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Genetic markers of Restless Legs Syndrome in Parkinson Disease

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Abstract

Introduction—Several studies proposed that Restless Legs Syndrome (RLS) and Parkinson disease (PD) may be clinically and/or etiologically related. To examine this hypothesis, we aimed to determine whether the known RLS genetic markers may be associated with PD risk, as well as with PD subtype.

Methods—Two case-control cohorts from Tel-Aviv and New-York, including 1,133 PD patients and 867 controls were genotyped for four RLS-related SNPs in the genes *MEIS1*, *BTBD9*, *PTPRD*

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and *MAP2K5/SKOR1*. The association between genotype, PD risk and phenotype was tested using multivariate regression models.

Results—None of the tested SNPs was significantly associated with PD risk, neither in any individual cohort nor in the combined analysis after correction for multiple comparisons. The *MAP2K5/SKOR1* marker rs12593813 was associated with higher frequency of tremor in the Tel-Aviv cohort (61.0% vs. 46.5%, p=0.001, dominant model). However, the risk allele for tremor in this gene has been associated with reduced RLS risk. Moreover, this association did not replicate in Tremor-dominant PD patients from New-York.

Conclusion—RLS genetic risk markers are not associated with increased PD risk or subtype in the current study. Together with previous genetic, neuropathological and epidemiologic studies, our results further strengthen the notion that RLS and PD are likely to be distinct entities.

Introduction

Restless Legs Syndrome (RLS) and Parkinson disease (PD) are common disorders, affecting millions of individuals worldwide. The prevalence of RLS is estimated as 5-15% of the general population, [1] while PD occurs in about 1-2% of individuals older than 65 years. [2] Dopaminergic mechanisms play an important role in both disorders, yet the exact pathophysiology is still unclear. [3]

There is some debate whether RLS and PD are related, and if they are, what is the nature of this relation; do they share common pathogenic mechanisms, or can symptoms of one disease mimic the other? Several case-control studies reported that RLS is more prevalent in PD patients than in controls, while others did not demonstrate this association (reviewed in reference [4]). These studies all used the IRLSSG (International RLS Study Group) criteria, but with different methods and approaches of exclusion and inclusion criteria, therefore the inconclusive association may be a result of these differences. Of note, reliability of the IRLSSG criteria has not been tested in PD cohorts. [4]

For both RLS and PD, there are well-validated genetic risk factors that are associated with each condition. Shared genetic loci may suggest shared pathophysiology, however, there is no known overlap among these RLS and PD loci. [5, 6] In PD, estimates of the genetic susceptibility component range from 27% to 60%, yet known genetic markers can only explain up to 7% of PD cases, [7-9] suggesting that there are still other genetic variants that are either rare or with a small effect size, that can influence PD risk. [10] Could RLS genetic markers be some of these genetic variants that were undetected in PD GWAS?

Another possibility is that there are specific genetic markers for specific subtypes of PD, such as tremor dominant (TD) and postural instability/gait difficulty (PIGD) PD, preventing these variants from being discovered in GWAS based only on dichotomous diagnosis of PD.

Herein, we aimed to examine whether RLS loci are associated with PD, by analyzing two case-control PD cohorts for the four well-validated RLS markers in the genes *MEIS1*, *BTBD9, PTPRD* and *MAP2K5/SKOR1*. [6] We further examined whether these loci are associated with specific PD symptoms and subtypes - TD and PIGD PD.

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Methods

Population

The study included two case-control cohorts, including 1,133 PD patients and 867 controls, from Tel-Aviv (TA) and New-York (NY). The cohort from TA was consecutively recruited at the Movement Disorders Unit of Tel-Aviv Medical Center, and included 600 PD patients and 600 controls. The TA PD patient population was composed of unrelated Ashkenazi-Jewish patients, 62.8% men, with an average age at enrollment of 68.2±10.1 years. More details on the recruitments, questionnaires and procedures were previously published. [11] The TA controls included 300 unrelated, sex- and age- matched Ashkenazi Jews (60.7% men, p=0.52 age 67.6±10.0, p=0.38), and additional 300 anonymous samples that represent other Jewish communities from three different regions: 100 Moroccan Jews (North-Africa), 100 Iraqi Jews (Middle-East) and 100 Bukhara Jews (Middle-Asia). The cohort from NY Included 533 unrelated PD patients and 267 spouse controls, consecutively recruited in the Center for Parkinson's disease at Columbia University Medical Center in New York, NY. The control population was composed mainly of spouses of the patients, and was therefore not matched for sex (63.6% vs. 35.2% men in patients and controls, respectively, p<0.05), but matched for age at enrollment (66.0±10.4 years vs. 65.1±9.9 years, p=0.21). In both centers, patients were diagnosed using the United Kingdom PD brain bank criteria, except for the inclusion of PD cases with strong family history. In the NY cohort, TD and PIGD were defined by their Unified Parkinson Disease Rating Scale (UPDRS) scores as was previously described. [12] All participants signed an informed consent form before entering the study, and the study protocols were approved by the institutional review boards.

Selection of SNPs and genotyping

Four known RLS associated SNPs were selected: rs2300478 (located in the *MEIS1* gene), rs9357271 (*BTBD9*), rs1975197 (*PTPRD*) and rs12593813 *MAP2K5/SKOR1*). [6] DNA was extracted from blood using a standard salting out protocol. All four SNPs were genotyped by TaqMan SNP genotyping assays (assay IDs: C__15754717_10, C__30244102_10, C__12094576_10 and C__31739685_10, respectively) following the manufacturer's instructions. PCR amplifications were performed in 384 well plate format with 5 μl reaction volume and following the program: 95°C for 10 minutes, then 95°C for 15 seconds and 60°C for 1 minute for a total of 40 cycles. The genotypes were called using the the QuantStudio™ 7 Flex Real-Time PCR System and Software (v 1.0) or by the StepOne RT-PCR system, (Applied Biosystems). Success rates of genotyping of the four markers combined were 100% in the Tel-Aviv cohort and 99% in the New-York cohort. The two cohorts were previously genotyped for founder *GBA* and *LRRK2* mutations. [13, 14]

Statistical Analysis

Continuous variables are presented as mean (± SD), and categorical variables are presented in percentages. Allele frequencies are presented as a range of 0–1, after applying the Goodness of-fit test to examine deviation from the Hardy-Weinberg equilibrium. Binary logistic regression was applied to examine the association between the four selected SNPs and PD, with the status of the disease as the dependent variable. When patients and controls were not matched for sex, it was added as covariates to adjust for the effect of gender. The

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presence of *GBA* and *LRRK2* mutations were also added as covariates. Regression was performed for allele frequency, and for dominant and recessive models of effect, and Bonferroni correction for multiple comparisons was applied. Differences of continuous variables were tested using analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis or Mann-Whitney ANOVA when the analysis included a small number of individuals. χ^2 or Fisher exact test was used for comparison of categorical variables. SPSS software v. 22 (IBM) was used for all data analysis. Supplementary Table 1 details the frequencies of RLS markers in the Jewish controls from Tel-Aviv of four different Jewish origins: Ashkenazi, Moroccan, Iraqi and Bukhara Jews. None of the markers deviated from Hardy-Weinberg equilibrium after Bonferroni correction (corrected *p* value = 0.0125). The frequencies of the four RLS markers are generally comparable to those reported in other populations. [6]

Results

Table 1 details the logistic regression models comparing patients and controls from both the TA and NY centers, separately and combined. There were no significant associations after Bonferroni correction for multiple comparisons. The trend observed in the TA cohort, with the allele frequencies of *BTBD9* rs9357271 SNP being 0.28 vs. 0.22 among patients and controls, respectively (OR = 1.41, 95% CI = 1.05-1.90, uncorrected *p* value = 0.02), was in the opposite direction of the known association of this SNP with RLS. However, the trend in the combined analysis of the *PTPRD* rs1975197 SNP (Allele frequencies of 0.113 and 0.098 among PD patients and controls, respectively, OR = 1.31, 95% CI = 1.02-1.69, uncorrected *p* value = 0.03) was in the same direction of the association in RLS. When comparing the 600 PD patients from Tel-Aviv to all 600 Jewish controls (including the 300 non-Ashkenazi controls) no significant differences were found (data not shown). Overall, there is no evidence supporting association of RLS genetic markers to PD in the current study.

We further compared demographic and clinical data according to the genotypes of the RLS SNPs in both dominant and recessive models in the Ashkenazi-Jewish PD patient population from TA (Table 2). Since this analysis (using both dominant and recessive models) increased the number of comparisons, a more strict Bonferroni correction of p=0.0031 was applied, adjusting for 16 comparisons. Interestingly, the *MAP2K5/SKOR1* rs12593813 was associated with higher frequency of tremor in the dominant model (61.0% vs. 46.5%, p=0.001). Accordingly, in the same model, carriers of the *MAP2K5/SKOR1* rs12593813 SNP had less gait difficulties as a presenting symptom (13.8% vs. 21.5%, uncorrected p=0.02, not significant after Bonferroni correction). This association with tremor as a presenting symptom prompted us to examine whether RLS markers might be associated with subtypes of PD, more specifically with TD-PD.

UPDRS scores were available only for the NY cohort, therefore we used it for further analysis of the possible associations between the *MAP2K5/SKOR1* rs12593813 and the other RLS markers and TD-PD (Table 3), or PIGD-PD (data not shown). No significant associations were found in both analyses, most likely ruling out the possibility that RLS genetic markers are associated with any sub-type of PD. In addition, we examined whether the RLS markers may be associated with genetic sub groups of PD, since both cohorts were

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genotyped for *GBA* and *LRRK2* mutations as was previously described. [11, 12] None of the RLS markers was associated with either *LRRK2*- or *GBA*-associated PD in both cohorts (data not shown).

Discussion

The current study suggests that the four well-validated RLS genetic risk markers have no clear role in PD susceptibility. This analysis holds among all PD patients and defined clinical sub-groups. The minor alleles of the *BTBD9* and *MAP2K5/SKOR1* SNPs were in fact more common among PD patients than in controls, while in RLS patients they are less frequent than controls. [6] Hence, the minor allele of *MAP2K5/SKOR1* SNP rs12593813, which is associated here with tremor, is associated with a reduced risk for RLS, not supporting the association between the two diseases. A previous report that examined three RLS loci, *MEIS1*, *BTBD9*, and *MAP2K5/SKOR1*, in a PD cohort of 369 patients and 403 controls, also demonstrated no association. [15] Recently, the α-synuclein Rep1 allele 2, which is more frequent among PD patients than controls, was demonstrated to be less frequent among RLS patients. [16] The PD associated *MAPT* SNP rs1052553 was also not associated with RLS in another case-control study. [17] In two families with *PARK2 (Parkin)* mutations and RLS, the mutations did not segregate with RLS and had no effect on RLS phenotype. [18] Altogether, with the lack of overlap between GWAS loci of RLS and PD, [5, 6] these genetic findings suggest a distinct genetic background of RLS and PD. Such different genetic background, may suggest an alternative pathogenesis for each disease.

Neuropathological and imaging findings further support this lack of association between the two conditions. Post-mortem studies of patients who had idiopathic RLS, with no other neurologic disorders, demonstrated the absence of PD pathological hallmark, α-synuclein accumulation and Lewy-bodies. [19] In a large family that presented with Parkinsonism, Essential Tremor, RLS and depression, only sparse Lewy-bodies were found. [20] In addition, while iron is elevated the substantia nigra of PD patients, it is decreased in the substantia nigra of RLS patients. [21] The clear decrease in [18 F]-dopa uptake and [123I]-ß-CIT binding in PD, is not clearly evident in RLS imaging studies, [22-24] and results from studies that did show some reduction in putamen uptake of [18 F]-dopa in RLS patients, demonstrated that they are unlikely to result from loss of striatal neurons. [25] Sonographic studies of patients with RLS only, co-morbid PD and RLS patients, PD only patients and controls revealed a decreased echogenicity of the substantia nigra in those with only RLS, but increased echogenicity among PD or PD and RLS patients. [26-28]

To conclude, the current findings further support a lack of pathophysiological association between RLS and PD. Although the central dopaminergic system appears to be involved in both RLS and PD, and these two conditions can co-occur, it seems less likely that they share a common mechanism. It is possible that the increased co-occurrence of RLS in PD patients that was described in several studies is the result of mimicry of RLS symptoms, created by the motor restlessness typical to Parkinsonism. Alternative explanation may be that the dopaminergic treatment in PD leads to augmentation/unmasking of subclinical RLS, however, more studies are needed to confirm these hypotheses.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

RLS SNPs and risk estimates for PD in the Tel-Aviv and New-York cohorts

SNP	Gene	MAF		Allele Frequency			Dominant model			Recessive model		
		Patients	Controls	OR	(95%CI)	*p* value	OR	(95%CI)	*p* value	OR	(95%CI)	*p* value
Tel Aviv Cohort [a]		**N=600**	**N=300**									
rs2300478	*MEIS1*	0.24	0.23	0.91	0.67-1.24	0.54	0.96	0.71-1.30	0.79	1.51	0.80-2.84	0.20
rs9357271	*BTBD9*	0.28	0.22	1.18	0.86-1.60	0.28	1.41	1.05-1.90	0.02	1.56	0.80-3.02	0.19
rs1975197	*PTPRD*	0.09	0.08	1.12	0.72-1.75	0.62	1.20	0.80-1.78	0.38	1.29	0.31-5.32	0.73
rs12593813	*MAP2K5/SKOR1*	0.47	0.45	1.30	1.00-1.69	0.05	1.22	0.89-1.68	0.21	1.01	0.71-1.43	0.96
New-York cohort [b]		**N=533**	**N=267**									
rs2300478	*MEIS1*	0.26	0.23	1.21	0.92-1.59	0.17	1.21	0.87-1.67	0.26	1.82	0.90-3.66	0.10
rs9357271	*BTBD9*	0.26	0.29	0.86	0.66-1.12	0.25	0.98	0.71-1.36	0.92	0.72	0.40-1.29	0.26
rs1975197	*PTPRD*	0.14	0.12	1.35	0.95-1.91	0.09	1.35	0.92-1.97	0.12	3.39	0.74-15.6	0.12
rs12593813	*MAP2K5/SKOR1*	0.42	0.39	1.07	0.84-1.36	0.59	1.08	0.78-1.51	0.64	1.27	0.82-1.95	0.28
Combined analysis [b]		**N=1,133**	**N=567**									
rs2300478	*MEIS1*	0.25	0.23	1.07	0.89-1.29	0.46	1.00	0.81-1.26	0.96	1.52	0.95-2.43	0.08
rs9357271	*BTBD9*	0.27	0.25	1.07	0.90-1.28	0.45	1.19	0.96-1.49	0.12	1.11	0.72-1.71	0.64
rs1975197	*PTPRD*	0.113	0.098	1.32	1.02-1.69	0.03	1.31	0.99-1.72	0.06	2.09	0.76-5.78	0.15
rs12593813	*MAP2K5/SKOR1*	0.44	0.42	1.03	0.88-1.21	0.69	1.19	0.95-1.50	0.13	1.09	0.83-1.42	0.55

[a] adjusted for the presence of *GBA* and *LRRK2* mutations.

[b] adjusted for the presence of *GBA* and *LRRK2* mutations and for gender.

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Table 2

Variable	rs2300478			rs9357271			rs1975197			rs12593813		
	Mut	**WT**	**p**	**Mut**	**WT**	**p**	**Mut**	**WT**	**p**	**Mut**	**WT**	**p**
Dominant model												
Age at onset, yrs [a]	62.1	60.5	0.14	61.0	61.3	0.79	61.9	60.9	0.49	61.4	60.5	0.50
± SD	±11.0	±10.9		±10.8	±11.1		±10.2	±11.1		±11.0	±10.9	
Age at enrollment, yrs [a]	69.8	68.9	0.34	68.7	69.7	0.32	70.2	69.0	0.35	69.4	68.8	0.58
± SD	±9.6	±10.0		±10.0	±9.7		±10.1	±9.8		±10.0	±9.5	
% of women	37.9	36.7	0.77	36.5	37.8	0.73	43.8	35.8	0.12	35.7	40.7	0.26
Family history of PD[b]**, %**	28.3	23.7	0.21	25.3	25.9	0.86	24.8	25.8	0.83	27.4	21.2	0.12
History of smoking, %	37.8	42.9	0.21	38.2	43.2	0.22	35.2	42.0	0.20	40.0	42.9	0.51
Initial Symptoms[c]**, %**												
Tremor	58.8	55.5	0.41	56.3	57.4	0.78	59.0	56.4	0.61	**61.0**	**46.5**	**0.001**
Rigidity	23.9	28.3	0.23	28.1	25.0	0.39	26.7	26.5	0.97	25.7	28.5	0.48
Bradykinesia	14.0	18.2	0.17	17.0	16.0	0.75	13.3	17.2	0.34	15.2	19.8	0.17
Gait difficulties	16.9	15.4	0.63	16.3	15.7	0.84	19.0	15.4	0.35	**13.8**	**21.5**	**0.02**
Recessive model												
Age at onset, yrs[a]	64.4	60.9	0.10	63.3	61.0	0.30	70.6	61.0	0.05[d]	62.3	60.8	0.24
± SD	±10.1	±11.0		±9.2	±11.0		±8.4	±10.9		±11.8	±10.7	
Age at enrollment, yrs[a]	69.9	69.2	0.70	68.3	69.3	0.61	78.0	69.1	0.05[d]	70.0	69.0	0.41
± SD	±10.4	±9.8		±9.7	±9.9		±7.0	±9.8		±11.1	±9.4	
% of women	28.3	37.9	0.19	39.6	37.0	0.76	42.9	37.1	0.72	36.4	37.4	0.83
Family history of PD[b]**, %**	37.8	24.6	0.07	14.6	26.6	0.08	28.6	25.6	1.0	30.8	24.1	0.13
History of smoking, %	37.8	41.1	0.75	39.6	41.0	0.88	42.9	40.8	1.0	36.6	42.0	0.27
Initial Symptoms[c]**, %**												
Tremor	47.8	57.6	0.22	52.1	57.2	0.54	57.1	56.8	1.0	58.3	56.4	0.69
Rigidity	28.3	26.4	0.73	27.1	26.4	1.0	14.3	26.6	0.68	20.5	28.2	0.08

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Variable	rs2300478			rs9357271			rs1975197			rs12593813		
	Mut	WT	p	Mut	WT	p	Mut	WT	p	Mut	WT	p
Bradykinesia	17.4	18.4	0.84	27.1	15.6	0.07	14.3	16.5	1.0	16.7	16.5	0.95
Gait difficulties	21.7	15.5	0.29	20.8	15.6	0.31	14.3	16.0	1.0	18.2	15.4	0.44

Mut, in the dominant model - carrier of at least one allele with the SNP, in the recessive model – Homozygous carrier of the SNP; WT, in the dominant model - homozygous carrier of the wild-type allele, in the recessive model – carrier of at least one wild-type allele; SD, standard deviation; yrs, years

[a] Calculated after excluding carriers of *GBA* and *LRRK2* mutations that affect the age at onset and age at enrollment

[b] Family history in this study is defined as having at least one first- or second-degree relative with PD

[c] Only symptoms with prevalence of ≥10% were included to avoid unstable estimates

[d] Since there were only five homozygous carriers of the rs1975197 SNP, a non-parametric Mann-Whitney test was done

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Table 3
RLS SNPs and risk estimates in Tremor-Dominant PD patients

SNP	MAF		Dominant model			Recessive model		
	Patients (n=167)	Controls (n=267)	OR[a]	(95%CI)	*p*	OR[a]	(95%CI)	*p*
rs2300478	0.27	0.23	1.22	0.80-1.85	0.36	1.98	0.86-4.56	0.11
rs9357271	0.24	0.29	0.88	0.58-1.33	0.54	0.49	0.21-1.15	0.10
rs1975197	0.15	0.12	1.46	0.90-2.36	0.12	4.04	0.74-22.1	0.11
rs12593813	0.40	0.39	0.94	0.61-1.44	0.77	1.03	0.59-1.81	0.92

MAF, Minor Allele Frequency; OR, Odds Ratio

[a]Adjusted for the presence of *GBA* and *LRRK2* mutations, and for the age at enrollment, since in this analysis patients and control were still matched for sex but not for age.