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Assessment of *LIN28A* variants in Parkinson's disease in Large European Cohorts

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Abstract

Parkinson's disease (PD) is a complex neurodegenerative disease with a strong genetic component. To date, several genes have been associated with monogenic forms of the disease but these only explain a small fraction of the observed familial aggregation in PD. Recently, a heterozygous loss-of-function variant in *LIN28A* was associated with PD pathogenesis in the Asian population. Here, we comprehensively investigate the role of *LIN28A* variants in PD patients of European ancestry and assess susceptibility using individual-level genotyping data from 14,671 PD cases and 17,667 controls, as well as whole-genome sequencing data from 1,647 PD patients and 1,050 controls. Additionally, we further assessed the summary statistics from the most recent GWAS meta-analyses to date for PD risk and age at onset. After evaluating these data, we did not find evidence to support a role for *LIN28A* as a major causal gene for PD. However, additional large-scale familial and case-control studies in non-European ancestry populations are necessary to further evaluate the role of *LIN28A* in PD etiology.

Keywords

Parkinson's disease; risk factor; *LIN28A*; loss of function

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Disclosure statement

The authors declare that they have no conflict of interest.

Introduction

Parkinson's disease (PD) is a complex neurodegenerative disorder with a strong genetic component in which both rare and common genetic variants contribute to disease risk, onset and progression. Mutations in several genes have been associated with familial forms of disease, most of which are highly penetrant rare variants resulting in early onset presentation. Although significant progress in understanding the genetic basis of PD has been made, validation of novel genes associated with PD remains extremely challenging (Bandres-Ciga et al., 2020; Blauwendraat et al., 2020).

We read with great interest the recently published article by *Chang and colleagues* in which the authors report and claim that a rare variant in *LIN28A*, which encodes for a highly conserved RNA-binding protein (Lin28a), contributes to PD pathogenesis (Chang et al., 2019). Through whole exome sequencing, the authors identified a heterozygous missense variant in *LIN28A* (p.Arg192Gly, rs558060339), shown to result in gene loss-of-function (LOF), in two patients of East Asian ancestry with early-onset PD (EOPD). In addition, midbrain dopamine (mDA) neurons differentiated from patient-derived human embryonic stem cells showed developmental defects and PD-specific pathological features that could be rescued by expression of wild-type Lin28a. Indeed, previous work from the same group had shown that loss of *LIN28A* function could ultimately lead to a PD-like phenotype coupled with mDA neuronal loss (Rhee et al., 2016).

There are some known similarities and differences in the genetic contributions of PD on a population genetics scale. An example of this is the identification of the p.Arg1628Pro and p.Gly2385Arg *LRRK2* mutations found only in Asian-specific cohorts, whereas European cohorts identified the p.G2019S *LRRK2* variant. Studies looking at the genetic risk of PD in European populations identified the *MAPT* H2 haplotype as protective, which was absent in Asian populations (Foo et al. 2020). Here, we sought to investigate the role of *LIN28A* variants in PD susceptibility in Europeans as part of the International Parkinson's Disease Genomics Consortium (IPDGC) efforts to screen reported risk factors for PD.

Methods

Cohorts description

We used publicly available whole-genome sequencing (WGS) data including 1,647 PD patients without known disease-causing mutations (mean age at onset (AAO) 64.2 ± 9.6) and 1,050 controls (mean age 60.3 ± 11.9) from the Accelerating Medicines Partnership - Parkinson's disease initiative (AMP-PD; www.amp-pd.org). All individuals were of white European descent and were obtained from three different cohorts (Biofind (https://biofind.loni.usc.edu/), Parkinson's Disease Biomarker Program (PDBP; https://pdbp.ninds.nih.gov/), and Parkinson's Progression Markers Initiative (PPMI; https://www.ppmi-info.org/) included in the AMP-PD resource. Among the PD cases, 145 (8.8%) had EOPD (mean AAO 45.2 ± 4.6) and 527 (32%) had a family history. More detailed cohort characteristics as well as quality control procedures are further described in https://amp-pd.org/whole-genome-data.

Additionally, we analyzed individual-level genotyping data from genome-wide association studies (GWAS) from the International Parkinson's Disease Genomics Consortium (IPDGC) consisting of 14,671 PD cases and 17,667 neurologically healthy individuals of white European ancestry. Quality control procedures on both individual and variant levels were performed before imputation as previously described (Nalls et al. 2019; Blauwendraat et al. 2019). We further assessed data from the most recent GWAS meta-analyses to date (excluding 23andMe data) for PD risk consisting of 15,056 PD cases, 18,618 UK Biobank proxy-cases (i.e., subjects with a first degree relative with PD) and 449,056 controls (Nalls et al., 2019), as well as for AAO comprising 17,996 PD cases (Blauwendraat et al., 2019). All participants were of European ancestry and details on genotyping quality and imputation are described in the original sources (Blauwendraat et al., 2019; Nalls et al., 2019). Summary statistics used in this study are public and available via https://pdgenetics.org.

For both sequencing and genotyping and datasets, the entire *LIN28A* region (NM_024674) was annotated using ANNOVAR (Wang et al., 2010) and variant allele frequencies were determined using PLINK v1.9 (Chang et al., 2015). Additional allele frequencies were obtained from the Genome Aggregation Database (gnomAD v2.1.1; https://gnomad.broadinstitute.org/) (Lek et al., 2016).

Statistical analyses

Association analyses of *LIN28A* variants and risk for PD were done using single-variant score tests in RVTESTS package v.2.1.0 (Zhan et al., 2016) for sequencing data and logistic regression for genotyping data. All analyses were adjusted by sex, age, family history, education level, 10 principal components (PCs) to account for population stratification, and cohort to control for chip bias.

To assess the cumulative effect of multiple rare variants on the risk for PD, we performed gene-based burden analyses (SKAT, sequence Kernel association tests; and SKAT-O, optimized SKAT) on WGS data. We used the RVTESTS package v.2.1.0 following default parameters (Zhan et al, 2016) and adjusted for covariates age, gender, 10 PCs and dataset to control for chip bias. All code used can be found here https://github.com/ipdgc/IPDGC-Trainees/blob/master/LIN28A.md.

Results

We identified a total of 83 variants within the *LIN28A* gene in WGS data, of which only three were coding, including two synonymous (p.Lys127Lys, p.Pro205Pro) and one non-synonymous variant (p.Thr189Ile) (Table 1). All three coding variants are extremely rare (MAF ≤ 1%) according to public databases. Case-control association testing using single-variant score test adjusted by covariates (including sex, age, family history, education level, 10 principal components, and cohort) showed no significant differences in the frequency of *LIN28A* variants between PD cases and controls after Bonferroni correction (significance threshold = 0.05/83 = 6.02E-04) (Table 1, Supplementary Table 1). Gene-based burden analyses did not detect an enrichment of rare variants in PD cases versus controls (Supplementary Table 2).

Similarly, we performed a case-control association analysis using logistic regression in imputed individual-level genotyping data from IPDGC. All 34 *LIN28A* variants found were non-coding, including 28 intronic and six in the 3'-untranslated region (3'-UTR). None of the variants were significantly associated with the disease (Supplementary Table 3). We further analyzed summary statistics from the above-mentioned risk and AAO PD GWAS meta-analyses. No evidence for an association between *LIN28A* common genetic variation and PD was detected for either PD risk or AAO (Supplementary Figure 1; Supplementary Table 4). Among the 97 *LIN28A* variants identified within summary statistics, 81 were intronic, 14 were in the 3'-UTR, and only two were coding non-synonymous variants (p.Arg123Gln, p.Thr189Ile) (Supplementary Table 4). A total of 46 variants (47.4%) were rare (MAF ≤ 3%). Of note, we did not find the original reported mutation (NC_000001.10:g.26752893C>G, p.Arg192Gly) by *Chang and colleagues* in any of our datasets.

We also explored the frequency of LOF variants in *LIN28A* by looking at the gnomAD database v.2.1.1 (https://gnomad.broadinstitute.org/), which comprises a total of 125,748 exomes and 15,708 genomes. Six LOF variants were identified (four stop-gain and two frameshift, all heterozygous) resulting in an estimate of 0.0056% of the population having haploinsufficiency for *LIN28A*. The reported p.Arg192Gly variant was identified in six Korean individuals out of a total of 1909 included, setting the allele frequency at 0.15% in the general population.

Discussion

In conclusion, based on the current data presented, our analyses do not support a role for *LIN28A* variants as a major causal gene or risk factor for PD. However, the vast majority of our data is of European ancestry, in which the p.Arg192Gly variant is not present according to the gnomAD database, and we have a relatively small number of EOPD cases in the cohorts used. Noteworthy the frequency of this variant is relatively high in the Korean population (0.15%). Overall, we cannot rule out a potential role of very rare variants in *LIN28A* in PD among other populations. Additional large-scale familial and case-control studies in non-European ancestry populations are necessary to further evaluate the role of *LIN28A* in PD etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

LIN28A coding variants in whole-genome sequencing data from PD and controls.

Variant	rsID	Frequency (gnomAD)*	Frequency (cases)	Frequency (controls)	OR	95% CI	P-value
p.Lys127Lys	rs138964629	0.0008	0.0009107	0	NA	NA	NA
p.Thr189Ile	rs201124162	0.0007	0.0009107	0.0004762	2.26	−1.70 – 3.33	0.524
p.Pro205Pro	rs201055562	0.0009	0.0006072	0.0009524	0.45	−3.21 – 1.63	0.521

Key: CI, confidence interval; OR, odds ratio.

*Allele frequencies according to Non-Finnish European gnomAD exomes.