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Parkinson's disease susceptibility variants and severity of Lewy body pathology

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Abstract

Introduction—Meta-analyses of genome-wide association studies (GWAS) have established common genetic risk factors for clinical Parkinson's disease (PD); however, associations between these risk factors and quantitative neuropathologic markers of disease severity have not been well-studied. This study evaluated associations of nominated variants from the most recent PD GWAS meta-analysis with Lewy body disease (LBD) subtype (brainstem, transitional, or diffuse) and pathologic burden of LB pathology as measured by LB counts in five cortical regions in a series of LBD cases.

Methods—547 autopsy-confirmed cases of LBD were included and genotyped for 29 different GWAS-nominated PD risk variants. LB counts were measured in middle frontal (MF), superior temporal (ST), inferior parietal (IP), cingulate (CG), and parahippocampal (PH) gyri.

Results—None of the variants examined were significantly associated with LB counts in any brain region or with LBD subtype after correcting for multiple testing. Nominally significant ($P < 0.05$) associations with LB counts where the direction of association was in agreement with that observed in the PD GWAS meta-analysis were observed for variants in *BCKDK/STX1B* (MF, ST, IP) and *SNCA* (ST). Additionally, *MIR4697* and *BCKDK/STX1B* variants were nominally associated with LBD subtype.

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Conclusion—The lack of a significant association between PD GWAS variants and severity of LB pathology is consistent with the generally subtle association odds ratios that have been observed in disease-risk analysis. These results also suggest that genetic factors other than the susceptibility loci may determine quantitative neuropathologic outcomes in patients with LBD.

Keywords

Lewy body disease; Parkinson's disease; genetics; neuropathology

Introduction

Lewy body disease (LBD) refers to neurodegenerative diseases that are defined by the presence of Lewy bodies (LBs) and Lewy neurites in vulnerable brain regions that also show neuronal loss and gliosis. Depending on the severity of LB pathology, as well as the amount of concomitant Alzheimer's disease (AD) type pathology (as well as other less common types of pathology), LBD can present with several distinct clinical syndromes, the most common of which is Parkinson's disease (PD). [1]

Our understanding of the genetics of PD has advanced greatly over the past 20 years, with the discovery of a number of disease-causing mutations and also the identification of common risk-modifying variants through genome-wide association studies (GWAS). [2] Recently, several groups have used meta-analysis of PD GWAS data to definitively identify common variants that are associated with PD risk. [3, 4] In the largest of these analyses, Nalls et al studied 19,081 PD patients and 100,833 controls and identified a total of 28 independent genetic risk variants. [4]

Given that Lewy-related pathology is a neuropathological hallmark of PD, studies of how PD genetic risk factors relate to severity of LB pathology have potential to provide insight into how these variants modulate disease risk. To date, such investigations have focused on α -synuclein (*SNCA*), microtubule-associated protein tau (*MAPT*), and glucocerebrosidase (*GBA*) in varying patient populations, and sample sizes have generally been relatively small. [5–12] In this study we evaluated the associations of PD susceptibility variants identified in the most recent GWAS meta-analysis with pathologic burden of LB pathology and LBD subtype in a large series of autopsy-confirmed LBD.

Materials and Methods

Case material

A total of 547 autopsy-confirmed LBD cases from the Mayo Clinic Jacksonville brain bank for neurodegenerative disorders were included in this study. The brain bank operates under procedures approved by the Mayo Clinic Institutional Review Board, and research on autopsy tissue is considered exempt from Human Subject Research regulations. Autopsies were performed after informed consent of the next-of-kin or someone with legal authority to grant permission. Cases of amygdala predominant LBs in the setting of advanced AD were excluded, as were cases with significant coexisting non-AD pathology (e.g. progressive supranuclear palsy, corticobasal degeneration, Pick's disease, or multiple system atrophy),

and cases without a LB count measure in any of the brain regions assessed (see Neuropathologic assessment section). Based on patient identification information and medical record review, all subjects were unrelated, non-Hispanic, and Caucasian. Individuals with a known pathogenic mutation in the α -synuclein gene (*SNCA*; p.A53T, p.A30P, p.E46K, p.H50Q, p.G51D, *SNCA* duplications, and *SNCA* triplications were assessed) or the leucine-rich repeat kinase 2 gene (*LRRK2*; p.N1437H, p.R1441C, p.R1441G, p.R1441H, p.Y1699C, p.G2019S, and p.I2020T were assessed) were excluded. Mean age at death was 79 years (Range: 50 – 99 years) and 327 cases (60%) were male.

Neuropathologic assessment

The methods used in the neuropathologic assessment have been described in detail previously. [13] Briefly, neuroanatomical sampling and thioflavin-S fluorescence microscopy was performed using procedures of Terry and colleagues, with manual counts of neurofibrillary tangles (NFTs) and senile plaques in 6 cortical regions, as well as 4 sectors of the hippocampus and 2 regions of the amygdala. [14] Formalin-fixed, paraffin-embedded tissue from cortical and limbic regions were cut at a 5 μ m thickness and mounted on glass slides. LB pathology was assessed using an α -synuclein antibody (NACP, 1:3000 rabbit polyclonal, Mayo Clinic antibody) and was processed using the DAKO Autostainer (DAKO Auto Machine Corporation, Carpinteria, CA) with DAKO Envision+ HRP System. LB counts were assessed in middle frontal (MF), superior temporal (ST), inferior parietal (IP), cingulate (CG), and parahippocampal (PH) gyri. The staging scheme of Kosaka and colleagues was used to classify the distribution of LB pathology as brainstem, transitional, or diffuse. [15] The distributions of NFTs and amyloid plaques were used to assign a Braak NFT stage [16] and Thal amyloid phase [17], respectively. A summary of neuropathologic measures is shown in Table 1.

Genetic analysis

Genomic DNA was extracted from brain tissue using an automated process performed by the Autogen 245T (Autogen, Holliston, Ma). Genotyping was based on a combination of the Agena Bioscience Mass Array system (Agena Bioscience, San Diego, CA) and ABI Taqman genotyping assays (Applied Biosystems, ThermoFisher, Waltham, MA). Primers were designed by using Assay Design 3.1 software (available upon request). Typer 4.0 software was used to analyze acquired genotype data. We selected all 28 variants that were independently associated with risk of PD in the replication phase of a recent GWAS meta-analysis by Nalls et al. [4] for inclusion in this study (Table 2). Additionally, we also included the *TCEANC2* rs10788972 variant due to an association with PD in a recent GWAS based on neuropathologically-confirmed PD patients and controls [18]. We also calculated a PD genetic risk score as described by Nalls et al. [4] by combining information from all 28 variants included from that study. This was only calculated for the 495 patients with genotype information for all 28 variants. The mean PD genetic risk score was 3.04 (Range: 1.67 – 4.81). All genotype call rates were >95%. There was no evidence of a departure from Hardy-Weinberg equilibrium for any variants (all $P > 0.01$) with the exception of *HLA-DQB1* rs13201101 ($P < 0.001$), which was driven by an excess of rare homozygotes. As this could be due to an association between this variant and LBD rather than a

genotyping error, the rs13201101 variant was retained for use in the analysis. Genotype counts and frequencies are provided in Supplementary Table 1.

Statistical analyses

Continuous variables were summarized with the sample mean, minimum, first quartile (i.e. 25th percentile), third quartile (i.e. 75th percentile), and maximum. Separately for each brain region, the association between each variant and the LB count in the given brain region was evaluated using a negative binomial regression model [19] adjusted for age at death and sex. Given the relatively small number of rare homozygotes for many of the variants, we considered each variant under a dominant model (i.e. presence vs. absence of the minor allele). Multiplicative increases and 95% confidence intervals (CIs) were estimated, and are interpreted as the multiplicative increase in mean LB count in the given brain region corresponding to presence of the minor allele of the given variant.

Associations between each variant and LBD subtype (brainstem, transitional, or diffuse) were examined using proportional odds logistic regression models [19] in order to account for the ordered nature of this categorical outcome measure. These proportional odds logistic regression models were adjusted for age at death and sex, and odds ratio (OR) estimates are interpreted as the multiplicative increase in the odds of a more widespread distribution of LB pathology (i.e. diffuse more than transitional more than brainstem) corresponding to presence of the minor allele. Associations of PD genetic risk score with LB counts and LBD subtype were examined using the aforementioned negative binomial regression and proportional odds logistic regression models, where PD genetic risk score was evaluated as a continuous variable and also as a categorical variable based on sample quartiles. We utilized a Bonferroni correction to adjust for multiple testing separately for each different outcome in single-variant analysis, after which p-values of 0.0017 or lower were considered as statistically significant. All statistical analysis was performed using SAS (version 9.4; SAS Institute, Inc., Cary, North Carolina).

Results

Associations between each variant and LB counts in each of the five different brain regions that were assessed are displayed in Supplementary Tables 2–6. After adjusting for multiple testing, no variant was significantly associated with LB counts in any individual brain region. Nominally significant ($P \leq 0.05$) associations were observed for *BST1* rs11724635, *SNCA* rs3910105, *LRRK2* rs76904798, *BCKDK/STX1B* rs14235, and *MAPT* rs17649553, and these are displayed in Table 3. Specifically, increases in mean LB count corresponding to presence of the minor allele were 1.19 to 1.27-fold for *BST1* (ST, IP, CG), 0.77-fold for *LRRK2* (IP), 1.17 to 1.36-fold for *BCKDK/STX1B* (MF, ST, IP), ~1.25-fold for *MAPT* (MF, IP), and 0.83-fold for *SNCA* (ST) variants. Of note, for these nominally significant findings, the direction of association with LB count (higher or lower) was in agreement with the direction of association in the GWAS meta-analysis (risk or protective) only for the variants in *BCKDK/STX1B* and *SNCA* (Table 3).

We also examined the association between each variant and LBD subtype (brainstem, transitional, or diffuse), and these results are shown in Table 4. Similar to the analysis of LB

counts, no significant associations were identified with LBD subtype that survived correction for multiple testing. However, several nominally significant associations were observed that aligned with the direction of association in the original PD GWAS meta-analysis, and these occurred for *MIR4697* rs329648 (OR: 1.41, P=0.041) and *BCKDK/STX1B* rs14235 (OR: 1.45, P=0.032).

Finally, we combined information across variants in order to create a PD genetic risk score [4] and assessed associations with LB outcomes. There was no statistically significant evidence of an association between PD genetic risk score and LB count in any brain region (all P 0.15) or LBD subtype (all P 0.39) (data not shown).

Discussion

Common genetic variation that is associated with risk of PD has been relatively well defined with the large sample sizes that have been generated by meta-analyses of PD GWAS data. With the establishment of common PD genetic susceptibility factors, it becomes important to understand how these variants impact specific markers of disease severity in PD and other LB disorders.

To address this critical gap in knowledge, in this study we genotyped 28 variants that have previously been shown to be associated with PD risk in PD GWAS meta-analysis (as well as one additional variant associated with pathologically-confirmed PD), and assessed associations with LBD subtype and burden of LB pathology from manual LB counts in a relatively large series of autopsy-confirmed LBD. We did not identify any major associations that withstood correction for multiple testing, however this is perhaps not unexpected for several reasons. First, despite displaying genome-wide significant associations with PD risk, association odds ratios for these variants are in general relatively modest. [4, 18] Second, although the LBD patient population that we examined is clinically heterogeneous, it is more homogenous from a pathological disease severity standpoint compared to studies that also include healthy controls, which naturally limits effect sizes regarding severity of LB pathology.

Although only nominally significant and therefore requiring validation, we did observe several associations of interest that are concordant with the previously observed associations regarding PD risk. The *BCKDK/STX1B* rs14235 variant showed the greatest degree of association with severity of LB pathology, displaying nominally significant associations with LB counts in three different brain regions (IP, MF, ST), one of which (IP, P=0.0026) was the strongest association observed in the study. Furthermore, this *BCKDK/STX1B* variant was also nominally associated with LBD subtype. Interestingly, the minor allele (A) for rs14235 is observed at a much higher frequency (~90%) in Asians than what was observed in our study (42%) that involved only Caucasians. Further study of this *BCKDK/STX1B* variant in relation to severity of LB pathology may be warranted in Caucasian and Asian populations, and additionally *BCKDK/STX1B* rs14235 would be a reasonable candidate to study in relation to risk of DLB and PD with dementia (PDD) where more severe LB pathology is found.

The nominally significant association between *SNCA* rs3910105 and temporal LB count also deserves mention, as common *SNCA* variation has a well-known role in susceptibility to PD [2] and has recently been linked to susceptibility to DLB. [20] This association is biologically plausible given that α -synuclein is the major protein component of LBs. Associations between *SNCA* variants and greater LB pathology have been observed by other groups, specifically in the context of occurrence of diffuse LBD in population-based subjects [5] and presence of LBs in AD [6]. The relatively subtle association between *SNCA* rs3910105 and LB counts in one of the five studied brain regions in our study suggests that although common *SNCA* variation could play a limited role in the degree to which LB pathology progresses after it initially develops, it is likely more involved in the initial appearance of the pathology. Interestingly, *SNCA* variants (and PD risk variants in general) have also not been observed to strongly associate with clinical features such as age-at-onset as may be expected given findings from *SNCA* multiplication families [21–23]. The *MAPT* H1 haplotype, another very well-replicated risk factor for PD, has been inconsistently associated with severity of LB pathology [7–9]. Our findings do not support such an association, as the *MAPT* variant that was examined, where the minor allele corresponds to the H2 haplotype, was nominally associated with LB counts in the opposite direction to the H1-risk association of PD.

Several limitations of this study should be acknowledged. First, although the sample size is relatively large and power to detect associations is improved by the fact that the outcomes studied (LB counts and LBD subtype) are numerical or ordinal as opposed to binary as in case-control disease-risk studies, the possibility of a type II error (i.e. a false-negative finding) is still important to consider given the $P = 0.0017$ significance threshold that was utilized after correction for multiple testing; emphasis should be placed on 95% confidence limits when interpreting the results of our association analysis. Second, given that all of the variants studied have been associated specifically with PD, it may initially seem most intuitive to examine associations between these variants and burden of LB pathology in only those patients with antemortem clinical diagnosis of PD. However, this is challenging in an autopsy series where most cases are studied because of atypical clinical syndromes, notably dementia (PDD and DLB) rather than de novo PD. Our approach of studying pathologically-defined LBD is reasonable since PD is fundamentally a LB disorder and the studied variants are all therefore plausible candidates as disease modifiers in LBD. Finally, although all subjects were non-Hispanic Caucasians living in the United States at the time of death, without available genome-wide population control markers, population stratification could still have had an impact on our results.

In conclusion, our findings do not indicate that currently known PD genetic risk variants have a major impact on severity of LB pathology or LBD subtype in individuals with LBD. These results are in agreement with previous PD disease-association studies, where despite any statistical significance, effect sizes for these common variants (in the form of association odds ratios) have been mostly relatively small [4]. The nominally significant results involving *BCKDK/STX1B*, *SNCA*, and *MIR4697* may deserve further study, both in the context of association with severity of LB pathology and association with other LB disorders such as DLB and PDD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- McCann H, Stevens CH, Cartwright H, Halliday GM. α -Synucleinopathy phenotypes. *Parkinsonism Relat Disord.* 2014; 20(Suppl 1):S62–67. [PubMed: 24262191]
- Soto-Ortolaza AI, Ross OA. Genetic susceptibility variants in parkinsonism. *Parkinsonism Relat Disord.* 2016; 22(Suppl 1):S7–11. [PubMed: 26414118]
- International Parkinson Disease Genomics Consortium. Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, Saad M, Simón-Sánchez J, Schulte C, Lesage S, Sveinbjörnsdóttir S, Stefánsson K, Martinez M, Hardy J, Heutink P, Brice A, Gasser T, Singleton AB, Wood NW. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet.* 2011; 377:641–649. [PubMed: 21292315]
- Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M, Schulte C, Keller MF, Arepalli S, Letson C, Edsall C, Stefansson H, Liu X, Pliner H, Lee JH, Cheng R, International Parkinson's Disease Genomics Consortium (IPDGC); Parkinson's Study Group (PSG) Parkinson's Research: The Organized GENetics Initiative (PROGENI); 23andMe; GenePD; NeuroGenetics Research Consortium (NGRC); Hussman Institute of Human Genomics (HIHG); Ashkenazi Jewish Dataset Investigator; Cohorts for Healthy and Aging Research in Genetic Epidemiology (CHARGE); North American Brain Expression Consortium (NABEC); United Kingdom Brain Expression Consortium (UKBEC); Greek Parkinson's Disease Consortium; Alzheimer Genetic Analysis Group. Ikram MA, Ioannidis JP, Hadjigeorgiou GM, Bis JC, Martinez M, Perlmutter JS, Goate A, Marder K, Fiske B, Sutherland M, Xiromerisiou G, Myers RH, Clark LN, Stefansson K, Hardy JA, Heutink P, Chen H, Wood NW, Houlden H, Payami H, Brice A, Scott WK, Gasser T, Bertram L, Eriksson N, Foroud T, Singleton AB. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* 2014; 46:989–993. [PubMed: 25064009]
- Peuralinna T, Oinas M, Polvikoski T, Paetau A, Sulkava R, Niinistö L, Kalimo H, Hernandez D, Hardy J, Singleton A, Tienari PJ, Myllykangas L. Neurofibrillary tau pathology modulated by genetic variation of alpha-synuclein. *Ann Neurol.* 2008; 64:348–352. [PubMed: 18661559]
- Lutz MW, Saul R, Linnertz C, Glenn OC, Roses AD, Chiba-Falek O. A cytosine-thymine (CT)-rich haplotype in intron 4 of SNCA confer risk for Lewy body pathology in Alzheimer's disease and affects SNCA expression. *Alzheimers Dement.* 2015; 11:1133–1143. [PubMed: 26079410]
- Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, Lashley T, Kallis C, Williams DR, Silva Rde, Lees AJ, Tevesz T. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? *Brain.* 2011; 134:1493–1505. [PubMed: 21596773]
- Wider C, Ross OA, Nishioka K, Heckman MG, Vilariño-Guell C, Jasinska-Myga B, Erketin-Taner N, Rademakers R, Graff-Radford NR, Mash DC, Papapetropoulos S, Duara R, Uchikado H, Wszolek ZK, Farrer MJ, Dickson DW. An evaluation of the impact of MAPT, SNCA, and APOE on

- the burden of Alzheimer's and Lewy body pathology. *J Neurol Neurosurg Psychiatry*. 2013; 83:424–429.
9. Colom-Cadena M, Gelpi E, Martí MJ, Charif S, Dols-Icardo O, Blesa R, Clarimón J, Lleó A. MAPT H1 haplotype is associated with enhanced α -synuclein deposition in dementia with Lewy bodies. *Neurobiol Aging*. 2013; 34:936–942. [PubMed: 22819391]
 10. Clark LN, Katsaklis LA, Gilbert RWolf, Dorado B, Ross BM, Kisselev S, Verbitsky M, Mejia-Santana H, Cote LJ, Andrews H, Vonsattel JP, Fahn S, Mayeux R, Honig LS, Marder K. Association of glucocerebrosidase mutations with dementia with lewy bodies. *Arch Neurol*. 2009; 66:578–583. [PubMed: 19433657]
 11. Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, Li A, Holton J, Guerreiro R, Paudel R, Segarane B, Singleton A, Lees A, Hardy J, Houlden H, Revesz T, Wood NW. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain*. 2009; 132:1783–1794. [PubMed: 19286695]
 12. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, Morris CM, Theuns J, Crosiers D, Cras P, Engelborghs S, Deyn PPDe, Broeckhoven CVan, Mann DM, Snowden J, Pickering-Brown S, Halliwell N, Davidson Y, Gibbons L, Harris J, Sheerin UM, Bras J, Hardy J, Clark L, Marder K, Honig LS, Berg D, Maetzler W, Brockmann K, Gasser T, Novellino F, Quattrone A, Annesi G, Marco EVDe, Rogaeva E, Masellis M, Black SE, Bilbao JM, Foroud T, Ghetti B, Nichols WC, Pankratz N, Halliday G, Lesage S, Klebe S, Durr A, Duyckaerts C, Brice A, Giasson BI, Trojanowski JQ, Hurtig HI, Tayebi N, Landazabal C, Knight MA, Keller M, Singleton AB, Wolfsberg TG, Sidransky E. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA Neurol*. 2013; 70:727–735. [PubMed: 23588557]
 13. Murray ME, Cannon A, Graff-Radford NR, Liesinger AM, Rutherford NJ, Ross OA, Duara R, Carrasquillo MM, Rademakers R, Dickson DW. Differential clinicopathologic and genetic features of late-onset amnesic dementias. *Acta Neuropathol*. 2014; 128:411–421. [PubMed: 24899141]
 14. Terry RD, Hansen LA, DeTeresa R, Davies P, Tobias H, Katzman R. Senile dementia of the Alzheimer type without neocortical neurofibrillary tangles. *J Neuropathol Exp Neurol*. 1987; 46:262–268. [PubMed: 2881985]
 15. Kosaka K, Yoshimura K, Ikeda K, Budka H. Diffuse type of Lewy body disease: progressive dementia with abundant cortical Lewy bodies and senile changes of varying degree—a new disease? *Clin Neuropathol*. 1984; 3:185–192. [PubMed: 6094067]
 16. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. 1991; 82:239–259. [PubMed: 1759558]
 17. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology*. 2002; 58:1791–1800. [PubMed: 12084879]
 18. Beecham GW, Dickson DW, Scott WK, Martin ER, Schellenberg G, Nuytemans K, Larson EB, Buxbaum JD, Trojanowski JQ, Van Deerlin VM, Hurtig HI, Mash DC, Beach TG, Troncoso JC, Pletnikova O, Frosch MP, Ghetti B, Foroud TM, Honig LS, Marder K, Vonsattel JP, Goldman SM, Vinters HV, Ross OA, Wszolek ZK, Wang L, Dykxhoorn DM, Pericak-Vance MA, Montine TJ, Leverenz JB, Dawson TM, Vance JM. PARK10 is a major locus for sporadic neuropathologically confirmed Parkinson disease. *Neurology*. 2015; 84:972–980. [PubMed: 25663231]
 19. Agresti, A. *Categorical Data Analysis*. second. John Wiley & Sons; New York: 2002.
 20. Bras J, Guerreiro R, Darwent L, Parkkinen L, Ansorge O, Escott-Price V, Hernandez DG, Nalls MA, Clark LN, Honig LS, Marder K, Van Der Flier WM, Lemstra A, Scheltens P, Rogaeva E, St George-Hyslop P, Londos E, Zetterberg H, Ortega-Cubero S, Pastor P, Ferman TJ, Graff-Radford NR, Ross OA, Barber I, Braae A, Brown K, Morgan K, Maetzler W, Berg D, Troakes C, Al-Sarraj S, Lashley T, Compta Y, Revesz T, Lees A, Cairns N, Halliday GM, Mann D, Pickering-Brown S, Dickson DW, Singleton A, Hardy J. Genetic analysis implicates APOE, SNCA, and suggests lysosomal dysfunction in the etiology of dementia with Lewy bodies. *Hum Mol Genet*. 2014; 23:6139–6146. [PubMed: 24973356]
 21. Hill-Burns EM, Ross OA, Wissemann WT, Soto-Ortolaza AI, Zarepari S, Siuda J, Lynch T, Wszolek ZK, Silburn PA, Mellick GD, Ritz B, Scherzer CR, Zabetian CP, Factor SA, Breheny PJ, Payami H. Identification of genetic modifiers of age-at-onset for familial Parkinson's disease. *Hum Mol Genet*. 2016; 25:3849–3862. [PubMed: 27402877]

22. Escott-Price V, International Parkinson's Disease Genomics Consortium; Nalls MA, Morris HR, Lubbe S, Brice A, Gasser T, Heutink P, Wood NW, Hardy J, Singleton AB, Williams NM, IPDGC consortium members. Polygenic risk of Parkinson disease is correlated with disease age at onset. *Ann Neurol*. 2015; 77:582–591. [PubMed: 25773351]
23. Ross OA, Braithwaite AT, Skipper LM, Kachergus J, Hulihan MM, Middleton FA, Nishioka K, Fuchs J, Gasser T, Maraganore DM, Adler CH, Larvor L, Chartier-Harlin MC, Nilsson C, Langston JW, Gwinn K, Hattori N, Farrer MJ. Genomic investigation of alpha-synuclein multiplication and parkinsonism. *Ann Neurol*. 2008; 63:743–750. [PubMed: 18571778]

Highlights

- We studied 547 autopsy-confirmed cases of Lewy body disease (LBD).
- 29 different GWAS-nominated Parkinson's disease (PD) risk variants were genotyped.
- Outcome measures were LBD subtype and LB counts in five cortical regions
- No significant associations between the PD risk variants and outcomes were identified.
- Currently known PD genetic risk variants do not appear to have a major impact on severity of LB pathology in patients with LBD.

Table 1

Neuropathological characteristics

Variable	Summary (N=547)
LBD subtype	
Brainstem	61 (11.2%)
Transitional	198 (36.2%)
Diffuse	288 (52.7%)
LB counts	
Middle frontal gyrus	4.4 (0, 1, 6, 35)
Superior temporal gyrus	9.8 (0, 3, 15, 50)
Inferior parietal gyrus	3.4 (0, 1, 5, 30)
Cingulate gyrus	9.9 (0, 4, 15, 35)
Parahippocampal gyrus	14.8 (0, 7, 22, 45)
Braak NFT stage	
0	11 (2.0%)
I	18 (3.3%)
II	61 (11.2%)
III	130 (23.8%)
IV	102 (18.6%)
V	99 (18.1%)
VI	126 (23.0%)
Thal amyloid phase	
0	61 (11.2%)
1	51 (9.3%)
2	27 (5.0%)
3	112 (20.5%)
4	47 (8.6%)
5	248 (45.4%)

LB=Lewy body; LBD=Lewy body disease; NFT=neurofibrillary tangle. The sample mean (minimum, first quartile (i.e. 25th percentile), third quartile (i.e. 75th percentile), maximum) is given for continuous variables. Information was unavailable regarding middle frontal LB count (N=1), superior temporal LB count (N=2), inferior parietal LB count (N=2), cingulate gyrus LB count (N=8), parahippocampal LB count (N=61), and Thal amyloid phase (N=1).

Table 2

Summary of genetic variants examined

Variant	Nearby gene	Chr.	Position	Type of variant	MA	MAF from the current study	MAF in a normal Caucasian population ^c	OR (p-value) from Nalls et al. study [4] ^d
rs10788972 ^a	<i>TCEANC2</i>	1	54572243	Intronic	A	47.5%	44.0%	0.64 (6.2×10^{-8})
rs35749011	<i>GBA/SYT11</i>	1	155135036	Intergenic	A	2.7%	2.2%	1.82 (1.4×10^{-29})
rs114138760 ^b	<i>GBA/SYT11</i>	1	154925709	Intronic	C	1.8%	1.2%	1.57 (3.8×10^{-7})
rs823118	<i>RAB7L1/NUCKS1</i>	1	205723572	Intergenic	C	44.8%	46.5%	0.89 (1.7×10^{-16})
rs10797576	<i>SIPA1L2</i>	1	232664611	Intronic	T	13.9%	13.8%	1.13 (4.9×10^{-10})
rs6430538	<i>ACMSD/TMEM163</i>	2	135539967	Intergenic	T	43.4%	49.7%	0.88 (9.1×10^{-20})
rs1955337	<i>STK39</i>	2	168272635	Intergenic	A	12.5%	11.8%	1.21 (1.2×10^{-20})
rs12637471	<i>MCCC1</i>	3	182762437	Intronic	A	20.3%	19.8%	0.84 (2.1×10^{-21})
rs34311866	<i>TMEM175/GAK/DGKQ</i>	4	951947	Missense	C	24.4%	18.7%	1.27 (1.0×10^{-43})
rs34884217 ^b	<i>TMEM175/GAK/DGKQ</i>	4	950422	Missense	G	8.4%	9.6%	0.80 (1.1×10^{-6})
rs11724635	<i>BST1</i>	4	15737101	Intronic	C	43.3%	44.0%	0.89 (9.4×10^{-18})
rs6812193	<i>FAM47E/SCARB2</i>	4	77198986	Intronic	T	37.6%	39.0%	0.91 (3.0×10^{-11})
rs356182	<i>SNCA</i>	4	90626111	Intergenic	G	35.4%	36.2%	1.32 (4.2×10^{-73})
rs3910105 ^b	<i>SNCA</i>	4	89761420	Intronic	C	49.1%	44.3%	0.84 (7.1×10^{-19})
rs9275326	<i>HLA-DQB1</i>	6	32666660	Intergenic	T	8.5%	10.4%	0.83 (1.2×10^{-12})
rs13201101 ^b	<i>HLA-DQB1</i>	6	32375827	Intergenic	T	5.2%	6.9%	1.19 (3.8×10^{-6})
rs199347	<i>GPNMB</i>	7	23293746	Intronic	C	38.5%	38.3%	0.90 (1.2×10^{-12})
rs591323	<i>FGF20</i>	8	16697091	Intergenic	T	25.6%	28.9%	0.92 (6.7×10^{-8})
rs117896735	<i>INPP5F</i>	10	119776815	Intronic	A	1.2%	1.3%	1.62 (4.4×10^{-13})
rs329648	<i>MIR4697</i>	11	133765367	Intergenic	T	35.5%	33.3%	1.11 (9.8×10^{-12})
rs76904798	<i>LRRK2</i>	12	40614434	Intergenic	T	13.5%	12.7%	1.16 (5.2×10^{-14})
rs11060180	<i>CCDC62</i>	12	123303586	Intronic	G	48.0%	43.5%	0.90 (6.0×10^{-12})
rs11158026	<i>GCHI</i>	14	55348869	Intronic	T	32.7%	31.1%	0.90 (5.9×10^{-11})
rs2414739	<i>VPS13C</i>	15	61994134	Intergenic	G	28.3%	27.9%	0.90 (1.3×10^{-11})
rs14235	<i>BCKDK/STX1B</i>	16	31121793	Synonymous	A	41.5%	38.2%	1.10 (2.4×10^{-12})
rs11868035	<i>SREBF1/RAI1</i>	17	17715101	Intergenic	A	31.6%	33.8%	0.94 (6.0×10^{-5})

Variant	Nearby gene	Chr.	Position	Type of variant	MA	MAF from the current study	MAF in a normal Caucasian population ^c	OR (p-value) from Nalls et al. study [4] ^d
rs17649553	<i>MAPT</i>	17	43994648	Intronic	A	22.8%	24.1%	0.77 (2.4×10^{-48})
rs12456492	<i>RTT2</i>	18	40673380	Intronic	G	30.9%	33.8%	1.11 (7.7×10^{-12})
rs55785911	<i>DDRGKI</i>	20	3168166	Intergenic	A	37.2%	38.7%	1.11 (3.0×10^{-11})

MA=minor allele; MAF=minor allele frequency; OR=odds ratio.

^a rs10788972 was included not due to a significant association in the Nalls et al. GWAS meta-analysis [4], but rather due to an association observed by the GWAS performed by Beecham et al. [18] which utilized neuropathologically confirmed PD patients and controls.

^b Indicates a variant included due to a significant association with risk of Parkinson's disease risk that was independent of the other variant in the given gene that had the strongest association signal.

^c MAF in a normal Caucasian population was obtained using 1000 Genomes Project Phase 3 data.

^d To be consistent with the results of our study, the OR from the Nalls et al. study that we provide is that associated with the minor allele of the given variant in our study.

Table 3

Summary of all nominally significant associations with Lewy body counts

Variant	Nearest gene	Chr.	Position	MA	MAF	Cortical region	Minor allele not present		Minor allele present		Association with LB count in the given brain region	
							Mean (Q1, Q3) LB count	N	Mean (Q1, Q3) LB count	N	Multiplicative increase (95% CI)	P-value
rs11724635	<i>BST1</i>	4	15737101	C	43.3%	Superior temporal	8.5 (3, 12)	164	10.4 (3, 16)	360	1.19 (1.01, 1.39)	0.033
rs11724635	<i>BST1</i>	4	15737101	C	43.3%	Inferior parietal	2.7 (0, 4)	165	3.7 (1, 5)	359	1.27 (1.03, 1.57)	0.028
rs11724635	<i>BST1</i>	4	15737101	C	43.3%	Cingulate	8.5 (4, 12)	162	10.5 (4, 16)	358	1.20 (1.04, 1.38)	0.010
rs3910105	<i>SNCA</i>	4	89761420	C	49.1%	Superior temporal	11.1 (4, 16)	136	9.3 (3, 14)	403	0.83 (0.71, 0.97)	0.023
rs76904798	<i>LRRK2</i>	12	40614434	T	13.5%	Inferior parietal	3.6 (1, 5)	406	2.7 (0, 4)	138	0.77 (0.62, 0.96)	0.021
rs14235	<i>BCKDK/STX1B</i>	16	31121793	A	41.5%	Middle frontal	3.7 (1, 5)	191	4.8 (1, 7)	355	1.28 (1.06, 1.54)	0.011
rs14235	<i>BCKDK/STX1B</i>	16	31121793	A	41.5%	Superior temporal	8.8 (2, 14)	190	10.3 (4, 15)	355	1.17 (1.01, 1.35)	0.041
rs14235	<i>BCKDK/STX1B</i>	16	31121793	A	41.5%	Inferior parietal	2.7 (0, 4)	191	3.8 (1, 5)	354	1.36 (1.11, 1.65)	0.0026
rs17649553	<i>MAPT</i>	17	43994648	A	22.8%	Middle frontal	4.0 (1, 6)	331	5.0 (1, 7)	213	1.25 (1.04, 1.49)	0.016
rs17649553	<i>MAPT</i>	17	43994648	A	22.8%	Inferior parietal	3.1 (1, 4)	330	3.8 (1, 6)	213	1.24 (1.02, 1.50)	0.029

Chr=chromosome; MA=minor allele; MAF=minor allele frequency; Q1=first quartile (i.e. 25th percentile); Q3=third quartile (i.e. 75th percentile); CI=confidence interval; LB=Lewy body. Multiplicative increases, 95% CIs, and p-values result from negative binomial regression models adjusted for age at death and sex. Multiplicative increases are interpreted as the multiplicative increase in mean Lewy body count in the given brain region corresponding to presence of the minor allele of the given variant. After applying a Bonferroni correction for multiple testing, p-values of 0.0017 or lower are considered as statistically significant. Nominally significant associations were those with a p-value of 0.05 or lower.

Table 4

Association between each variant and LBD subtype (brainstem, transitional, or diffuse)

Variant	Nearest gene	Chr.	Position	MA	MAF	Fraction (%) of patients with diffuse LBD		Association with LBD subtype	
						Minor allele not present	Minor allele present	OR (95% CI)	P-value
rs10788972	<i>TCEANC2</i>	1	54572243	A	47.5%	86/159 (54.1%)	201/387 (51.9%)	0.97 (0.68, 1.38)	0.85
rs35749011	<i>GBA/SYTI1</i>	1	155135036	A	2.7%	268/517 (51.8%)	20/30 (66.7%)	1.75 (0.82, 3.75)	0.15
rs114138760	<i>GBA/SYTI1</i>	1	154925709	C	1.8%	275/524 (52.5%)	11/20 (55.0%)	1.11 (0.46, 2.65)	0.82
rs823118	<i>RAB7L/NUCKS1</i>	1	205723572	C	44.8%	88/170 (51.8%)	200/377 (53.1%)	1.09 (0.77, 1.55)	0.62
rs10797576	<i>SIPA1L2</i>	1	232664611	T	13.9%	215/402 (53.5%)	73/145 (50.3%)	0.87 (0.60, 1.25)	0.44
rs64303538	<i>ACMSD/TMEM163</i>	2	135539967	T	43.4%	103/186 (55.4%)	185/361 (51.2%)	0.93 (0.66, 1.31)	0.69
rs1955337	<i>STK39</i>	2	168272635	A	12.5%	216/417 (51.8%)	72/130 (55.4%)	1.14 (0.78, 1.67)	0.50
rs12637471	<i>MCCC1</i>	3	182762437	A	20.3%	187/347 (53.9%)	101/200 (50.5%)	0.93 (0.67, 1.31)	0.68
rs34311866	<i>TMEM175/GAK/DGKQ</i>	4	951947	C	24.4%	168/319 (52.7%)	119/224 (53.1%)	1.07 (0.77, 1.50)	0.67
rs34884217	<i>TMEM175/GAK/DGKQ</i>	4	950422	G	8.4%	238/459 (51.9%)	50/88 (56.8%)	1.17 (0.75, 1.82)	0.50
rs11724635	<i>BST1</i>	4	15737101	C	43.3%	78/165 (47.3%)	196/361 (54.3%)	1.20 (0.84, 1.71)	0.32
rs6812193	<i>FAM47E/SCARB2</i>	4	77198986	T	37.6%	116/214 (54.2%)	172/333 (51.7%)	0.91 (0.65, 1.27)	0.56
rs356182	<i>SNCA</i>	4	90626111	G	35.4%	117/223 (52.5%)	169/321 (52.6%)	0.95 (0.68, 1.33)	0.77
rs3910105	<i>SNCA</i>	4	89761420	C	49.1%	79/138 (57.2%)	205/403 (50.9%)	0.79 (0.54, 1.16)	0.23
rs9275326	<i>HLA-DQB1</i>	6	32666660	T	8.5%	240/456 (52.6%)	47/90 (52.2%)	0.95 (0.61, 1.47)	0.81
rs13201101	<i>HLA-DQB1</i>	6	32375827	T	5.2%	261/496 (52.6%)	26/49 (53.1%)	1.04 (0.59, 1.84)	0.90
rs199347	<i>GPNMB</i>	7	23293746	C	38.5%	113/206 (54.9%)	174/339 (51.3%)	0.92 (0.66, 1.29)	0.63
rs591323	<i>FGF20</i>	8	16697091	T	25.6%	162/309 (52.4%)	125/237 (52.7%)	1.04 (0.75, 1.44)	0.81
rs117896735	<i>INPP5F</i>	10	119776815	A	1.2%	268/508 (52.8%)	5/12 (41.7%)	0.61 (0.21, 1.79)	0.37
rs329648	<i>MIR4697</i>	11	133765367	T	35.5%	109/226 (48.2%)	179/321 (55.8%)	1.41 (1.01, 1.96)	0.041
rs76904798	<i>LRRK2</i>	12	40614434	T	13.5%	224/407 (55.0%)	63/139 (45.3%)	0.67 (0.46, 0.97)	0.034
rs11060180	<i>CCDC62</i>	12	123303586	G	48.0%	80/149 (53.7%)	208/398 (52.3%)	0.97 (0.67, 1.40)	0.86
rs11158026	<i>GCHI</i>	14	55348869	T	32.7%	124/241 (51.5%)	164/306 (53.6%)	1.09 (0.79, 1.51)	0.61
rs2414739	<i>VPS13C</i>	15	61994134	G	28.3%	141/270 (52.2%)	147/277 (53.1%)	0.94 (0.68, 1.30)	0.69
rs14235	<i>BCKDK/STX1B</i>	16	31121793	A	41.5%	90/191 (47.1%)	198/356 (55.6%)	1.45 (1.03, 2.03)	0.032
rs11868035	<i>SREBF/RAI1</i>	17	17715101	A	31.6%	136/258 (52.7%)	152/289 (52.6%)	1.00 (0.72, 1.38)	0.98
rs17649553	<i>MAPT</i>	17	43994648	A	22.8%	164/331 (49.5%)	123/214 (57.5%)	1.39 (0.99, 1.95)	0.054

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Variant	Nearest gene	Chr.	Position	MA	MAF	Fraction (%) of patients with diffuse LBD		Association with LBD subtype	
						Minor allele not present	Minor allele present	OR (95% CI)	P-value
rs12456492	<i>RIT2</i>	18	40673380	G	30.9%	131/257 (51.0%)	156/289 (54.0%)	1.14 (0.83, 1.59)	0.42
rs55785911	<i>DDRGK1</i>	20	3168166	A	37.2%	116/215 (54.0%)	169/328 (51.5%)	0.87 (0.62, 1.22)	0.42

Chr=chromosome; MA=minor allele; MAF=minor allele frequency; OR: odds ratio; CI=confidence interval. LB=Lewy body. ORs, 95% CIs, and p-values result from proportional odds logistic models adjusted for age at death and sex. ORs are interpreted as the multiplicative increase in the odds of a more widespread distribution of LB pathology (i.e. diffuse more than brainstem) corresponding to presence of the minor allele. The proportion of patients with diffuse LBD is given for illustrative purposes, however LBD subtype (brainstem, transitional, diffuse) was analyzed as an ordered categorical variable in proportional odds logistic regression analysis. After applying a Bonferroni correction for multiple testing, p-values of 0.0017 or lower are considered as statistically significant. Variants shown in bold are those with a nominally significant (P 0.05) association.