Lack of Causal Effects or Genetic Correlation between Restless Legs Syndrome and Parkinson's Disease

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Abstract

Background: Epidemiological studies have reported association between Parkinson’s disease (PD) and restless legs syndrome (RLS).

Objectives: We aimed to use genetic data to study whether these two disorders are causally linked or share genetic architecture.

Methods: We performed two-sample Mendelian randomization (MR) and linkage disequilibrium score regression (LDSC) using summary statistics from recent genome-wide meta-analyses of PD and RLS.

Results: We found no evidence for a causal relationship between RLS (as the exposure) and PD (as the outcome, inverse variance-weighted; b=-0.003, se=0.031, p=0.916, F-statistic=217.5). Reverse MR also did not demonstrate any causal effect of PD on RLS (inverse variance-weighted; b=-0.012, se=0.023, p=0.592, F-statistic=191.7). LDSC analysis demonstrated lack of genetic correlation between RLS and PD (rg=-0.028, se=0.042, p=0.507).

Conclusions: There was no evidence for a causal relationship or genetic correlation between RLS and PD. The associations observed in epidemiological studies could be, in part, attributed to confounding or non-genetic determinants.
Introduction

Restless legs syndrome (RLS) and Parkinson’s disease (PD) are common neurological disorders with a prevalence of 1.9-4.6% and 0.1-2.9% in Europeans, respectively.\textsuperscript{1, 2} Epidemiological studies suggest that RLS is more common than expected in PD patients, and PD affects RLS patients more frequently than matched controls or the general population.\textsuperscript{3} Some studies suggest that RLS may be an early clinical manifestation of PD,\textsuperscript{4-6} whereas other studies found no association between RLS and PD.\textsuperscript{3} A recent meta-analyses showed a higher odds for RLS in PD patients compared to controls.\textsuperscript{3} In this study, the previous contradictory results were explained by different inclusion and diagnostic criteria and differences in sex distribution.\textsuperscript{3} However, there are major differences between RLS and PD including clinical, ultrasonographic, functional and neuroimaging aspects, which do not support an association between RLS and PD.\textsuperscript{7-10}

Therefore, the true nature of the association between RLS and PD remains unclear. Mendelian Randomization (MR) may help mitigate some of the bias introduced by reverse causation and confounding in traditional observational studies.\textsuperscript{11} In addition, genetic correlation using linkage disequilibrium (LD) score regression (LDSC) may help determine whether different traits have overlapping genetic background, which may explain some of the observed associations between traits.

Here, we used bidirectional MR and LDSC to seek evidence for a causal relationship and/or shared genetic architecture between RLS and PD.
Methods

Study population and genetic data

To perform MR and LDSC, we used summary statistics from two recent genome-wide association study (GWAS) meta-analyses of RLS and PD. The RLS summary statistics included data from 15,126 patients and 95,725 controls, and the PD summary statistics included data from 33,674 cases (15,056 PD patients and 18,618 proxy-cases), and 449,056 controls. A subset of data (23andMe data) was not included in the PD summary statistics to avoid potential overlap with the RLS data which included 23andMe data. 23andMe participants provided informed consent and participated in the research under a protocol approved by the external AAHRPP-accreted IRB, Ethical & Independent Review Services (E&I Review). The full GWAS summary statistics for the 23andMe discovery data set will be made available through 23andMe to qualified researchers under an agreement with 23andMe that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/collaborate/#dataset-access/ for more information and to apply to access the data. Information on recruitment procedures and diagnostic criteria is detailed in the original publications. All cases and controls in this study were of European ancestry.

Power calculation

Power was calculated for detecting an effect size of odds ratio of 1.2 on RLS and PD risk, using online sample size and power calculator for Mendelian randomization with a binary outcome (https://sb452.shinyapps.io/power/). For all analyses the power was estimated at >80%.

Mendelian randomization
We performed bidirectional MR, i.e. examining whether RLS is a causal risk factor (exposure) for PD (outcome) and if PD is a causal risk factor for RLS. For each MR analysis, we constructed multi-variant instruments from the independent (“index”) GWAS significant SNPs (p<=5e-08) from the exposure GWAS. In brief, index SNPs were obtained by clumping all GWAS significant SNPs within each LD block using an R^2 threshold of 0.001 or a distance of 10,000 kb from the index SNP. This process increased the independence of each index SNP based on the above parameters. Additional details regarding the instrument construction and the code used for the analysis are available at https://github.com/gan-orlab/MR_LDSC_RLS-PD.

To calculate the proportion of variability in the exposure explained by the SNPs and to test the strength of the instrument variables (IVs), we used the statistical power for MR analyses (the coefficient of determination, R^2) and F-statistics tests, as previously described.15 In order to perform MR, an estimate of the individual effect of SNPs on the exposure and outcome (RLS and PD, interchangeably) was used to calculate the Wald ratio. Then, the effect estimates were combined using the Inverse-variance weighted (IVW) method, which is a weighted mean of the Wald ratio estimates obtained from each individual SNP separately.16

**Sensitivity analyses**

To explore whether IVW results might be biased due to violations of MR assumptions and to evaluate the robustness of the results, we used weighted median and MR Egger16 estimators as sensitivity analyses. The weighted median estimate provides a reliable pooled estimate assuming that at least half of the weight of the SNPs in the instrument are valid. MR Egger assesses directional pleiotropy similarly to the IVW approach except that the regression slope y-intercept is not constrained to pass through the origin. For each approach, we constructed funnel plots to detect outliers. We evaluated the heterogeneity statistics Q for IVW and Q′ for MR-Egger.
Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO)\textsuperscript{17} was used to examine outlier SNPs which might occur in the presence of horizontal pleiotropy and correct pooled estimates. Steiger filtering was used to discard SNPs that explain more variance in the outcome than in the exposure. To find all the SNPs that are in LD with the index SNP, the LDmatrix module on the LDlink web tool was used.\textsuperscript{18}

Genetic correlation analyses

To assess the genetic correlation between RLS and PD, we performed LDSC after computing z-scores and formatting data from the two GWASs as previously described.\textsuperscript{19, 20} In brief, LDSC calculates genetic correlation between two traits by incorporating LD scores (the more variants in LD with a SNP, the higher the LD score) and GWAS summary statistics (z scores) in a regression model.
Results

In total, 20 and 55 index SNPs for RLS and PD, respectively, were initially used as IVs for exposure. These IVs explain 3.5% and 2.1% of the risk in RLS and PD. All SNPs were strong instruments for MR analysis as measured by F-statistics (RLS F-statistics=217.5; PD F-statistics=191.7). There was no overlap between the genes where the clumped SNPs are located in both meta-analyses (Supplementary Table 1).

We then performed MR analyses to assess the bidirectional causal relationship between RLS and PD. RLS, as the exposure, was not causally associated with PD (IVW; b=-0.051, se=0.037, \( p = 0.172 \)). However, the \( p \) values of IVW-Q and MR Egger-Q' tests were 0.034 and 0.025, respectively, raising the possibility of pleiotropic SNP(s) in our dataset, which violates MR assumptions. MR-PRESSO\(^{17}\) was applied and a pleiotropic index SNP, rs11860769 \( (p=0.02) \) was identified when RLS was used as exposure. This SNP has an opposite effect on risk of RLS and PD as was previously shown.\(^{21}\) After removing the pleiotropic index SNP (rs11860769), 19 index SNPs were used as IVs for RLS, respectively. Again, there was no causal effect of RLS on PD \( (b=-0.003, \ se=0.031, \ p=0.916) \) or of PD on RLS \( (b=-0.012, \ se=0.023, \ p=0.592) \) with 55 IVs, and the results of sensitivity analyses suggested that there were no additional deviations from the MR assumptions (Table 1, Figure 1, Supplementary Figure 1,2).

We then sought to examine whether there is genetic correlation between RLS and PD that may explain the overrepresentation of these disorders in one another. There was no genetic correlation between RLS and PD \( (r_g=-0.028, \ se=0.042, \ p=0.507) \).
Discussion

Our findings suggest lack of causal relationship between RLS and PD, and lack of genetic correlation. One locus on chromosome 16, including the genes *TOX3* and *CASC16*, is pleiotropic with opposite direction of effect, as SNPs associated with increased risk of RLS are associated with reduced risk of PD, as previously reported.\(^\text{21}\) Therefore, this locus also cannot explain the observed increased frequency of PD in RLS and of RLS in PD.

Although RLS and PD co-occur at a rate higher than expected and share several traits such as dopaminergic treatment response, multiple lines of evidence have shown differences between RLS and PD from a pathophysiological perspective. PD arises from the loss of dopaminergic neurons in the substantia nigra, whereas in RLS there is no loss of dopaminergic cells, no reduced levels of dopamine,\(^\text{22}\) and instead increased presynaptic dopaminergic activity.\(^\text{23}\) The neuronal loss may explain the elevated level of iron (seen as hyperechogenicity in transcranial sonography) and impairment in motor performance in PD versus reduced iron content (hypoechogenicity in transcranial sonography) and normal motor function in idiopathic RLS.\(^\text{3, 24, 25}\).

Our LDSC analyses showed lack of genetic correlation between RLS and PD. Similarly, various genetic studies found no association between known RLS-associated variants and PD in the *BTBD9*,\(^\text{26, 27}\) *MAP2K5/SKOR1*,\(^\text{26, 27}\) *MEIS1*,\(^\text{26, 27}\) and *PTPRD*\(^\text{26}\) loci. In a study of two Italian families, 10 of 20 RLS patients carried compound heterozygous or single heterozygous *PRKN* variants. It is not clear if these variants are pathogenic, and the clinical symptoms did not differ between RLS patients with and without *PRKN* variants in these 2 families, indicating that their presence was likely random.\(^\text{28}\) In an Asian cohort of 80 PD patients, one patient with a homozygous *PINK1* mutation presented features of RLS, but two other unrelated PD patients
with *PINK1* mutations in the same cohort did not show RLS features.\(^{29}\) In a study of 258 RLS patients vs 235 healthy controls, the authors reported that the *SNCA* Rep1 allele was associated with reduced risk of RLS.\(^{30}\) However, this association was not replicated by the much larger RLS GWAS.\(^{12}\) Overall, genetic studies, including the current study, do not support a genetic overlap between RLS and PD.

Our study has some limitations. We could not exclude PD patients with RLS and RLS patients with PD in the datasets used for this analysis, which would have made the results cleaner, since these data was not available. In addition, this study focused on individuals of European ancestry. Studies from multiple ethnicities are required to further study PD, RLS and the association between them. It is possible that rare or structural variants outside of what can be detected with current GWAS technologies are contributing to a shared genetic etiology.

In light of the current and previous findings, it is likely that confounding factors such as treatment, closer neurological follow up and others may have contributed to the observed epidemiological association between RLS and PD. While additional studies are required to identify these potential confounders, the observed association between RLS and PD should not be considered causal on current evidence.
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Authors’ Roles

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

MAE: 1A, 1B, 1C, 2A, 2B, 3A
KS: 1C, 2A, 2B, 2C, 3B
EY: 1C, 2B, 2C, 3B
PV: 2B, 2C, 3B
LK: 2A, 3B

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SB: 2B, 3B
AJN: 1C, 2B, 2C, 3B
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References

1. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Movement disorders 2014;29(13):1583-1590.
2. Ohayon MM, O'Hara R, Vitiello MV. Epidemiology of restless legs syndrome: a synthesis of the literature. Sleep medicine reviews 2012;16(4):283-295.
3. Alonso-Navarro H, García-Martín E, Agúndez JA, Jiménez-Jiménez FJ. Association between restless legs syndrome and other movement disorders. Neurology 2019;92(20):948-964.
4. Gao X, Schwarzschild MA, O'Reilly EJ, Wang H, Ascherio A. Restless legs syndrome and Parkinson's disease in men. Movement disorders 2010;25(15):2654-2657.
5. Wong JC, Li Y, Schwarzschild MA, Ascherio A, Gao X. Restless legs syndrome: an early clinical feature of Parkinson disease in men. Sleep 2014;37(2):369-372.
6. Szatmari Jr S, Bereczki D, Fornadi K, Kalantar-Zadeh K, Kovesdy CP, Molnar MZ. Association of restless legs syndrome with incident Parkinson’s disease. Sleep 2017;40(2):zw065.
7. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JA. Neurochemical features of idiopathic restless legs syndrome. Sleep medicine reviews 2019;45:70-87.
8. Ryu JH, Lee MS, Baik JS. Sonographic abnormalities in idiopathic restless legs syndrome (RLS) and RLS in Parkinson’s disease. Parkinsonism & related disorders 2011;17(3):201-203.
9. Alberts J, Adler S, Saling M, Stelmach G. Prehension patterns in restless legs syndrome patients. Parkinsonism & related disorders 2001;7(2):143-148.
10. Ferini-Strambi L, Carli G, Casoni F, Galbiati A. Restless legs syndrome and Parkinson disease: a causal relationship between the two disorders? Frontiers in neurology 2018;9:551.
11. Kia DA, Noyce AJ, White J, et al. Mendelian randomization study shows no causal relationship between circulating urate levels and Parkinson's disease. Annals of neurology 2018;84(2):191-199.
12. Schormair B, Zhao C, Bell S, et al. Identification of novel risk loci for restless legs syndrome in genome-wide association studies in individuals of European ancestry: a meta-analysis. The Lancet Neurology 2017;16(11):898-907.
13. Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. The Lancet Neurology 2019;18(12):1091-1102.
14. Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. International journal of epidemiology 2014;43(3):922-929.
15. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. International journal of epidemiology 2011;40(3):755-764.
16. Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: A review. Research synthesis methods 2019;10(4):486-496.
17. Verbanck M, Chen C-y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nature genetics 2018;50(5):693-698.
18. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics 2015;31(21):3555-3557.
19. Bulik-Sullivan BK, Loh P-R, Finucane HK, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nature genetics 2015;47(3):291-295.
20. Bulik-Sullivan B, Finucane HK, Anttila V, et al. An atlas of genetic correlations across human diseases and traits. Nature genetics 2015;47(11):1236.
21. Mohtashami S, He Q, Ruskey JA, et al. TOX3 variants are involved in restless legs syndrome and Parkinson’s disease with opposite effects. Journal of Molecular Neuroscience 2018;64(3):341-345.
22. Pittock SJ, Parrett T, Adler CH, Parisi JE, Dickson DW, Ahlskog JE. Neuropathology of primary restless leg syndrome: Absence of specific \( \tau \) and \( \alpha \) synuclein pathology. Movement disorders: official journal of the Movement Disorder Society 2004;19(6):695-699.
23. Connor JR, Wang X-S, Allen RP, et al. Altered dopaminergic profile in the putamen and substantia nigra in restless leg syndrome. Brain 2009;132(9):2403-2412.
24. Connor JR, Boyer P, Menzies S, et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. Neurology 2003;61(3):304-309.
25. Connor JR, Wang X, Patton S, et al. Decreased transferrin receptor expression by neuromelanin cells in restless legs syndrome. Neurology 2004;62(9):1563-1567.
26. Gan-Or Z, Alcalay RN, Bar-Shira A, et al. Genetic markers of restless legs syndrome in Parkinson disease. Parkinsonism & related disorders 2015;21(6):582-585.
27. Vilariño-Güell C, Soto A, Young J, et al. Susceptibility genes for restless legs syndrome are not associated with Parkinson disease. Neurology 2008;71(3):222-223.
28. Adel S, Djarmati A, Kabakci K, et al. Co-occurrence of restless legs syndrome and Parkin mutations in two families. Movement disorders 2006;21(2):258-263.
29. Tan EK, Yew K, Chua E, et al. PINK1 mutations in sporadic early-onset Parkinson's disease. Movement disorders 2006;21(6):789-793.
30. Lahut S, Vadasz D, Depboylu C, et al. The PD-associated alpha-synuclein promoter Rep1 allele 2 shows diminished frequency in restless legs syndrome. Neurogenetics 2014;15(3):189-192.
31. Di Angelantonio E, Thompson SG, Kaptoge S, et al. Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. The Lancet 2017;390(10110):2360-2371.
Legends

**Figure 1.** Forest plots showing results from the Mendelian randomization study to evaluate the potential causal relationships between RLS and PD. **A.** Forest plot showing point estimates of RLS as an exposure on PD (outcome). In total, 19 index SNPs were left after excluding the pleiotropic SNPs to construct instrument variables. The black dots represent the causal estimate ($b = \log \text{odds ratio}$) of each SNP on the risk of PD. Red dots represent the causal estimate when combining all SNPs together, using MR Egger and IVW methods. Horizontal lines denote 95% CI. **B.** Forest plot showing point estimates of PD as an exposure on RLS (outcome). The instrument variables were constructed by 55 index SNPs. The black dots represent the causal estimate ($b = \log \text{odds ratio}$) of each SNP on the risk of RLS. Red dots represent the causal estimate when combining all SNPs together, using MR Egger and IVW methods. Horizontal lines denote 95% CI.
Supplementary Tables and Figures

Supplementary Table 1. SNPs selected as instrumental variables after clumping.

Supplementary Figure 1. Plots showing results from the Mendelian randomization study to evaluate the potential effect of RLS on PD. A. Funnel plot indicating estimates of the exposure (RLS) by comparison of results using different MR methods. The effect estimate of the SNP-exposure (RLS) association and the SNP-outcome (PD) associations with standard error bars. Each line corresponds to causal estimates calculated by each MR method. B. Funnel plot showing the heterogeneity across the estimates when RLS is exposure. SNPs are represented by dots. IVW and MR Egger computed the average causal effect of all the SNPs. C.

Supplementary Figure 2. Plots showing results from the Mendelian randomization study to evaluate the potential effect of PD on RLS. A. Funnel plot indicating estimates of the exposure (PD) by comparison of results using different MR methods. The effect estimate of the SNP-exposure (PD) association and the SNP-outcome (RLS) associations with standard error bars. Each line corresponds to causal estimates calculated by each MR method. B. Funnel plot showing the heterogeneity across the estimates when PD is exposure. SNPs are represented by dots. IVW and MR Egger computed the average causal effect of all the SNPs.
Table 1. MR analysis between RLS and PD.

| Exposure | Outcome | F     | R²   | MR-PRESSO | Inverse variance weighted | MR Egger | Weighted median |
|----------|---------|-------|------|-----------|---------------------------|----------|-----------------|
|          |         |       |      |           | b  | se | P  | Q test | b  | se | P  | Q test |
| RLS      | PD      | 217.58 | 0.035 | 0.832     | -0.003 | 0.031 | 0.916 | 0.780 | -0.019 | 0.064 | 0.767 | 0.729 | -0.020 | 0.043 | 0.632 |
| PD       | RLS     | 191.79 | 0.0315 | 0.662     | -0.012 | 0.023 | 0.592 | 0.300 | -0.002 | 0.050 | 0.958 | 0.269 | -0.011 | 0.036 | 0.749 |

RLS, restless legs syndrome; PD, Parkinson’s disease; F, ‘strength’ of the genetic instrumental variable; R², proportion of variance in exposure variable explained by SNPs; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; MR, Mendelian randomization; b, beta; se, standard error; Q, Cochran’s Q test.
