LRRK2 protective haplotype and full sequencing study in REM sleep behavior disorder

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Abstract

Background: Individuals with rapid eye movement (REM)-sleep behavior disorder (RBD) are likely to progress to synucleinopathies, mainly Parkinson's disease (PD), dementia with Lew-bodies (DLB) and multiple system atrophy (MSA). The genetics of RBD only partially overlaps with PD and DLB, and the role of *LRRK2* variants in risk for RBD is still not clear.

Methods: The full coding sequence, exon-intron boundaries and 5' and 3' untranslated regions of *LRRK2* were sequenced using targeted next-generation sequencing. A total of 350 RBD patients and 869 controls were sequenced, and regression and burden models were used to examine the association between *LRRK2* variants and RBD.

Results: No pathogenic mutations that are known to cause PD were identified in RBD patients. The p.N551K-p.R1398H-p.K1423K haplotype was associated with a reduced risk for RBD (OR=0.66, 95% CI 0.44-0.98, p=0.0055 for the tagging p.N551K substitution). A common variant, p.S1647T, was nominally associated with risk for RBD (OR=1.28, 95% CI 1.05-1.56, p=0.029). Burden analysis identified associations with domains and exons that were derived by the variants of the protective haplotype, and no burden of other rare variants was identified.

Conclusions: Carriers of the *LRRK2* p.N551K-p.R1398H-p.K1423K haplotype have a reduced risk for developing RBD, yet PD-causing mutations probably have minor or no role in RBD. Additional work is needed to confirm these results and to identify the mechanism associated with reduced risk for RBD.

Introduction

Individuals with rapid eye movement (REM) sleep behavior disorder (RBD) suffer from loss of muscle atonia during REM sleep, and therefore enact their dreams. RBD is likely to be a prodromal synucleinopathy, as it often progresses to either Parkinson's disease (PD), dementia with Lewy-bodies (DLB) or multiple system atrophy (MSA) In long-term follow-up, more than 80% of RBD patients will progress into one of these synucleinopathies.[1]

The genetic background of RBD is still not fully understood, but it is clear that it does not fully overlap with that of PD or DLB. *GBA* mutations are probably the most common genetic risk factor for PD,[2] are also common in DLB,[3] and strongly associated with RBD.[4,5] However, other genetic variants that are associated with PD or DLB are not associated with RBD. For example, the *APOE* ε4 haplotype, one of the most important risk factors for DLB, was not associated with RBD in a recent study.[6] Mutations in *LRRK2* are among the most common genetic causes of PD, but are probably not involved in DLB or MSA. However, it is possible that other variants in *LRRK2* which do not cause PD, are associated with reduced risk for DLB or MSA.[7,8] Two studies examined whether specific *LRRK2* mutations that are known to cause PD (the p.G2019S and pR1441G/C/H substitutions) are also associated with RBD, and in both none of these variants were identified in RBD patients.[4,9]

In the current study, we performed targeted next generation sequencing of *LRRK2* that covered the entire coding region in a larger cohort of RBD patients, in order to examine whether there are RBD-specific *LRRK2* variants, and to examine the role of common *LRRK2* variants in RBD.

Methods

Population

The study population included individuals diagnosed with idiopathic RBD (n=350) and controls (n=869), all unrelated, all of French-Canadian and French ancestry. Patients were diagnosed with RBD using clinical interview and polysomnography according to the International Classification of Sleep Disorders, version 2 (ICSD-2) criteria. At recruitment, RBD patients had no neurological symptoms or dementia. In the RBD group, the male:female ratio was 3.6:1 (data was not available for 13 patients), and average age was 67.3±6.6 years. In the control group, the male:female ratio was 1.06:1, and it was composed of two sub-groups: 263 elderly controls (average age 60.02±7.9) and 606 young controls (average age 34.4±4.8). Since there were no differences in frequencies of common or rare *LRRK2* variants between these two sub-groups, they could be combined for a sex- and age-adjusted analysis. Controls were consecutively and randomly recruited, and no selection of controls were performed. At recruitment, the controls did not have reported manifestations of RBD, PD or dementia. All participants signed informed consent forms at enrollment, and the study protocol was approved by the institutional review boards.

Targeted next generation sequencing

DNA was extracted from peripheral blood lymphocytes using standard protocols. To capture the coding regions, 5' and 3' untranslated regions (UTR), and the exon-intron boundaries (±50bps) of the *LRRK2* gene (NM_198578), 151 molecular inversion probes (MIPs) spanning the 51 exons of the gene, were designed (supplementary Table 1). MIPs were designed using an online MIP design tool (http://krishna.gs.washington.edu/mip_pipeline), and to optimize the capture, MIPs were selected based on their inferred quality, coverage and overlap. A total of 100 ng of DNA was used for the MIPs capture. The library was sequenced using the Illumina HiSeq 2500 platform. Reads

of MIPs data were mapped to Human Reference Genome v37 using the Burrows-Wheeler Aligner (BWA) (v0.7.5), and GATK tools and ANNOVAR were used to call and to annotate variants.

Statistical analysis

Quality control: Only variants with depth-of-coverage of >50X were considered in the analysis, to avoid false positive calls. We performed the analyses twice, once after removing variants with >10% missing calls across all samples, and once after removing variants with >20% missing calls. Deviation from Hardy–Weinberg equilibrium (HWE) was assessed with 0.05 as threshold, and variants were removed if deviated from HWE in the controls.

Statistical analysis: Fisher exact tests and age- and sex-adjusted binary logistic regression were performed using PLINK v1.90 to test for association of single variants. Linkage disequilibrium (LD) between common variants was also assessed using PLINK. Correction for multiple comparisons was performed using Bonferroni correction, and nominal *p* values were considered significant only if they were previously reported. To test for burden of multiple variants, optimal Sequence Kernel association test (SKAT-O) was performed using R. SKAT-O was performed on the entire gene level, domain-by-domain level and exon-by-exon level, on coding and non-coding variants, and comparing synonymous, non-synonymous, stop, frameshift and splicing variants.

Results

No known-PD causing variants were detected in the RBD cohort

Two controls were removed from the analysis due to insufficient coverage, and the average coverage of the coding region of the gene was 811X, with 96.7% of >10X, 95.4% >20X, and 92.2% >50X. A total of 83 variants were included in the analysis after quality control (≥90% of samples with a call with coverage of >50X, not deviating from HWE), including 23 non-synonymous variants, 15 synonymous variants, one stop variant, two frameshift deletions, one intronic splicing variant, 37 intronic variants and 4 variants at the 3' UTR of *LRRK2* (Table 1 details the 41 coding variants with reads in >90% of samples, all other variants are in Supplementary Table 2). No known PD-causing *LRRK2* mutations were identified in our RBD cohort. Some rare variants were found only in one or two patients and not in controls (p.S901L, p.R1483X, p.Q1586H, p.S1636fs, p.M1869T, p.R1943Q, detailed in Table 1), and while there is some possibility that they may be associated with RBD, we could not determine it with the current data.

LRRK2 protective haplotype is associated with reduced risk for RBD

In the age- and sex-adjusted regression model, two non-synonymous and one synonymous coding variants, were associated with RBD: p.N551K (p=0.0055), p.S1647T (p=0.029) and p.K1423K (p=0.0017). The p.N551K and p.K1423K variants were in almost full LD (D'=0.98, r^2=0.96), and belong to a haplotype known to be associated with a reduced risk for PD.[10] The allele frequency of this haplotype was 0.05 in RBD patients and 0.074 in controls (OR=0.66, 95% CI 0.44-0.98, age- and sex- adjusted p=0.0055, for the tagging p.N551K substitution). When the QC threshold was reduced to include variants with >80% of samples with a read of >50X (Supplementary Table 3), two more variants were added to this haplotype, p.R1398H and the

intronic variant rs201235847, also with almost full LD with p.N551K and p.K1423K (r^2>0.93 between all SNPs). In addition, a common variant which is not a part of the protective haplotype, p.S1647T, had an with allele frequencies of 0.33 in RBD patients and 0.28 in controls (OR=1.28 , 95% CI 1.05-1.56, nominal *p*=0.029, not significant after correction for multiple comparison), and it is not in LD with the other variants.

Subsequently, to determine whether there is a burden of other *LRRK2* variants associated with RBD, we performed SKAT-O analyses (Table 2). When including all 83 variants, there was a significant association between *LRRK2* and RBD (*p*=0.001), however when including only rare variants, there was no association between *LRRK2* and RBD. Analysis of domain-by-domain identified a significant burden at the ROC domain (*p*=0.026) and in variants found outside of the known domains (*p*=0.028). However, these associations were driven by the p.K1423K and p.N551K, respectively, and when including only rare variants, there was no burden association between any of the LRRK2 domains and RBD. Similarly, in the exon-by-exon analysis, exons that carried these variants were associated with RBD in the SKAT-O analysis, but were no longer associated with RBD when including only rare variants. Hence all these associations were driven by the p.N551K-p.R1398H-p.K1423K haplotype.

Discussion

Our results demonstrate that the p.N551K-p.R1398H-p.K1423K *LRRK2* haplotype is associated with a reduced risk for RBD. While this association is similar to the association of this haplotype with PD,[10] our study also demonstrated that the known PD-causing *LRRK2* mutations seem to play a minor role in RBD, or no role at all. Interestingly, this protective haplotype demonstrated a similar trend in DLB, with marginal results (OR=0.76, p=0.061), and the allele frequencies in our RBD patients and controls (0.05 and 0.074, respectively) were nearly identical to those in the DLB study (0.055 and 0.076 for the p.N551K tagging variant in DLB patients and controls, respectively).[8] Of note, PD-causing *LRRK2* mutations are not associated with dementia,[8] and RBD is associated with higher rates of dementia.[1] Therefore, the lack of PD-causing *LRRK2* mutations in RBD cohorts may provide further support for lack of association of these mutations with dementia. Furthermore, the results of the current study are limited to the *LRRK2* gene only, and do not necessarily imply that other genes may or may not be involved in RBD.

It is still not clear how *LRRK2* mutations lead to PD, or how the protective *LRRK2* haplotype exerts its protective properties. The leading paradigm is that the pathogenic *LRRK2* mutations lead to gain-of-function of its kinase activity, which in turn leads to PD, through a yet unknown mechanism.[11] The mechanism underlying the protective effect of the *LRRK2* p.N551K-p.R1398H-p.K1423K haplotype is still unknown. However, a recent study on Crohn's disease, in which this haplotype confer protection as well, suggested that only the p.R1398H variant affects the function of the LRRK2 protein, by deactivating its kinase activity,[12] which may explain the reduced risk for RBD and PD.

Our study has several limitations. First, although it is the largest and most comprehensive study of *LRRK2* genetics in idiopathic RBD performed to date,[4,9] it is still relatively small

compared to the large PD genetic studies. Therefore, these results will need to be confirmed in additional populations. A second limitation is that we could not determine the effect of the protective haplotype on progression from RBD to the different synucleinopathies. If this is indeed a protective haplotype, we would expect that carriers of this allele who did develop RBD may progress slower, and might have less dementia. However, since this protective allele frequency in RBD patients is only 0.05, and since only about 30% of these patients have converted, the total numbers are too small to perform a powered statistical analysis. Similarly, the association of the protective haplotype with development of dementia could not be estimated. Therefore, it will be crucial to continue follow up on these patients and examine the association of this haplotype and other genetic variants on progression to dementia or PD, when a larger portion of the cohort had converted, as well as in additional populations. Another potential limitation may be the differences in age of the control groups, which in genetic studies ay lead to a bias. However, in our young and elderly controls there were no differences in the protective haplotype frequency (0.07 in both), and the statistical analysis was adjusted for age, therefore age could not have affected the results. The high male:female ratio in the current study (3.6:1) is typical to RBD,[1] and it could be due to more violent dream content in men which leads to higher rates of RBD diagnosis, or due to a yet unknown biological reason.

Overall, the current data, together with previous results, may suggest that genetic factors that are relevant to both DLB and PD, such as *GBA* mutations and the *LRRK2* protective haplotype are important in RBD as well.[4,5] On the other hand, genetic variants that are relevant in only PD or DLB, such as the *APOE* e4 allele in DLB and *LRRK2* mutations in PD, have no role in RBD.[4,6,9] Therefore, it is possible that RBD represents a sub-population of both disorders, which is somewhat distinct by its genetic background.

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Table 1. *LRRK2* coding variants in RBD patients and controls.

Exon	Variant	AF RBD	AF control	*p* value[a]
5	c.T457C, p.L153L	0.001497	0	NA
6	c.G633A, p.A211A	0.393	0.3756	0.677
8	c.C856G, p.L286V	0	0.001227	NA
8	c.C867T, p.N289N	0.00165	0	NA
8	c.G919A, p.A307T	0	0.0006053	NA
8	c.G936T, p.A312A	0	0.001211	NA
11	c.C1256T, p.A419V	0.001608	0.001224	0.3612
14	c.A1572G, p.T524T	0.001543	0	NA
14	c.C1653G, p.N551K	0.05	0.07416	0.005512
16	c.C1891T, p.L631L	0.001661	0	NA
18	c.A2167G, p.I723V	0.09568	0.08163	0.3668
21	c.T2701C, p.S901P	0.00152	0	NA
21	c.C2702T, p.S901L	0.00152	0	NA
21	c.G2769C, p.Q923H	0.00152	0.001172	0.712
23	c.G2918A, p.S973N	0	0.001188	NA
29	c.G3974A, p.R1325Q	0	0.0006105	NA
29	c.T3993G, p.P1331P	0.001623	0	NA
29	c.C4057A, p.Q1353K	0.003257	0.001829	0.6851
30	c.G4269A, p.K1423K	0.05254	0.07967	0.00168
31	c.G4352A, p.G1451D	0	0.0006165	NA
31	c.C4447T, p.R1483X	0.001582	0	NA
33	c.A4758C, p.Q1586H	0.001582	0	NA
33	c.A4815G, p.K1605K	0.001582	0.001801	0.7735
34	c.C4872A, p.G1624G	0.3444	0.3669	0.1962
34	c.G4883A, p.R1628H	0.001553	0.0005896	0.9876
34	c.4908delA, p.S1636fs	0.003175	0	NA
34	c.A4911G, p.K1637K	0.4668	0.4767	0.753
34	c.T4937C, p.M1646T	0.009554	0.01852	0.2232
34	c.T4939A, p.S1647T	0.3302	0.278	0.02851
37	c.5438_5439del, p.L1813fs	0	0	NA
37	c.T5457C:p.G1819G	0.4495	0.47	0.6263
38	c.T5606C, p.M1869T	0.001543	0	NA
40	c.A5799T, p.I1933I	0	0.0005974	NA
40	c.G5828A, p.R1943Q	0.001548	0	NA
42	c.A6241G, p.N2081D	0.01505	0.007362	0.1764
43	c.G6324A, p.E2108E	0.3533	0.3036	0.08633
48	c.G7069A, p.V2357M	0	0.0005924	NA
48	c.A7155G, p.G2385G	0.1468	0.1449	0.2525

49	c.T7190C, p.M2397T	0.3404	0.3462	0.8428
49	c.T7256A, p.L2419H	0	0	NA
49	c.C7382T, p.A2461V	0	0.000591	NA

AF, allele frequency; RBD, REM-sleep behavior disorder; NA, not applicable

[a] Adjusted for age and sex

Table 2. SKAT-O burden analysis of all variants, and domain-by-domain burden analysis, with and without the variants of the protective *LRRK2* haplotype.

Domain	N	*p* value
All	**83**	**0.001162947**
Domain by domain all variants		
ANK	1	0.33669277
ARM	7	0.75154247
COR	11	0.11212408
G	**9**	**0.02838475**
MAPKKK	4	0.16164559
ROC	**4**	**0.02555945**
WD40	5	0.65280598
Domain by domain, rare variant only		
ANK	1	0.35509078
ARM	3	0.67701719
COR	7	0.08539317
G	6	0.04408134
MAPKKK	2	0.09322025
ROC	3	0.51581054
WD40	4	0.59650851

N, number of variants; ANK, ankyrin domain; ARM, armadillo repeat domain; COR, c-terminal of ROC; G, general, no specific domain; MAPKKK, Mitogen-activated protein kinase kinase kinase; ROC, ras of complex protein; WD40, tryptophan-aspartic acid repeat.