting with PSP pathology described in the literature, we performed a comparison between them and our two PSP mutation carriers newly identified in this study (patients #4 and patient #5 in supplementary tables 4a-c). The medical records of our two cases support that they had a typical PSP-RS clinical presentation which contrasts with the absence of classical PSP symptoms in the three previously reported cases. However, given that the medical history is recorded retrospectively in our cases, we cannot exclude that they presented a parkinsonian syndrome early in the course of their disease. Similar to the cases described by Ruffmann et al., our two PSP cases also had predominant subcortical tauopathy and no LB pathology but a detailed observation of the tauopathy severity indicates that our two cases had a more severe presentation compared to the three previous cases and fulfilled all neuropathological criteria for PSP.

We also identified a *LRRK2* p.R1707K mutation in a patient with autopsy-confirmed CBD. The clinical presentation was of progressive cognitive impairment with apraxia and language difficulties, but no other features to meet clinical criteria for CBD.[3] The only major

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differential diagnosis would have been frontotemporal dementia and parkinsonism linked to chromosome 17,[15] but this was excluded by genetic studies. To our knowledge, *LRRK2* p.R1707K is the first mutation other than *MAPT* mutations[30] to be reported in autopsy-confirmed CBD. In support of its pathogenicity the p.R1707K has not been observed in controls, is predicted to be damaging to the LRRK2 protein; and is at an evolutionarily conserved site. Additionally, the p.R1707K is located near the pathogenic p.Y1699C mutation and both mutations are located in LRRK2 COR domain (Figure 1A). However, different from the p.R1707K mutation, the p.Y1699C mutation has been associated with clinical PD, SN degeneration and absence of tauopathy.[22] . The p.R1707K mutation has been reported in the dbSNP database and, interestingly, this mutation carrier is affected with IBS. The fact that GWAS studies have also linked *LRRK2* variants to Crohn's disease, a form of IBS, supports the pleomorphic nature of *LRRK2* mutations.

Finally, using common variant association analyses, we showed that individual common *LRRK2* variants that have previously been associated with risk of PD (p.M1646T and the p.N551K-R1398H-K1423K haplotype) are not associated with PSP or CBD. Interestingly, we did observe a nominally significant association between p.L153L and risk of PSP (P=0.014), with a similar but non-significant association for CBD (P=0.065). Since these associations were not significant after adjustment for multiple testing, it will be important to further assess them in additional, independent PSP and CBD cohorts. We also detected a significant association between a haplotype involving all 18 common *LRRK2* variants and risk of CBD, but given that the specific haplotypes that appeared to differ between CBD patients and controls were all rare, examination of this finding in larger series will also be needed. Importantly, the possibility of type II error (i.e. a false-negative association) also cannot be excluded and emphasis should be placed on 95% confidence limits when interpreting our results; especially in the smaller CBD series where association ORs are less precise.

In conclusion, our study emphasizes the importance of *LRRK2* as a rare causative gene in pathologically confirmed PSP and CBD. More phenotype-genotype correlative studies and association studies in larger cohorts are now needed to elucidate how *LRRK2* mutations result in different clinical and pathological manifestations and to clarify a possible link between *LRRK2* variation and tau hyperphosphorylation and aggregation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We would like to thank all those who have contributed to our research, especially the patients and their families who donated brains for this work. We would also like to acknowledge the work of Audrey Strongosky, BA who was instrumental in assisting us in collecting the blood specimens from controls and enteric the clinical data into the computer systems.

Full financial disclosure of all authors

PT is supported by a Jaye F. and Betty F. Dyer Foundation Fellowship in progressive supranuclear palsy research, a Max Kade Foundation postdoctoral fellowship, an Allergan Medical Educational Grant, and a gift from Carl

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Edward Bolch, Jr., and Susan Bass Bolch. OAR is supported by NS078086 and the Michael J. Fox Foundation. LIG received grants from the Movement Disorders Research Fund of Rutgers Robert Wood Johnson Medical School, the Rainwater Charitable Foundation, and from a Center of Excellence grant from the American Parkinson's Disease Association. ZKW is supported by the Udall National Institutes of Health (NIH)/National Institute of Neurological Disorders and Stroke P50NS40256, NIH/National Institute on Aging (NIA) P01AG017216, NIH/NIA R01AG015866, and Pacific Alzheimer Research Foundation C06-01 grants, and holds a patent on LRRK2 genetic variability. He is also partially funded by the Mayo Clinic Center for Regenerative Medicine, the Mayo Clinic Center for Individualized Medicine, the Mayo Clinic Neuroscience Focused Research Team, and the gift from The Sol Goldman Charitable Trust. DWD receives research support from the NIH (P50 AG016574; P01 AG003949); is an editorial board member of American Journal of Pathology, Annals of Neurology, Journal of Neuropathology and Experimental Neurology, and Brain Pathology; and is the editor-in-chief of American Journal of Neurodegenerative Disease and International Journal of Clinical and Experimental Pathology.

Funding sources for study: This study was supported by the CurePSP Brain Bank and the funding support from the Udall Center for Excellence in Parkinson's Disease Research (P50 NS072187, funding for MS-C, OR, ZKW, DWD, and RR).

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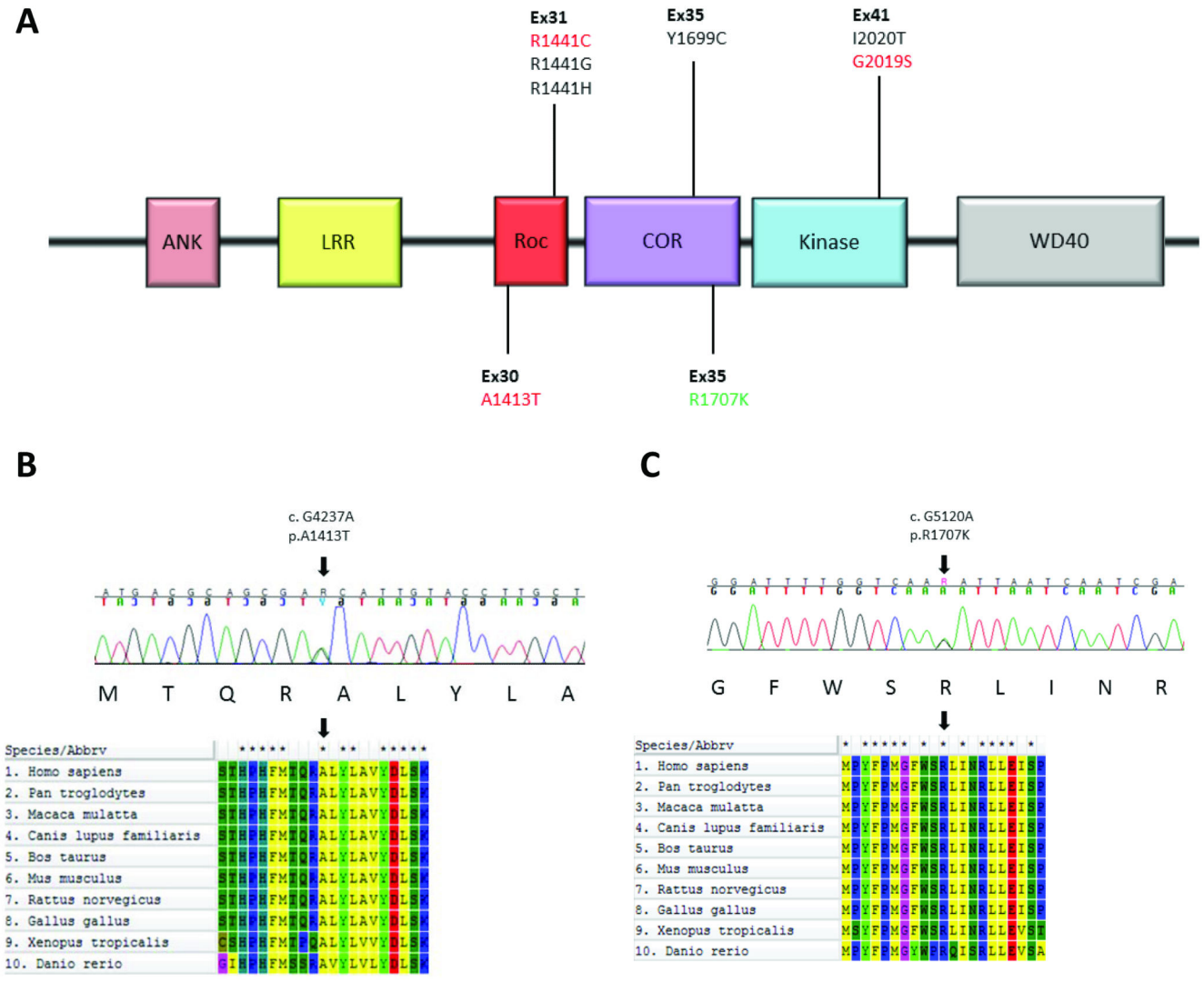

Figure 1.

LRRK2 functional domains and localization of known PD pathogenic mutations (top), mutations presenting as PSP (red) and the p.R1707K mutation found in a CBD patient (green) (**A**). Conservation analysis of LRRK2 p.A1413T (**B**) and p.R1707K mutations (**C**).

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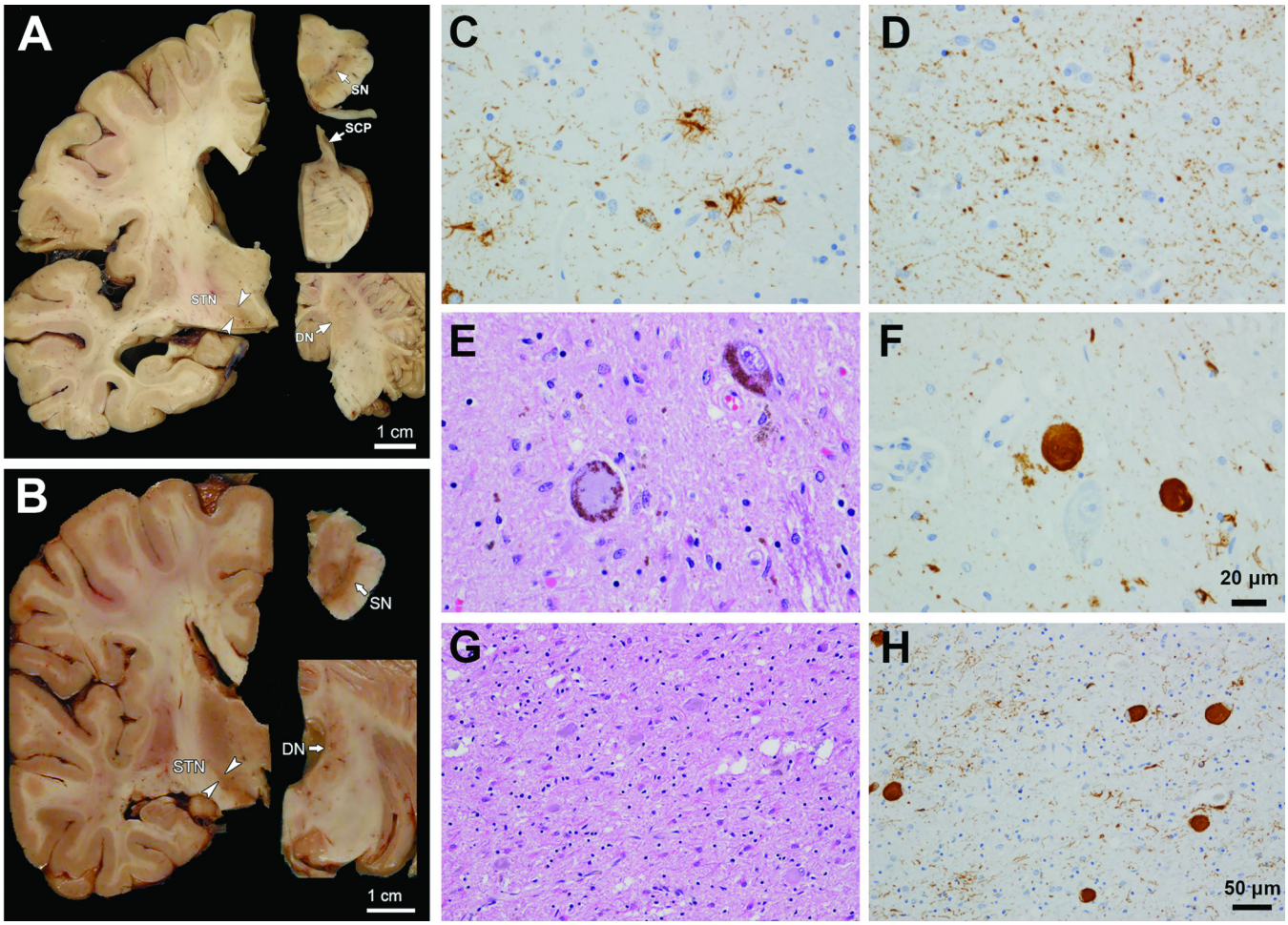

Figure 2.

Neuropathologic findings of two PSP patients carrying LRRK2 mutations. Coronal cerebral section of the LRRK2 p.G2019S (**A**) and the LRRK2 p.A1413T (**B**) patients. Microscopic findings in the LRRK2 p.A1413T patient (**C-H**). Phospho-tau immunostaining (**C, D, F**, **H**) and H&E stain (**E**, **G**).

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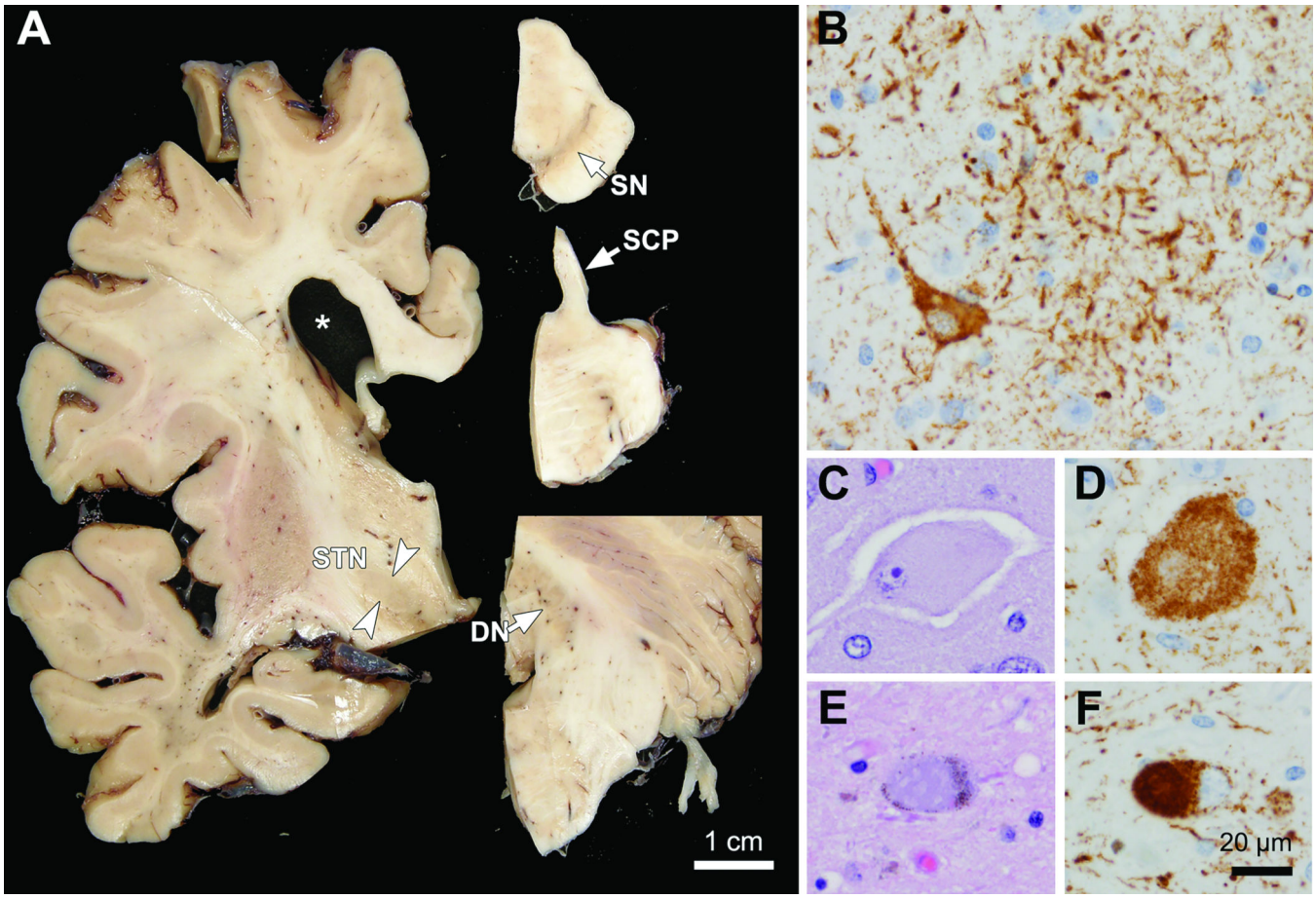

Figure 3.

Neuropathologic findings in a CBD patient with a LRRK2 p.R1707K mutation. Coronal section of the cerebrum (**A**). Microscopic findings with phospho-tau immunostaining (**B**, **D**, **F**) and H&E stain (**C - E**). Bar in panel (**F**) applies to panels (**B – E)**, as well.

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Table 1

Association of common *LRRK2* variants with risk of PSP and CBD

				PSP patients vs. controls			CBD patients vs. controls		
Variant	**Amino Acid**	**MA[a]**	**MAF[b] in controls (N=1790)**	**MAF[b] in PSP (N=996)**	**OR[c] (95% CI[d])**	**P-value**	**MAF[b] in CBD (N=134)**	**OR[c] (95% CI[d])**	**P-value**
rs10878245	L153L	T	38.2%	41.4%	1.22 (1.04, 1.44)	0.014	41.4%	1.42 (0.97, 2.08)	0.065
rs7308720	N551K	G	7.6%	7.1%	0.92 (0.73, 1.15)	0.44	8.6%	1.06 (0.65, 1.73)	0.81
rs10878307	I723V	G	7.7%	7.3%	0.93 (0.74, 1.16)	0.51	9.0%	1.13 (0.69, 1.85)	0.62
rs7966550	L953L	C	12.6%	12.7%	1.04 (0.86, 1.24)	0.72	13.4%	1.28 (0.85, 1.91)	0.24
rs7133914	R1398H	A	7.4%	7.1%	0.94 (0.75, 1.18)	0.60	8.6%	1.09 (0.67, 1.77)	0.74
rs11175964	K1423K	A	7.3%	7.1%	0.97 (0.77, 1.21)	0.76	8.2%	1.06 (0.64, 1.74)	0.83
rs35507033	R1514Q	A	0.8%	0.5%	0.59 (0.28, 1.26)	0.17	0.7%	0.99 (0.23, 4.29)	0.98
rs33958906	P1542S	T	2.8%	2.9%	1.03 (0.73, 1.44)	0.88	1.9%	0.62 (0.25, 1.57)	0.31
rs1427263	G1624G	C	35.1%	34.3%	1.00 (0.86, 1.17)	0.98	35.8%	1.13 (0.79, 1.63)	0.50
rs11176013	K1637K	A	45.1%	45.2%	1.07 (0.90, 1.26)	0.46	45.5%	1.08 (0.73, 1.59)	0.70
rs35303786	M1646T	C	1.3%	1.4%	1.03 (0.63, 1.68)	0.90	1.5%	1.12 (0.39, 3.19)	0.84
rs11564148	S1647T	A	30.4%	31.0%	1.02 (0.87, 1.19)	0.79	30.6%	0.98 (0.69, 1.40)	0.92
rs10878371	G1819G	T	45.1%	44.5%	1.01 (0.86, 1.20)	0.87	44.8%	1.01 (0.69, 1.48)	0.97
rs33995883	N2081D	G	1.9%	2.3%	1.24 (0.84, 1.82)	0.29	2.6%	1.51 (0.67, 3.39)	0.32
rs10878405	E2108E	A	31.0%	31.4%	1.02 (0.88, 1.20)	0.77	31.7%	1.16 (0.82, 1.66)	0.40
rs33962975	G2385G	G	14.1%	14.4%	1.01 (0.85, 1.21)	0.90	13.1%	0.86 (0.57, 1.31)	0.48
rs3761863	M2397T	T	34.5%	34.1%	1.04 (0.89, 1.21)	0.66	35.4%	1.20 (0.84, 1.72)	0.32
rs76904798	N/A	T	14.0%	14.8%	1.04 (0.87, 1.24)	0.69	10.5%	0.70 (0.45, 1.08)	0.10

LRRK2 variants were examined under a dominant model; ORs correspond to presence of the minor allele. After applying a single-step minP permutation adjustment for multiple testing, p-values ≤ 0.0036 (PSP vs. controls) and ≤ 0.0038 (CBD vs. controls) were considered as statistically significant.

[a]MA=minor allele;

[b]MAF=minor allele frequency;

[c]OR=odds ratio;

[d]CI=confidence interval. ORs, 95% CIs, and p-values result from logistic regression models adjusted for age (age at death in PSP/CBD patients, age at last follow-up in controls) and gender.

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Table 2

Association between a general haplotype including all 18 common *LRRK2* variants and risk of CBD

		Haplotype																	
Variant	**Amino acid**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
rs10878245	L153L	C	T	T	C	C	C	C	C	C	T	T	T	T	T	T	C	C	T
rs7308720	N551K	C	C	C	C	C	G	C	C	C	C	C	C	G	C	C	C	C	C
rs10878307	I723V	A	A	A	A	A	A	A	A	G	A	A	A	A	A	A	A	A	G
rs7966550	L953L	T	T	T	T	T	T	T	T	T	C	T	T	T	T	T	T	T	T
rs7133914	R1398H	G	G	G	G	G	A	G	G	G	G	G	G	A	G	G	G	G	G
rs11175964	K1423K	G	G	G	G	G	A	G	G	G	G	G	G	A	G	G	G	G	G
rs35507033	R1514Q	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
rs33958906	P1542S	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
rs1427263	G1624G	A	C	A	A	A	A	C	A	C	C	A	A	A	A	A	A	A	C
rs11176013	K1637K	G	A	A	G	G	G	A	G	A	A	G	A	G	G	G	G	G	A
rs35303786	M1646T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
rs11564148	S1647T	T	T	T	T	A	T	T	A	T	T	T	T	T	T	A	T	T	T
rs10878371	G1819G	C	T	T	C	C	C	T	C	T	T	C	T	C	C	C	C	C	T
rs33995883	N2081D	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	A	A	A
rs10878405	E2108E	G	G	G	A	G	G	G	A	G	G	G	G	G	G	A	G	A	G
rs33962975	G2385G	A	A	G	A	G	A	A	A	A	A	A	G	A	A	A	A	A	A
rs3761863	M2397T	C	T	C	C	C	C	T	C	T	T	C	C	C	T	C	C	C	T
rs76904798	N/A	T	C	C	T	C	C	C	C	C	C	C	C	C	T	C	C	C	C
% in CBD		3.3	1.8	1.1	1.9	3.4	0.9	7.3	22.1	3.9	10.4	0.7	7.3	5.7	2.2	2.3	3.4	2.3	4.0
% in controls		6.0	4.2	2.3	3.4	4.3	1.7	8.2	23.3	4.9	10.7	1.0	6.0	5.2	1.6	1.4	2.2	1.1	1.8
P-value		0.024	0.17	0.20	0.20	0.38	0.40	0.45	0.47	0.71	0.87	0.86	0.63	0.58	0.44	0.30	0.25	0.033	0.016

P-values result from score tests for association.

Mov Disord. Author manuscript; available in PMC 2018 January 01.