BRIEF REPORT

Rare Variants in Specific Lysosomal Genes Are Associated with Parkinson's Disease

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ABSTRACT: Objective: Impaired lysosomal degradation of α-synuclein and other cellular constituents may play an important role in Parkinson's disease (PD). Rare genetic variants in the glucocerebrosidase (*GBA*) gene were consistently associated with PD. Here we examine the association between rare variants in lysosomal candidate genes and PD.
Methods: We investigated the association between PD and rare genetic variants in 23 lysosomal candidate genes in 4096 patients with PD and an equal number of controls using pooled targeted next-generation DNA sequencing. Genewise association of rare variants in cases or controls was analyzed using the optimized sequence kernel association test with Bonferroni correction for the 23 tested genes.
Results: We confirm the association of rare variants in *GBA* with PD and report novel associations for rare variants in *ATP13A2*, *LAMP1*, *TMEM175*, and *VPS13C*.
Conclusion: Rare variants in selected lysosomal genes, first and foremost *GBA*, are associated with PD. Rare variants in *ATP13A2* and *VPC13C* previously linked to monogenic PD and more common variants in *TMEM175* and *VPS13C* previously linked to sporadic PD in genome-wide association studies are associated with PD. © 2020 International Parkinson and Movement Disorder Society

Key Words: *ATP13A2*; chaperone-mediated-autophagy; *GBA*; *LAMP1*; Lysosme; Parkinson's disease; *TMEM175*; *VPS13C*; α-synuclein

Parkinson's disease (PD) is a neurodegenerative disease characterized by the preferential degeneration of dopaminergic neurons in the substantia nigra. Pathological

hallmarks of PD are proteinaceous intraneuronal inclusions containing aggregated α-synuclein (ASYN), so-called Lewy bodies. Most PD cases are genetically complex, and genome-wide association studies have identified numerous common DNA variants associated with PD. Altogether, these variants are estimated to explain approximately 23% of the heritability of PD.[1] Lysosomal protein degradation and autophagy, including mitophagy, are strongly implicated in PD pathogenesis.[2] Whereas monomeric ASYN is degraded in the lysosome by chaperone-mediated autophagy,[3] aggregated ASYN inhibits chaperone-mediated autophagy and is degraded by macroautophagy, a process also involving the lysosome.[3] Heterozygosity for missense variants in the glucocerebrosidase (*GBA*) gene is a known risk factor for PD.[4] In fact, the loss of *GBA* activity leads to alterations of ASYN metabolism. Moreover, glucosylceramide, the substrate for *GBA*, has proaggregatory properties, and ASYN inhibits *GBA* activity. In addition, an association of rare variants in sphingomyelin phosphodiesterase 1 with PD has been reported.[5] These findings add to a plethora of other studies also suggesting a major role of lysosomal disruption in PD pathogenesis. Hence, there is good reason to hypothesize that rare variants in genes related to lysosomal function represent PD risk factors as well. In the present study, we sequenced the coding regions of 23 lysosomal candidate genes in 4096 sporadic PD patients and 4096 controls to assess the level of association between rare variants in the coding regions of these genes and common, genetically complex PD.

Methods

Sample Collection

We collected preexisting DNA and clinical data from 4476 sporadic PD patients and 5140 healthy controls of German origin (supplemental online material, Supplementary Table 1).[6-10] All participants had given written informed consent.

Details on the selection of the gene panel, quality control of DNA samples, pooling, DNA sequencing, variant mapping, variant calling, and variant annotation can be found in the supplemental online material.

Statistical Analysis

We analyzed only variants in the coding regions with a minor allele frequency < 0.01 in cases or in controls and in the subgroup thereof with a Phil's Read Editor (PHRED)-scaled combined annotation dependent depletion (CADD) score > 12.37 representing the genomewide 2% variants with the highest CADD scores. We used a combination of a variance-component test and a burden test implemented in the optimized sequence kernel association test (SKAT-O) provided in the R package SKAT v1.0.9 (R Foundation for Statistical Computing, Vienna, Austria).[11,12] The SKAT-O test requires individual-level genotypes and does not work with pooled data. To overcome this problem, we simulated genotypes for the individuals based on the observed minor allele counts in each pool. To take into account the linkage disequilibrium (LD) structure within each gene, we extracted pairwise r^2 for known variants from the Ldlink database (https://ldlink.nci.nih.gov/; 1000 Genomes project phase 3, version 5 reference population "European," excluding Finnish). The LD between 2 private variants each present only once in the whole dataset was set to 0. For the remaining pairs of single nucleotide variant (SNV) with unknown LD we used an r^2 of 0.8, assuming strong LD. Assuming high LD is conservative, because if the rare alleles of 2 SNV in high LD occur in 1 pool, these will be usually assigned to the same individual. Particularly under the alternative hypothesis (higher frequency in cases than controls) this may only underestimate the allele frequency difference between cases and controls and thus provide a conservative test. Haplotypes of each pool were simulated using a multivariate binomial distribution as implemented in the R package mipfp v3.2.1[13] with the observed minor allele frequencies of the variants for that particular pool and the square root of the r^2 matrix (= correlation matrix) as parameters. Genotypes of the individuals were defined based on 2 consecutive haplotypes. We generated 100 data sets per gene and applied the SKAT-O test to each set. Here, we calculate the mean *P* value per gene assuming a conservative r^2 of 0.8 between variant pairs in unknown LD obtained in the 100 simulations, both uncorrected and Bonferroni corrected for the 23 genes tested. In cases of genes with at least 1 prior study reporting a genewise multiple-testing corrected rare variant association with PD (Supplementary Table 2), we report the uncorrected *P* value as replication of the known association.

Results

The 4096 PD patients had a mean age of 67 years at the time of sampling, and a mean age at onset of 60 years; the controls were somewhat younger (mean age 61 years). A total of 1256 variants with a frequency in cases or controls below 1% and located within the protein-coding regions of 23 genes were included in the analysis. These variants were assigned to 1 of the following functional classes: nonsynonymous (n = 898), stopgain or stoploss (n = 65), and synonymous (n = 275). We performed the genewise SKAT-O tests in each of the 23 genes (Supplementary Table 2) for all variants <1% and for the subgroup with a Phred-scaled CADD score >12.37. Bonferroni-corrected statistically significant associations were detected for the genes *ATP13A2*, *GBA*, *LAMP1*, *TMEM175*, and *VPS13C* (Table 1). For

TABLE 1. Genes with a Bonferroni-Corrected Significant Optimized Sequence Kernel Association Test

Gene[1]	Group[2]	*P* Value[3]	*P* Value Bonferroni[4]
ATP13A2	<1%	0.000359	0.008257
	CADD	0.001015	0.023347
GBA	<1%	0.000265	0.006103
	CADD	0.000002	0.000048
LAMP1	<1%	0.013903	0.319775
	CADD	0.000561	0.012911
TMEM175	<1%	0.003425	0.078765
	CADD	0.000482	0.011083
VPS13C	<1%	0.000100	0.002296
	CADD	0.037822	0.869898

[1]Gene: The Human Genome Organisation gene symbol.
[2]Group: <1% all variants with a minor allele frequency < 1% in cases or controls. CADD: subgroup with a Phred-scaled CADD score > 12.37.
[3]*P* value: unadjusted *P* value of the optimized sequence kernel association test.
[4]*P* value Bonferroni: *P* value after Bonferroni correction for 23 genes tested.
CADD, combined annotation dependent depletion.

VPS13C, the subgroup of variants with a Phred-scaled CADD of >12.37 failed to reach significance, whereas for *LAMP1* and *TMEM175*, only these subgroups containing variants predicted to be among the genomewide most damaging 2% of variants were associated with PD. We failed to replicate the association of rare variants in *SMPD1* and PD (unadjusted *P* values 0.07708 for all variants with a minor allele frequency <1% and 0.05834 for the subgroup with a Phred-scaled CADD score > 12.37). A recent study reported an association between rare variants in *CTSD1*, also analyzed in this study and PD.[14] However, the reported association was not corrected for multiple testing. Hence, we did not consider the analysis of *CTSD1* as a replication. The uncorrected *P* values for cathepsin-D in our study were 0.00791 (corrected: 0.18188) for all variants with a minor allele frequency <1% and 0.02881 (corrected: 0.66269) for the subgroup with a Phred-scaled CADD score > 12.37.

Discussion

We examined the association between sporadic PD with an age of onset older than 45 years and rare variants with a minor allele frequency < 0.01 in 23 genes with a function in lysosomal degradation (Supplementary Table 2) and identified a Bonferroni-corrected significant association of rare variants with PD for first and foremost *GBA* and the genes *ATP13A2*, *LAMP1*, *TMEM175*, and *VPS13C* (Table 1). We failed to replicate the previously reported association of rare variants in *SMPD1* with PD.[5]

We chose the individual gene as the unit of analysis and did not perform any tests across all genes together because we believe that single genes are better functional units in the pathogenesis of PD than gene sets including genes with diverse functions. The association between rare variants in *GBA* and PD is well known.[15] Of 5 genes, 4 identified in this study are well-established genes in the pathogenesis of monogenic PD (*ATP13A2*,[16] *VPS13C*[17]) and/or SNV in these genes are associated with genetically complex PD (*GBA*, *TMEM175*, *VPS13C*).[18] In contrast, only 2 of the 16 remaining genes (*SMPD1*, *SCARB2*) that did not meet our Bonferroni-corrected significance threshold have been robustly associated with PD in previous studies. Common variants in *SCARB2* were found to be associated with PD in genome-wide association studies studies; no association of rare variants in *SCARB2* and PD have been reported.[1] A functional link to ASYN aggregation—the main pathological and pathphysiological hallmark of sporadic PD—has been reliably shown for *ATP13A2*, *GBA*, *TMEM175*, and *VPS13C*.[17,19-21] Autopsies of patients with *GBA* risk variants or monogenic PD caused by *VPS13C* mutations show ASYN pathology, and no autopsy data are available for *ATP13A2*. *LAMP1* has so far not genetically been linked to PD. *LAMP1* immunoreactivity has been reported to be significantly decreased within PD nigral neurons when compared with age-matched human controls with a significantly greater decrease in nigral neurons that contain ASYN inclusions.[22] *LAMP1*-deficient mice did not show brain phenotype except for a slight astrogliosis and reduced cathepsin-D staining.[23] Interestingly cathepsins are key-proteases in ASYN degradation.[24]

This is the second and so far largest study of the association between rare variants in lysosomal genes and PD. In contrast to our study, the first study by Robak and colleagues[14] focused solely on genes previously linked to lysosomal storage disease and reported all single-gene associations without correction for multiple testing. Only *GBA* would remain significant in a single-gene analysis after correction for the 54 genes in the study.[14] The main strengths of our study are the large and ethnically homogeneous samples, comprising 4096 patients and 4096 controls from Germany. The age at onset and the patient age distributions were typical for common sporadic PD. The main limitations of our study were threefold: first, the lack of a replication sample; second, the use of pooled sequencing that did not allow quality control by Sanger sequencing because variants could not be traced to individual patients; and third, the lack of methods to detect genomic rearrangements. We are aware that pooled sequencing that was dictated by the available budget has its limitations (inter alia: inability to assess the number of variants carried at the individual level), and therefore our main concern was to control the type I error. To exclude false-positive variant calls as much as possible, we used a variant-calling algorithm (CRISP – Comprehensive Read analysis for Identification of Single Nucleotide Polymorphisms (SNPs)

from Pooled sequencing) that was developed specifically to detect rare alleles in heterogeneous tumor samples and used stringent analytical thresholds and Bonferroni correction. It is likely that we missed an unkown number of rare variants. Following the common assumption that rare variants are more likely to be pathogenic, this will lead to an increase of type II error but help to control the type I error.

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Supporting Data

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(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the first draft, B. Review and Critique.

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D.B.: 3B
M.K.: 1A, 2A, 2C,3A, 3B
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