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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Comprehensive Analysis of Familial Parkinsonism Genes in Rapid-Eye-Movement Sleep Behavior Disorder

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ABSTRACT: Background: There is only partial overlap in the genetic background of isolated rapid-eye-movement sleep behavior disorder (iRBD) and Parkinson's disease (PD).
Objective: To examine the role of autosomal dominant and recessive PD or atypical parkinsonism genes in the risk of iRBD.
Methods: Ten genes, comprising the recessive genes *PRKN*, *DJ-1 (PARK7)*, *PINK1*, *VPS13C*, *ATP13A2*, *FBXO7*, and *PLA2G6* and the dominant genes *LRRK2*, *GCH1*, and *VPS35*, were fully sequenced in 1039 iRBD patients and 1852 controls of European ancestry, followed by association tests.
Results: We found no association between rare heterozygous variants in the tested genes and risk of iRBD. Several homozygous and compound heterozygous carriers were identified, yet there was no overrepresentation in iRBD patients versus controls.
Conclusion: Our results do not support a major role for variants in these genes in the risk of iRBD. © 2020 International Parkinson and Movement Disorder Society

Key Words: REM sleep behavior disorder; genetic analysis; Parkinson's disease

Isolated rapid-eye-movement sleep behavior disorder (iRBD) is a prodromal neurodegenerative disease. More than 80% of iRBD patients will eventually convert to an overt α-synucleinopathy,[1] either Parkinson's disease (PD), dementia with Lewy bodies (DLB), or multiple system atrophy.[2]

Currently, 90 independent risk factors of PD are known through genome-wide association studies (GWAS).[3] Other, rarer genetic variants have been implicated in familial forms of PD, including autosomal dominant (AD) inherited variants in genes such as *SNCA*, *LRRK2*, *GCH1*, and *VPS35*[4,5] and autosomal recessive (AR) inherited variants in *PRKN*, *PINK1*, and *PARK7*.[6] Biallelic mutations in other genes, including *ATP13A2*, *VPS13C*, *FBXO7*, and *PLA2G6*, may cause AR atypical syndromes with parkinsonism,[4,7] in some of which α-synucleinopathy has also been reported.[8-10]

The genetic background of iRBD has been only recently studied, with studies showing that there is no full genetic overlap between the genetic background of iRBD and that of PD or DLB. *GBA* mutations are associated with risk of iRBD, PD, and DLB,[11-15] but pathogenic *LRRK2* mutations seem to be involved only in PD and not in iRBD and DLB.[7,16,17] *MAPT* and *APOE* variants are important risk factors of PD and DLB, respectively,[18,19] but both genes are not associated with iRBD.[20,21] In the *SNCA* locus, there are independent risk variants of PD, DLB, and iRBD.[22] Within the *TMEM175* locus, there are two independent risk factors of PD, but only one of them, the coding polymorphism p.M393T, has been associated with iRBD.[23]

Here, because *GBA* and *SNCA* have been studied previously,[12,22] we aimed at thoroughly examining the roles of *PRKN*, *PINK1*, *PARK7* (*DJ-1*), *VPS13C*, *ATP13A2*, *FBXO7*, *PLA2G6*, *LRRK2*, *GCH1*, and *VPS35* in iRBD.

Methods

Population

This study comprised 1039 unrelated iRBD patients and 1852 unrelated controls, all of European ancestry (confirmed by principal component analysis of GWAS data). Additional information about the study population can be found in the Supplementary Data. All patients signed an informed consent form before participating in the study, and the study protocol was approved by the institutional review boards.

Genetic Analysis

Complete details on the genetic analysis and quality control can be found in the Supplementary Data. The coding sequences and 5′ and 3′ untranslated regions of *PRKN*, *PINK1*, *DJ-1*, *VPS13C*, *ATP13A2*, *FBXO7*, *PLA2G6*, *LRRK2*, *GCH1*, and *VPS35* were captured using molecular inversion probes designed as previously described,[24] and the full protocol is available at https://github.com/gan-orlab/MIP_protocol.

Data and Statistical Analysis

Complete details on data and statistical analysis can be found in the Supplementary Data. We used different approaches to examine the effect of multiple variants on iRBD risk. To examine whether there is a burden of rare (MAF < 0.01) heterozygous variants in each of our targeted genes, we used optimized sequence Kernel association test (SKAT-O, R package)[25] and burden tests for different types of variants: all rare variants, potentially functional rare variants (nonsynonymous, frame-shift, stop-gain, and splicing), rare loss-of-function variants (frame-shift, stop-gain, and splicing), and rare nonsynonymous variants only. We then examined the association between variants predicted to be pathogenic based on the combined annotation-dependent depletion (CADD) score of ≥12.37 (representing the top 2% of potentially deleterious variants) and iRBD. Because copy number variants (CNVs) are frequent in the *PRKN* gene,[26] we included CNVs when we analyzed the association of *PRKN* variants with iRBD, identified as recently described.[27]

Availability of Data and Materials

Data used for the analysis are available in the supplementary tables. Anonymized raw data can be shared on request from any qualified investigator.

Results

Quality of Coverage

The average coverage of the 10 genes analyzed was >144X for all genes, and the coverage of 8 of the genes was >900X. The per-gene coverage for all 10 genes, although not perfect, is better than the coverage of these specific genes in gnomAD. Supplementary Table S2 presents the average coverage and the percentage of nucleotides covered at 20X and 50X for each gene.

Rare Homozygous and Compound Heterozygous Variants Are Not Enriched in iRBD Patients

To examine whether homozygous or compound heterozygous variants in our genes of interest may cause iRBD, we compared the carrier frequencies of very rare (MAF < 0.001) biallelic variants between iRBD patients and controls. Only three carriers (one patient and two controls) were identified with homozygous variants across all genes. All three carried homozygous noncoding variants that are not likely to cause a disease.

For the analysis of compound heterozygous carriers, because phasing could not be performed, we considered carriers of two rare variants as compound heterozygous carriers, with two exceptions: (1) when variants were physically close, we could determine their phase based on the sequence reads, and (2) if the same combination of very rare variants appeared more than once, we assumed that the variants are likely on the same allele. We found 9 patients and controls, presumably compound heterozygous carriers, in the studied genes (Table 1). Three affected and three unaffected carriers of compound heterozygous variants in *VPS13C* were identified, with no overrepresentation in iRBD patients (Fisher's test, $P = 1$).

TABLE 1. Summary of all samples carrying two nonsynonymous variants detected in the present study

Gene	Sample	Sex	AAS	dbSNP	Allele*	Substitution	F_A	F_C	gnomAD ALL	gnomAD NFE
PRKN	C	M	46	rs137853054	G/A	p.T212M	0	0.0005504	0.0004	0.0003
				rs9456735	T/G	p.M192L	0	0.001101	0.0043	0.0003
PINK1	C	M	57	rs370906995	C/T	p.T257I	0	0.0002756	7.02E-05	0.0001
				rs372280083	C/G	p.L268V	0	0.0002756	9.34E-05	0.0001
VPS13C	A	M	75	15:62165489	C/A	p.D3469Y	0.0005092	0	–	–
				15:62204039	C/A	p.E2862D	0.0005139	0	–	–
VPS13C	C	F	60	rs746819519	C/T	p.G3172D	0	0.001096	1.76E-05	0.00003753
				rs202056315	A/C	p.V2235G	0	0.0002744	4.06E-05	0.00001793
VPS13C	C	M	30	rs780081183	C/G	p.A2368P	0	0.0002738	1.24E-05	0.00002724
				15:62302740	C/G	p.E271D	0	0.0002738	–	–
VPS13C	C	M	52	rs767080349	A/G	p.M2344T	0	0.0002738	1.87E-05	0.0000187
				rs370832130	T/C	p.M1416V	0	0.0002738	0.0001	0.0001
VPS13C	A	M	64	rs760460320	C/G	p.D1496H	0.0005081	0	1.75E-05	0.00002803
				rs765303583	G/C	p.Q660E	0.0005081	0	0	0
VPS13C	A	M	59	rs141515062	A/T	p.S522T	0.001016	0	0.0002	0.0004
				rs376219715	T/C	p.Y365C	0.001016	0	1.63E-05	0.00003598
LRRK2	C	M	63	rs886344692	A/T	p.R1282S	0	0.000275	1.63E-05	2.69E-05
				rs202179802	A/G	p.T2310A	0	0.000275	4.47E-05	7.17E-05

*Allele, reference allele/mutant allele.
A, affected; C, control; M, male; F, female; AAS, age at sampling; dbSNP, single nucleotide polymorphism database; F_A, frequency in affected patients; F_C, frequency in controls; gnomAD ALL, exome allele frequency in all populations; gnomAD NFE, exome allele frequency in non-Finnish European.

Rare Heterozygous Variants Are Not Enriched in Any of the Studied Genes

To further study the role of rare (MAF < 0.01) heterozygous variants, we performed SKAT-O and burden tests, repeated twice for variants detected at a coverage depth of >30X and variants detected at >50X (see Supplementary Data). All rare heterozygous variants identified in each gene are detailed in Supplementary Table S3. We performed SKAT-O and burden tests at five different levels: all rare variants, all potentially functional variants (nonsynonymous, splice-site, frameshift, and stop-gain), loss-of-function variants (frameshift, stop-gain, and splicing), nonsynonymous variants only, and variants with CADD score ≥ 12.37 (Table 2). The Bonferroni corrected *P*-value for statistical significance was set at $P < 0.001$. We found no statistically significant association between iRBD and any of the variant types in any of the genes, suggesting that these genes either have no role in iRBD or have a minor role that we could not detect with this sample size. We did not identify any iRBD patient with known biallelic pathogenic variants in *PARK7*, *PINK1*, *VPS13C*, and *ATP13A2* or heterozygous pathogenic variants in *LRRK2*, *GCH1*, and *VPS35*. Two controls were found with the pathogenic *LRRK2* p.G2019S variant.

Analysis of CNVs in *PRKN*

We further examined the association between deletions and duplications in *PRKN* and risk for iRBD. Using ExomeDepth, 7 patients (0.7%) and 17 controls (0.9%, $P = 0.53$) were found to carry CNVs in *PRKN*, and none of the patients were found to have an additional nonsynonymous variant. Therefore, there were no homozygous or compound heterozygous carriers of rare *PRKN* variants among the iRBD patients. Supplementary Table S4 lists all the CNVs found in our cohort.

Discussion

The present study provides the first large-scale, full-sequencing analysis to examine the role of the dominant and recessive parkinsonism genes *PRKN*, *PARK7*, *PINK1*, *VPS13C*, *ATP13A2*, *FBXO7*, *PLA2G6*, *LRRK2*, *GCH1*, and *VPS35* in iRBD. We did not find evidence for association of any of these genes with iRBD. In the recessive genes, there was no overrepresentation of carriers of homozygous or compound heterozygous variants in iRBD patients and no single patient with biallelic pathogenic variants. In the dominant genes, we did not find any known pathogenic variants in these genes, and SKAT-O and burden analyses did not identify burden of rare heterozygous variants in any of these 10 genes. Overall, these results suggest that iRBD is more likely to be associated with the sporadic, multifactorial forms of PD rather than with the monogenic forms of parkinsonism.

Whether heterozygous carriage of mutations in recessive PD or atypical parkinsonism-related genes is a risk factor for PD is still controversial.[28] *PRKN*-associated PD is characterized by pure nigral degeneration without α-synuclein accumulation,[29] and reports on synucleinopathy and Lewy bodies in *PINK1*-associated PD are inconclusive, as some studies identified Lewy

TABLE 2. Summary of results from burden analyses of rare heterozygous variants

		All rare (*P*-value)		Rare functional (*P*-value)		Rare LOF (*P*-value)		Rare NS (*P*-value)		Rare CADD (*P*-value)	
DOC	Gene	SKAT-O	SKAT Burden	SKAT-O	SKAT Burden	SKAT-O	SKAT Burden	SKAT-O	SKAT Burden	SKAT-O	SKAT Burden
30x	Recessive genes										
	PRKN	0.4316	0.484	0.388	0.240	NV	NV	0.508	0.331	1	0.889
	PARK7	0.104	0.254	0.008	0.369	0.175	0.174	0.005	0.005	NV	NV
	PINK1	0.703	0.505	0.117	0.605	NV	NV	0.117	0.605	0.124	0.494
	Recessive (atypical) genes										
	ATP13A2	0.543	0.383	0.379	0.227	NV	NV	0.379	0.227	0.201	0.121
	FBXO7	0.525	0.562	0.266	0.140	0.163	0.252	0.327	0.160	0.228	0.279
	PLA2G6	0.325	0.859	0.222	0.663	0.260	0.193	0.243	0.948	0.196	0.688
	VPS13C	0.018	0.047	0.334	0.206	0.237	0.137	0.343	0.207	0.468	0.834
	Dominant genes										
	GCH1	0.361	0.217	0.730	0.804	0.730	0.804	NV	NV	NV	NV
	LRRK2	0.601	0.827	0.578	0.888	0.134	0.199	0.590	0.966	0.610	0.871
	VPS35	0.159	0.111	0.161	0.247	0.382	0.522	0.161	0.247	0.434	0.807
50x	Recessive genes										
	PRKN	0.085	0.084	0.452	0.609	NV	NV	0.452	0.609	0.771	0.564
	PARK7	0.180	0.288	0.017	0.436	NV	NV	0.010	0.010	NV	NV
	PINK1	0.572	0.546	0.050	0.133	NV	NV	0.050	0.133	0.050	0.133
	Recessive (atypical) genes										
	ATP13A2	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV
	FBXO7	0.618	0.624	0.209	0.125	0.331	0.613	0.256	0.148	0.540	0.309
	PLA2G6	0.528	0.853	0.360	0.680	0.680	0.452	0.360	0.680	0.680	0.452
	VPS13C	0.101	0.055	0.073	0.038	0.777	0.971	0.149	0.082	0.332	0.227
	Dominant genes										
	GCH1	0.901	0.817	0.734	0.760	0.734	0.760	NV	NV	NV	NV
	LRRK2	0.030	0.019	0.279	0.173	0.062	0.088	0.525	0.377	0.527	0.365
	VPS35	0.453	0.549	NV	NV	NV	NV	NV	NV	NV	NV

DOC, depth of coverage; CADD, combined annotation-dependent depletion; NS, nonsynonymous; LOF, loss of function; SKAT-O, optimized sequence kernel association test; SKAT, Kernel association test; NV, no variants were found for this filter.

bodies, whereas others did not.[30,31] Because iRBD is a prodromal synucleinopathy, it is not surprising that we did not identify biallelic mutations or burden of heterozygous variants in any of these genes.

Recently, we have shown that the *SNCA* locus is important in RBD, yet with different and distinct variants that are associated with risk of PD.[22] In the same study, *SNCA* was fully sequenced, and no known PD-causing variants were found in iRBD patients. We and others have previously reported that pathogenic *LRRK2* variants were not identified in smaller cohorts of iRBD,[17] which was further confirmed in the current study. In addition, several studies of PD patients with and without RBD have shown reduced prevalence of RBD[32-35] or reduced scores in RBD questionnaires among *LRRK2* mutation carriers. *VPS35* mutations have not been identified in iRBD in the current study, although pathogenic *VPS35* mutations are generally rare.[36,37] Altogether, these results provide no evidence that known, well-validated familial gene mutations involved in PD (including *SNCA*, *LRRK2*, *VPS35*, *PRKN*, *PINK1*, and *PARK7*) are also involved in iRBD. *GBA* is the only gene in which strong risk variants associated with PD are also associated with iRBD.[11] We did not exclude *GBA* mutation carriers in the current analysis, yet exclusion of these carriers did not change the results.

Our study has some limitations. Although it is the largest genetic study of iRBD to date, it may still be underpowered to detect rare variants in familial PD-related genes. Therefore, our study does not completely rule out the possibility that variants in these genes may lead to iRBD in very rare cases. Another potential limitation of the study design is the earlier age, the different sex distribution in the control population, and the fact that they have not been tested for iRBD. However, because iRBD is not common, found in about 1% of the population,[2] age would have a minimal or no effect on the results. The differences in sex ratios are less likely to have an effect, because in AD and AR Mendelian diseases, the risk is typically similar for men and women.

To conclude, the lack of association between different PD and parkinsonism genes may suggest either that iRBD is an entity more affected by environmental factors or that there are other, yet-undetected genes that may be involved in iRBD. Our study also suggests that screening for variants in the tested genes in iRBD will have a very low yield.

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Supporting Data

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