Comprehensive Analysis of LIN28A in Chinese Patients with Early Onset Parkinson's Disease

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Research Article

Keywords: LIN28A, Early onset Parkinson's disease, Rare variants, Burden analysis

Posted Date: March 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-326030/v1

Abstract

A loss-of-function variant in Lin-28 Homolog A gene (*LIN28A* p.R192G, rs558060339) has been identified in two East Asian ancestry patients with early-onset PD (EOPD). Functional studies revealed that such variant could lead to developmental defects and PD-related phenotype, and the phenotypes could be rescued after correction of the variant. The aim of the study was to screen the variants of *LIN28A* in Chinese patients with EOPD. A total of 682 EOPD patients were sequenced with whole exome sequencing and the coding and flanking region of *LIN28A* were analyzed. We identified a rare coding variant-p.P182L of *LIN28A* in a Chinese patient with EOPD. Moreover, we also found a 3'-UTR polymorphism(rs4659441) to be associated with an increased risk for PD. However, our rare variant burden analysis did not support a role for *LIN28A* as a major causal gene for PD.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease with a complex spectrum of etiologies including aging, environmental factors and genetic causes[1]. So far, more than 30 genes have been identified to be causative genes for PD, however, which could only explain a small proportion of patients[2].

With the development of genetic sequencing methods and bioinformatic analysis algorithms, novel PD causative genes have been extensively explored in recent years, and Lin-28 Homolog A gene (*LIN28A*) is one of them. LIN28A is a highly conserved RNA-binding protein, which is mainly expressed in the nervous system during early embryonic and fetal development and involving in the neuronal differentiation, tissue repair and the maintenance of synaptic plasticity[3, 4]. Moreover, previous studies have also indicated that LIN28A might play a role in the pathogenesis of PD. For example, a previous study showed that LIN28A exhibit a strong therapeutic potential in the cell model and mouse model of PD[5]; a loss-of-function variant in the *LIN28A* (p.R192G, rs558060339) was identified in two East Asian ancestry patients with early-onset PD (EOPD)[6]; developmental defects and PD-related phenotype due to the variant was rescued after correction of the variant[6]. However, a subsequent study conducted in a large cohort of PD patients of European ancestry failed to find the evidence supporting the causative role of *LIN28A* in PD[7]. There exists genetic heterogeneity among different regions. EOPD is more susceptible to genetic factors. Therefore, it is necessary to further investigate the role of *LIN28A* in EOPD.

Methods

Patients

A total of 682 EOPD patients (age of onset ≤ 45y) admitted to the Department of Neurology, West China Hospital were recruited into the study. All the PD patients were diagnosed by experienced neurologists based on the established clinical diagnostic criteria for PD [8, 9]. Data of demographic, clinical characteristics and rating scales assessments for patients were collected by face-to-face interview as previously described[10]. Written informed consent was obtained from all participants. The study was approved by the ethics committee of West China Hospital, Sichuan University.

Variant selection

Genomic DNA was collected from peripheral blood leukocytes and underwent whole exome sequencing (WES). Procedures of WES and variants annotation were performed as previously described[10]. All variants in the coding region and the flanking region of *LIN28A* captured by WES was included in the analysis. For the pathogenicity analysis, patients with mutations in known PD causative genes were excluded first.

Rare variants burden analysis

Controls were from the gnomAD East Asian population (https://gnomad.broadinstitute. org/). All the rare coding variants annotated as "missense", "splice donor", "splice acceptor", "splice region", "stop-gained" or "in-frame deletion" were included. Five different algorithms method were used for burden analysis independently (***Supplementary Methods***).

Results

The coding region and the flanking region of *LIN28A* were captured by the WES. The likely pathogenic variant p.R192G was not detected in our cohort. Totally, 9 variants were detected in our cohort, including 6 intronic variants, a variant in the 3'-untranslated region(3'-UTR), a synonymous variant in exon 3 (c.270T>A, p.G90=) and a missense variant in exon 4 (c.545C>T, p.P182L) (***Table 1***). The frequency of the missense variant is 0.0001(2/17248) in the gnomAD East Asian controls. The variant was predicted to be tolerated among several in silico prediction tools. Therefore, it was considered to be a variant of uncertain significance (VUS) [11]. The patient carrying the p.P182L developed tremor and rigidity at the age of 16. He first visited our clinic after disease onset 7 years, and was comprehensively assessed. His UPDRS Part III score was 50, Horhn-Yahr stage was stage 3, and cognition was normal.

Besides the coding variants, there were 7 non-coding variants in the flaking region being identified. In the allelic level, rs4659441 in the 3'-UTR were found to be both associated with an increased risk for EOPD. However, in the gene-based rare variant burden analysis, there was no significant enrichment of rare coding variants in Chinese patients with EOPD when compared with the gnomAD East Asian controls (***Supplementary table 1 and 2***).

Discussion

In the current study, we identified a rare coding variant p.P182L in the *LIN28A* in a Chinese patient with juvenile onset PD. Moreover, we found a polymorphism in the 3'-UTR to be associated with an increased risk for PD. However, rare coding variant burden analysis did not support *LIN28A* is a major causative gene for PD.

The rare coding variant p.P182L identified in our study, together with the previously described loss-of-function variant p.R192G found in 2 East Asian ancestry patients with EOPD[6], and the rare coding variant p.T189I identified in a European PD patient [7], were all located in the exon 4 of *LIN28A*, which is the C-terminal domain, distal to an RNA- binding Zn-knuckle domain (residues 138–176)[6]. Therefore, exon 4 might be a mutated hot spot for *LIN28A* and more functional studies are needed. Our patient carrying p.P182L had a much early age of onset (at the age of 16) than that of the Korea patient carrying p.R192G (at the age of 23 and 60).

Besides the rare coding variants identified in the current study, rs4659441 in the 3'-UTR was found to be associated with an increased risk for EOPD. Although untranslated, the 3'-UTR is important in the regulation of mRNA-based processes including mRNA localization, mRNA stability and translation[12]. More specifically, 3'UTR can provide binding site to certain miRNAs and lead to the mRNA degradation, therefore inhibiting gene expression[12]. Via bioinformatic tools (http://www.mirbase. org/search-rnacentral.shtml)[13], we found that rs4659441 was a binding site for both hsa-miR-4476 and hsa-miR-505-5p. Interestingly, hsa-miR-505-5p, also known as hsa-miR-505*, has been found to be alter-regulated in a wide spectrum of neurological disorders including multiple sclerosis, myasthenic gravis, Alzheimer's disease, Fredrich Ataxia and Lacunar stroke (http://bio-bigdata.hrbmu.edu.cn/ nsdna/map.jsp?organism= Homo%20sapiens). In relation to PD, hsa-miR-505-5p has been found to be upregulated in the cell model of PD[14], and in the brain of progressive supranuclear palsy, a parkinsonism plus syndrome [15].Therefore, it can be speculated that the hsa-miR-505-5p could regulate the expression of *LIN28A* via binding to the polymorphism of rs4659441 in the 3'-UTR, and thus contributing to the pathogenesis of PD. However, further functional studies are needed to elucidate.

Last but not least, burden analysis is a method calculating the aggregated effect of a gene on some disease. However, our findings using mathematical methods did not indicate the enrichment of rare variants in *LIN28A* in EOPD patients ,which is in consistent with the negative findings from the European population[7].

Conclusions

In conclusion, our findings of a rare coding variant-p.P182L expanded the mutation spectrum of *LIN28A* in EOPD. Moreover, we also found a risk allele for EOPD in the 3'-UTR. However, our rare variant burden analysis together with the findings from the European population did not support that *LIN28A* plays a major causative role for PD. More studies in different genetic backgrounds are needed.

Declarations

Funding: This work was supported by the National Key Research and Development Program of China (Grant No.2016YFC0901504), the 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (ZYJC18038 and ZY2016203) and the Science Foundation of Chengdu Science and Technology Bureau (2019-YF05-00307-SN).

Conflicts of interest/competing interest: All the authors declared no conflict of interest.

Availability of data and material All the correspondence and material requests should be addressed to prof. Huifang Shang at hfshang2002@163.com.

Code availability: Not applicable.

Author roles

X.J.G: the conception **and** design of the work; the acquisition, analysis, interpretation of data; drafted the work;

Y.B.H: the acquisition, analysis and interpretation of data;

Y.P.C: the acquisition, analysis and interpretation of data;

R.W.O: the acquisition and analysis of data;

B.C: the acquisition and analysis of data;

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Y.W: the acquisition and analysis of data;

C.Y.L: the acquisition and analysis of data;

H.F.S: the conception **and** design of the work; interpretation of data; substantively revised the work.

Ethics approval: The study was approved by the ethics committee of West China Hospital, Sichuan University.

Consent to participate Written informed consent was obtained from all participants.

Acknowledgement

The authors thank all the patients for their participation. This work was supported by the National Key Research and Development Program of China (Grant No.2016YFC0901504), the 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (ZYJC18038 and ZY2016203) and the Science Foundation of Chengdu Science and Technology Bureau (2019-YF05-00307-SN).

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Tables

Table1: The description of variants in *LIN28A* identified in the EOPD patients.

	gDNA	AA change	dbSNP147	Frequency in patients	Frequency in gnomAD EAS	class	p	OR
1:26738110	C>G		rs202211941	0.00586(8/1364)	0.00013(27/15378)	intronic	0.006	3.354(1.521-7.398)
1:26738112	T>C	-	-	0.00073(1/1364)	-	intronic	-	-
1:26738117	G>A	-	-	0.00073(1/1364)	-	intronic	-	-
1:26738122	C>T		rs4623750	0.06871(94/1364)	0.08406(1250/14870)	intronic	0.052	0.806(0.649-1.002)
1:26738123	delA	-	-	0.00073(1/1364)	-	intronic	-	-
1:26751835	T>A	p.Gly90=	-	0.00073(1/1364)	-	synonymous	-	-
1:26751993	G>A		rs187064721	0.00147(2/1364)	0.00413(71/17184)	intronic	0.052	0.177(0.025-1.274)
1:26752864	C>T	p.P182L	rs769630938	0.00073(1/1364)	0.00011(2/17248)	missense	0.204	6.362(0.573-69.814)
1:26752992	T>C		rs4659441	0.15322(209/1364)	0.11879(1947/16390)	3'-UTR	<0.001	1.132(1.150-1.567)

Adjust p=0.05/9=0.0055.

Supplementary Files

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