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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.

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.

1. 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

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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.

2. Materials and methods

2.1. 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.

2.2. 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 μm 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.

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).

2.3. 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.

2.4. 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.

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