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Family-Based and Population-Based Association Studies Validate *PTPRD* as a Risk Factor for Restless Legs Syndrome

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Abstract

Objective—We previously mapped a genetic locus for restless legs syndrome (RLS) to chromosome 9p22–24 (*RLS3*) and a later genome-wide association study (GWAS) implicated the *PTPRD* gene at the *RLS3* locus as a susceptibility gene for RLS. However, from the standpoint of genetics, the GWAS association needs to be validated by independent studies. In this study, we used both family-based and population-based association studies to assess the association between *PTPRD* and RLS in an American Caucasian population.

Methods—We genotyped two intronic SNPs rs1975197 and rs4626664 in *PTPRD* in 144 family members from 15 families and a case control cohort of 189 patients and 560 controls. Direct DNA sequence analysis was used to screen coding exons and exon-intron boundaries of *PTPRD* for rare mutations.

Results—A family-based sibling transmission disequilibrium test showed association of RLS with SNP rs1975197 ($P = 0.015$), but not with rs4626664 ($P = 0.622$). The association with rs1975197 was significantly replicated by a population-based case control association study (allelic $P = 0.0004$, odds ratio 5.168; genotypic $P = 0.0013$ and 0.0003 for an additive and dominant model, respectively). One rare p.E1639D variant was identified in exon 39 in kindred RLS40005. The rare D1639 allele did not co-segregate with RLS in the family, suggesting that p.E1639D variant is not a causative mutation.

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Conclusions—This represents the first independent study to validate the association between *PTPRD* variants and RLS. Both family-based and population-based association studies suggest that *PTPRD* variant rs1975197 confers risk of RLS.

Keywords

PTPRD gene; sibling transmission/disequilibrium test; restless legs syndrome; family-based association study; case-control association study

Restless legs syndrome (RLS, MIM 102300) is a common neurological disorder characterized by an urge to move the limbs and is usually accompanied by uncomfortable sensations that worsen with rest, improve with movement, and are worse in the evening or night.¹ It can impair sleep and adversely affects health and quality of life. Epidemiological studies of RLS revealed a prevalence rate of 5 to 12% in Caucasian populations, which is much higher than the rate in Asian and African populations (0.1–0.6%), suggesting a possible role for racial or ethnic factors in the occurrence of RLS.² Furthermore, RLS is as much as twice more common in females than in males.³ RLS is associated with a positive family history in 45 to 65% of patients, and twin studies showed a high concordant rate in monozygotic twins.⁴ Our familial aggregation analysis showed a correlation rate of 0.17 between first degree relatives and heritability of 0.60.⁵ All these results suggest that genetic factors play an important role in the pathogenesis of RLS.

Genome-wide linkage studies have identified six genetic loci for RLS, including *RLS1* on chromosome 12q12–q21,⁶ *RLS2* on 14q13–21,⁷ *RLS3* on 9p24–p22,⁵ *RLS4* on 2q33,⁸ *RLS5* on 20p13, and *RLS6* on 16p12.1.^{9,10} Till date, no disease-causing mutations from these loci have been reported. A recent genome-wide association study (GWAS) in a Caucasian population showed that two single nucleotide polymorphisms (SNPs), including rs1975197 and rs4626664 located within introns of the *PTPRD* gene, were significantly associated with RLS.¹¹ The *PTPRD* gene is located within the *RLS3* locus reported by our group.⁵ In this study, we took advantage of the 15 large families used for identifying the *RLS3* locus to independently assess whether SNPs in *PTPRD* (ENST00000381196) are associated with RLS using a family-based sibling transmission disequilibrium test (Sib-TDT). We also evaluated the association using a population-based association study in a cohort of 189 sporadic patients and 560 controls. To the best of knowledge, this is the first study to replicate the association between variants in *PTPRD* gene and RLS. Furthermore, we scanned the sequences of all coding exons and boundaries between exons and introns of *PTPRD* gene in the 15 probands from the 15 different families to identify potential disease-causing mutations.

Subjects and Methods

Subjects

This study was approved by local institutional review boards on human subject research and written consent was obtained from the participants. All study subjects are Caucasians in the U.S. The ascertainment strategy was described in detail in our previous report.⁵ The diagnosis of study subjects was made by two expert neurologists (W.G.O. and N.F.S.) and followed the diagnostic criteria for RLS described in the 1995 IRLSSG and 2003 NIH reports.^{1,12–14} The 15 large and extended RLS families were initially used for identification of the *RLS3* locus on chromosome 9p24–22, and their detailed characteristics were described previously.⁵ The case control cohort consisted of a total of 189 RLS patients and 560 Caucasian controls. The controls were general population study subjects who were not specifically screened for the presence or absence of RLS.

Genotyping and DNA Sequencing Analyses

Genotyping of SNPs was carried out using the Taq-Man SNP genotyping assay as described by us previously.^{15–18}

All coding exons and exon-intron boundaries of the *PTPRD* gene were amplified from genomic DNA and sequenced as described previously.^{19–21}

Statistical Analysis

All SNP genotyping data were tested for Hardy-Weinberg equilibrium using a Chi-square test²² (<http://www.oege.org/software/hwe-mr-calc.sht ml>).

A sibling transmission and disequilibrium test (sib-TDT) was used to test the association between *PTPRD* SNPs and RLS in the 15 families.^{23,24} The sib-TDT was carried out for genotyping data of 15 multiplex families using the TDT/STDT program 1.1. The results from the sib-TDT were expressed as *Z* scores and *P* values that were determined by use of the two tailed normal distribution approximation.

Statistical analysis of the genotyping data from the case-control association study cohort was performed using a Pearson 2×2 or 2×3 contingency table Chi-square test for allelic association and genotypic association assuming three different inheritance models, additive, dominant or recessive, respectively (SAS version 9.0). Odds ratios (OR) and 95% confidence intervals (CI) were estimated using SAS version 9.0.

For all statistical analyses, a Bonferroni-corrected *P* value of 0.025 after adjustment for two SNPs (0.05/2) was considered to be significant.

Results

We designed a family-based study to test the previously reported association between SNPs rs1975197 and rs4626664 in the *PTPRD* gene and RLS by a population-based GWAS.¹¹ The two SNPs were genotyped in 144 study subjects from the 15 families used for linkage analysis to identify the *RLS3* locus, and a sib-TDT was used to test whether the risk allele of a SNP was preferentially transmitted to affected offspring. Our sib-TDT analysis showed that SNP rs1975197 was significantly associated with RLS ($P = 0.015$) (Table 1). However, SNP rs4626664 did not show association with RLS ($P = 0.62$) (Table 1). These results suggest that *PTPRD* SNP rs1975197 confers risk of RLS in a Caucasian population in the U.S.

We also used a population-based case control cohort of 189 RLS patients (including the 15 probands from the 15 large families) and 560 general population controls to further test the association between SNP rs1975197 and RLS. Genotypic distribution of two SNPs rs1975197 and rs4626664 in cases and controls was in Hardy-Weinberg equilibrium ($P = 0.308$ and 0.396 , respectively). Highly significant allelic association was identified between SNP rs1975197 and RLS with an odds ratio (OR) of 1.68 ($P = 0.0004$) (Table 2).

We analyzed genotypic association assuming an additive, dominant, or recessive inheritance model. SNP rs1975197 showed highly significant association with RLS in either an additive model ($P = 0.0013$) or dominant model ($P = 0.0003$), but not with a recessive model ($P = 0.2143$) (Table 3).

The allelic association between SNP rs4626664 and RLS reached a significant level ($P = 0.023$), but genotypic association did not ($P > 0.025$ in all three different models); (Table 3).

Because SNP rs1975197 in *PTPRD* showed highly significant association with RLS, we hypothesized that rare disease-causing mutations may be found in RLS families, in particular in families that showed linkage to the *RLS3* locus. The probands from the 15 families used for linkage analysis and family-based TDT were scanned for potential mutations in all coding exons and exon-intron boundaries of *PTPRD* (ENST00000381196) by direct DNA sequence analysis. We found 10 variants (Supporting Information Table 1). Five variants are located in exons and five in introns. Four of the 5 exonic variants and two of the 5 intronic variants were found in the Ensemble and Hap-Map databases. The other three intronic variants, including c.3056-111 C>G, c.3504+64delC, and c.3955-14 G>C, were not found in the HapMap database, but they were present in the general Caucasian population at a frequency of 6.1%, 13.1%, and 39.4% (minor allele, n = 312 chromosomes, Supporting Information Table 1), respectively, suggesting that the three new variants are not RLS-causing mutations.

The exonic, non-synonymous c.4917G>C variant (p.E1639D) in exon 39 was identified in only one of the 15 probands, a proband from kindred RLS40005, but not in 156 controls. We then sequenced all available DNA samples from RLS40005 and found that only 2 of 6 patients in the family, individuals 4 and 5 at the second generation, carried the rare allele (Fig. 1). The results suggest that p.E1639D variant does not cause RLS in the family.

Discussion

A recent GWAS in Germany by Schormair et al. identified two SNPs in the *PTPRD* gene, rs1975197 and rs4626664, that showed significant association with RLS.¹¹ In the same report, they sought to replicate the finding in a combined German, Austria, Czech, and Canadian population. Both SNPs showed significant association with RLS in the combined three populations, but only nominal significance in the Canadian population and a trend (not significant) for association in the Czech population. There has not been any independent replication study for this association from an external group. Here for the first time, we used the family-based association study to determine whether SNPs rs1975197 and rs4626664 are associated with RLS in 15 large and extended multiplex families. Because these 15 families are the original families used to identify the *RLS3* locus within which the *PTPRD* gene is located, they are particularly useful for evaluating the association between *PTPRD* and RLS. The results from our study share some similarities as well as differences from the GWAS. Similarly, SNP rs1975197 showed significant association with RLS by sib-TDT analysis. These results provide family-based evidence to support the association between *PTPRD* and RLS. However, in contrast, SNP rs4626664 was not associated with RLS in our family-based association studies. In fact, the German study showed that the association of SNP rs4626664 with RLS was stronger than rs1975197 with a higher OR (1.44 vs. 1.33) and a more significant *P* value (5.91×10^{-10} vs. 5.81×10^{-9}).¹¹ Based on the HapMap genotyping data (<http://hapmap.ncbi.nlm.nih.gov/>), rs1975197 and rs4626664 are located in different linkage disequilibrium (LD) blocks, it is possible that recombination events occurred between rs1975197 and rs4626664 in 15 multiplex U.S. families, but not in the German population, which may explain the difference between our results and those of the German study. It was reported that this region of the genome was prone to recombination for 17 haplotype boundaries between them.²⁵ Schormair et al. also reported that there was no significant interaction between two SNPs.¹¹

To substantiate our results from a family-based association study, we performed a population-based case control study using a U.S. cohort of 189 Caucasian RLS patients and 560 Caucasian general population controls. Interestingly, a significant Bonferroni-corrected *P* value was obtained for allelic association between SNP rs1975197 and RLS, but not for SNP rs4626664 (Table 3). Our results from the population-based association study reached a

similar conclusion as the family-based association study. Thus, we conclude that SNP rs1975197 in the *PTPRD* gene confers a significant risk of RLS in the U.S. population. Despite this significant association, we did not find any RLS-causing mutation in *PTPRD*, suggesting that a rare variant in *PTPRD* is unlikely to be a cause of RLS.

In summary, the results of our study provide the first independent genetic evidence that the *PTPRD* gene is a risk factor for RLS. In the study, we first replicated significant association between SNP rs1975197 and RLS in a family-based study. Furthermore, population-based association studies reached the identical conclusion as the family-based association studies. Therefore our study demonstrates that SNP rs1975197 in the *PTPRD* gene confers a significant risk of RLS in the U.S. population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Genotyping data of *PTPRD* variant p.E1639D in exon 39 in RLS family RLS40005. The affected individuals are shown as filled squares (males) or circles (females). Healthy individuals are shown with empty symbols, and deceased individuals are shown with “/.” The genotyping data for the *PTPRD* variant (p.E1639D) in exon 39 are shown below each symbol.

TABLE 1

Sib-TDT to assess association between two SNPs in *PTPRD* and RLS in 15 large and extended families

SNP	Genomic location	Gene	Minor allele	Z-score	P
rs1975197	Chr9:8,836,955	<i>PTPRD</i>	T	2.437	0.015
rs4626664	Chr9:9,251,737	<i>PTPRD</i>	A	0.493	0.622

TABLE 2
Analysis of allelic association of two SNPs in *PTPRD* with RLS in a case-control association study

SNP	Minor allele	OR (95% CI)	MAF		<i>P</i>
			Case	Control	
rs1975197	T	1.68 (1.26–2.25)	0.234	0.154	0.0004
rs4626664	A	0.66 (0.46–0.95)	0.112	0.16	0.0227

OR, odds ratio; CI, confidence interval; MAF, minor allele frequency.

TABLE 3 Analysis of genotypic association of two SNPs in *PTPRD* with RLS in a case-control association study

SNP	Model	P	OR (95% CI)
rs1975197	Additive	0.0013	
	Recessive	0.2143	1.8247 (0.6968–4.7781)
	Dominant	0.0003	1.8826 (1.3338–2.6571)
rs4626664	Additive	0.0774	
	Recessive	0.3263	0.5412 (0.1559–1.8785)
	Dominant	0.026	0.6380 (0.4287–0.9496)

OR, odds ratio; CI, confidence interval.