Enrichment of Rare Variants in E3 Ubiquitin Ligase Genes in Early Onset Parkinson’s Disease

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Research

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Abstract

Background

Dysfunction of the ubiquitination proteasome system (UPS) is important in the pathogenesis of Parkinson's disease (PD). Patients with early onset PD (EOPD) are more susceptible to genetic factors. We systematically examined the overlaps between E3 ubiquitin ligase genes and EOPD.

Methods

A total of 695 EOPD patients were sequenced with whole exome sequencing. Aggregate burden for rare variants (Minor allele frequency <0.001 and <0.0001) in a total of 44 E3 ubiquitin ligase genes causing disorders involved in the nervous system were analyzed.

Results

There was significant enrichment of the rare and rare damaging variants in the E3 ubiquitin ligase genes in EOPD patients. Detailly, at the gene-based level, the strongest associations were found in HERC1, IRF2BPL, KMT2D, RAPSN, RLIM, RNF168 and RNF216.

Conclusion

Our findings highlight the importance of the UPS mechanism in the pathogenesis of PD from the genetic perspective. Moreover, our study also expanded the susceptible gene spectrum for PD.

Background

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by cardinal motor symptoms including bradykinesia, rigidity, tremor and postural instability, as well as various non-motor symptoms such as cognitive decline, autonomic dysfunction and psychiatric problems[1]. PD is considered to be a complex disorder caused by genetic factors, environmental factors and aging together. So far, approximately 30 genes have been found to be causative genes for PD but can only explain a small proportion of the etiology of PD[2], which highlights the importance of discovering the remaining responsible genes for PD as well as exploring the underlying mechanisms.

The pathological hallmark of PD is the loss of dopamine neurons and the abnormal accumulation of α-synuclein protein in form of Lewy bodies[3]. Although the pathological mechanisms of PD remain largely unknown, protein degradation and quality control were implicated to be dysregulated in PD and ultimately leading to the abnormal protein deposition[4]. The ubiquitination proteasome system (UPS) is one of the important pathways for protein clearance[4]. Pathologically, ubiquitin, the critical modifier that tagged proteins for degradation, has been found in Lewy bodies[5]. Genetically, several PD causative genes have also been found to be involved in UPS, including Parkin, PINK1, UCHL1 and FBXO7[6]. Therefore, these evidences strongly implicated altered ubiquitin signaling and disrupted protein quality control involved in PD.

Ubiquitination is a biological process requiring the involvement of several integral components known as ubiquitin ligases. The initiation of protein ubiquitination typically requires an ATP-dependent enzymatic cascade that is initiated with the priming of a ubiquitin onto a ubiquitin activating enzyme (E1) and the transfer to a ubiquitin
conjugating enzyme (E2). Ubiquitin is then covalently attached to a lysine residue on the target protein by an E3 ubiquitin ligase (E3) and this process can be repeated to create a series of ubiquitin chains[7]. There are only 2 E1 and 30–50 E2 genes, while there are over 600 E3 ubiquitin ligase genes. The diversity of E3 genes have been linked to neurological disorders including neurodegenerative disease, neuro-developmental disorders, and intellectual disability, such as Alzheimer’s disease, PD, multiple sclerosis and Autism spectrum disorder [8].

Considering the important role of ubiquitination in the genetic, mechanical and pathological changes of PD, and early onset PD (EOPD) is more susceptible to genetic factors[9], in the current study, we aimed to systematically examine whether there were overlaps between E3 ubiquitin ligase genes and EOPD with a whole exome sequencing (WES) dataset.

Methods

Patients

A total of 695 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[10, 11]. Clinical characteristics items and rating scales for patients were collected by face-to-face interview. Written informed consent was obtained from all participants. The study was approved by the ethics committee of West China hospital, Sichuan University.

Sequencing, genes selection, variants filtration

DNA samples were collected according to the standard SDS-phenol-chloroform method. WES was applied as addressed in our previous study[12]. Total coverage < 10 and altered frequency < 20% represented poor sequencing quality and were excluded from the analysis. The E3 ubiquitin ligase genes were downloaded from the Epithelial Systems Biology Laboratory (http: hpcwebapps.cit.nih.gov/ ESBL/ Database/ E3-ligases). Then we focused on those 44 genes that can cause monogenic disease with the nervous system involved by searching Online Mendelian Inheritance in Man database (OMIM, https://omim.org/) (Supplementary table 1). Controls were from the gnomAD East Asian population (https://gnomad.broadinstitute.org/). Variants were filtered firstly based on two minor allele frequency (MAF) thresholds: (1) rare<0.001; and (2) extra rare<0.0001. Then, 3 categories of comparison were conducted: (1) non-synonymous; (2) likely damaging;(3) protein-truncating variants (PTVs, frameshift variants, stop-gain variants, stop-loss variants, start-gain variants and start-loss variants). Combined Annotation Dependent Depletion (CADD) integrates predictions from numerous bioinformatic algorithms into a single ‘C-score’ and ranks all possible nucleotide changes in the genome based on potential to disrupt gene/protein function[13]. In accordance with the previous study, we defined a stringent CADD C-score ≥ 12.37 as likely damaging variants, representing the top 2% most damaging of all possible nucleotide changes in the genome—this subset is enriched for known pathogenic alleles[14]. Moreover, the in-frame deletion/insertion variants lead to the change of the protein length and are classified as moderately strong evidence for pathogenicity[15]. Therefore, in-frame deletion/insertion was classified as damaging variants in the current study.

Statistical analysis

Genetic association analysis was performed with Chi square test or Fisher’s exact test in the whole E3 ubiquitin ligase genes dataset and each gene. And a p value < 0.0012(0.05/44) was considered as statistically significant after Bonferroni correction. Burden analysis was conducted with 5 algorithms with AssotesteR Package in the per
gene level, including Comprehensive Approach to Analyzing Rare Genetic Variants (CARV), Sum of Squared Score (SSU), Sum Test (SUM), Cumulative Minor Allele Test (CMAT), and Bayesian Score Test (BST)[16]. Moreover, in order to highlight those gene driving associations detected in the gene set, secondary analyses were also performed to evaluate variants in each E3 ubiquitin gene independently. Bonferroni-correction was applied for the multiple comparison and an adjusted p < 0.0012(0.05/44) was considered as statistically significant. The final results were considered significant if at least 3 algorithms were significant.

Results

Variants were extracted from 44 ubiquitin E3 ubiquitin ligase genes responsible for monogenic diseases with the nervous system affected (Supplementary Table 1), and filtered into nested categories based on two frequency thresholds (MAF < 0.001 and MAF < 0.0001) and 3 tiers of functional criteria (nonsynonymous, damaging and PTVs).

Among the 44 E3 ubiquitin ligase genes, no rare coding variants were found in MARCH6, TRIM71 and VPS11. The observed variants in above-mentioned 2 categories and 3 tiers are listed in Supplementary Table 2. Following adjustment for multiple comparisons, significant associations were detected for the E3 ubiquitin ligase gene set considering both non-synonymous variants, likely damaging variants and PTVs, when using the both the MAF of 0.001 and 0.0001 (Supplementary Table 2 and Fig. 1A). In general, our results implicated an enrichment of rare and rare damaging variants in E3 ubiquitin ligase gene in Chinese EOPD patients.

To determine which E3 ubiquitin ligase genes/variants may be responsible for the observed association with EOPD risk, we performed exploratory analyses to assess for potential contribution of variants within each gene separately. In these association analyses, combined frequency of rare variants in 6 genes and extra rare variants in 10 genes had the strongest aggregate associations with EOPD (Supplementary Table 3, and Fig. 1B and 1C). Moreover, when taking the predicted function into consideration, 6 genes with rare and extra rare damaging variants were found to be enriched in EOPD patients, those include HECT and RLD domain containing E3 ubiquitin protein ligase family member 1 gene (HERC1), interferon regulatory factor 2-binding protein-like gene (IRF2BPL), histone-lysine N-methyltransferase 2D gene (KMT2D), leucine rich repeat and sterile alpha motif containing 1 gene (LRSAM1), 43 kDa receptor-associated protein of the synapse isoform 2 gene (RAPSN), Ring finger protein, LIM domain interacting gene (RLIM), and ring finger protein 216 gene (RNF216) (Supplementary Table 4, Fig. 1D and 1E).

Furthermore, in the rare variants burden analyses, rare and extra rare variants in 7 genes were found to be enriched in the patients with EOPD, including HERC1, IRF2BPL, KMT2D, LRSAM1, RAPSN, RLIM and RNF216. Moreover, when taking the predicted function into consideration, rare and extra rare damaging variants in HERC1, IRF2BPL, KMT2D, RAPSN, RLIM, RNF168 and RNF216 were found to be enriched in the patients with EOPD (Table 1).
Table 1
Results of the 5 algorithms for the rare variants burden analyses for per gene.

| Rare variants | MAF   | CARV | SSU  | SUM  | CMAT | BST  | CARV | SSU  | SUM  | CMAT | BST  |
|---------------|-------|------|------|------|------|------|------|------|------|------|------|
| AFF4          | < 0.001 | 0.460 | 0.460 | 0.618 | 0.460 | 0.460 | 0.710 | 0.710 | 0.514 | 0.710 | 0.710 |
|               | < 0.0001 | 0.522 | 0.386 | 0.126 | 0.386 | 0.386 | 0.386 | 0.386 | 0.102 | 0.386 | 0.386 |
| HACE1         | < 0.001 | 1.000 | 0.798 | 0.344 | 0.798 | 0.798 | 0.766 | 0.604 | 0.208 | 0.604 | 0.604 |
|               | < 0.0001 | 0.408 | 0.172 | 0.088 | 0.172 | 0.172 | 0.420 | 0.176 | 0.076 | 0.176 | 0.176 |
| HECW2         | < 0.001 | 0.176 | 0.134 | 0.070 | 0.134 | 0.134 | 1.000 | 0.900 | 0.484 | 0.900 | 0.900 |
|               | < 0.0001 | 0.054 | 0.006 | 0.006 | 0.006 | 0.006 | 0.338 | 0.048 | 0.032 | 0.048 | 0.048 |
| HERC1         | < 0.001 | 0.018 | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 |
|               | < 0.0001 | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 |
| HERC2         | < 0.001 | 0.040 | 0.012 | 0.008 | 0.012 | 0.012 | 0.126 | 0.084 | 0.054 | 0.084 | 0.084 |
|               | < 0.0001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 |
| HUWE1         | < 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.036 | 0.000 | 0.000 | 0.000 | 0.000 |
|               | < 0.0001 | 0.104 | 0.006 | 0.006 | 0.006 | 0.006 | 0.162 | 0.006 | 0.006 | 0.006 | 0.006 |
| IRF2BPL       | < 0.001 | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.032 | 0.000 | 0.000 | 0.000 | 0.000 |
|               | < 0.0001 | 0.024 | 0.000 | 0.000 | 0.000 | 0.000 | 0.032 | 0.000 | 0.000 | 0.000 | 0.000 |
| KMT2C         | < 0.001 | 0.626 | 0.484 | 0.232 | 0.484 | 0.484 | 0.588 | 0.538 | 0.708 | 0.538 | 0.538 |
|               | < 0.0001 | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.024 | 0.002 | 0.002 | 0.002 | 0.002 |
| KMT2D         | < 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.008 | 0.000 | 0.000 | 0.000 | 0.000 |
|               | < 0.0001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 |

\[ P_{\text{adj}}^{} = 0.05/41 \approx 0.0012 \]
| Rare variants | Rare damaging variants |
|---------------|------------------------|
| LRSAM1 < 0.001| 0.014 0.000 0.000 0.000 0.000 0.102 0.010 0.008 0.010 0.010 |
| < 0.0001     | 0.024 0.000 0.000 0.000 0.000 0.264 0.008 0.008 0.008 0.008 |
| MID1 < 0.001 | 0.206 0.002 0.002 0.002 0.002 0.290 0.118 0.054 0.118 0.118 |
| < 0.0001     | 0.174 0.000 0.000 0.000 0.000 0.230 0.002 0.002 0.002 0.002 |
| MID2 < 0.001 | 0.236 0.014 0.014 0.014 0.014 0.268 0.024 0.024 0.024 0.024 |
| < 0.0001     | 0.388 0.002 0.002 0.002 0.002 0.394 0.000 0.000 0.000 0.000 |
| NEDD4L < 0.001| 0.396 0.186 0.062 0.186 0.186 0.276 0.090 0.040 0.090 0.090 |
| < 0.0001     | 0.734 0.734 0.254 0.734 0.734 0.984 0.732 0.226 0.732 0.732 |
| NHLRC1 < 0.001| 0.206 0.062 0.038 0.062 0.062 0.724 0.724 0.296 0.724 0.724 |
| < 0.0001     | 0.202 0.006 0.006 0.006 0.006 0.412 0.412 0.134 0.412 0.412 |
| PCGF2 < 0.001 | 0.522 0.498 0.616 0.498 0.498 0.658 0.468 0.600 0.468 0.468 |
| < 0.0001     | 1.000 0.722 0.262 0.722 0.722 0.984 0.732 0.226 0.732 0.732 |
| PEX10 < 0.001 | 0.236 0.082 0.052 0.082 0.082 1.000 0.780 0.264 0.780 0.780 |
| < 0.0001     | 0.202 0.006 0.006 0.006 0.006 0.438 0.024 0.024 0.024 0.024 |
| PEX12 < 0.001 | 0.120 0.016 0.014 0.016 0.016 0.158 0.022 0.016 0.022 0.022 |
| < 0.0001     | 0.256 0.000 0.000 0.000 0.000 0.308 0.002 0.002 0.002 0.002 |
| PEX2 < 0.001  | 0.772 0.772 0.254 0.772 0.772 0.728 0.728 0.420 0.728 0.728 |
| < 0.0001     | 0.280 0.038 0.038 0.038 0.038 0.422 0.422 0.120 0.422 0.422 |
| PLAG1 < 0.001 | 0.608 0.592 0.558 0.592 0.592 0.736 0.516 0.188 0.516 0.516 |
| < 0.0001     | 0.486 0.016 0.016 0.016 0.016 0.486 0.016 0.016 0.016 0.016 |

\[ P_{\text{adjust}} = 0.05/41 = 0.0012 \]
| Rare variants | Rare damaging variants |
|---------------|------------------------|
| RAG1 < 0.001  | 0.488 0.320 0.142 0.320 0.320 0.238 0.148 0.070 0.148 0.148 |
|               | 0.096 0.006 0.006 0.006 0.006 0.170 0.004 0.004 0.004 0.004 |
| RAPSN < 0.001 | 0.010 0.000 0.000 0.000 0.000 0.012 0.000 0.000 0.000 0.000 |
|               | 0.048 0.000 0.000 0.000 0.000 0.082 0.000 0.000 0.000 0.000 |
| RBCK1 < 0.001 | 0.718 0.718 0.558 0.718 0.718 0.656 0.652 0.226 0.652 0.652 |
|               | 0.000 0.250 0.620 0.250 0.250 0.000 0.104 0.692 0.104 0.104 |
| RLIM < 0.001  | 0.106 0.000 0.000 0.000 0.000 0.118 0.000 0.000 0.000 0.000 |
|               | 0.294 0.000 0.000 0.000 0.000 0.314 0.000 0.000 0.000 0.000 |
| RNF113A < 0.001 | 0.558 0.558 0.136 0.558 0.558 0.396 0.046 0.046 0.046 0.046 |
|               | 0.598 0.000 0.000 0.000 0.000 0.444 0.024 0.024 0.024 0.024 |
| RNF125 < 0.001 | 1.000 0.396 0.162 0.396 0.396 1.000 0.396 0.162 0.396 0.396 |
|               | 1.000 0.044 0.000 0.044 0.044 1.000 0.044 0.044 0.044 0.044 |
| RNF13 < 0.001 | 0.772 0.588 0.520 0.588 0.588 0.456 0.408 0.670 0.408 0.408 |
|               | 0.44 0.024 0.024 0.024 0.024 0.486 0.044 0.044 0.044 0.044 |
| RNF168 < 0.001 | 0.044 0.002 0.002 0.002 0.002 0.078 0.000 0.000 0.000 0.000 |
|               | 0.132 0.002 0.002 0.002 0.002 0.224 0.000 0.000 0.000 0.000 |
| RNF170 < 0.001 | 0.432 0.432 0.108 0.432 0.432 0.648 0.448 0.080 0.448 0.448 |
|               | 0.704 0.074 0.074 0.074 0.074 1.000 0.044 0.044 0.044 0.044 |
| RNF216 < 0.001 | 0.326 0.000 0.000 0.000 0.000 0.368 0.000 0.000 0.000 0.000 |
|               | 0.368 0.000 0.000 0.000 0.000 0.426 0.000 0.000 0.000 0.000 |

\( P_{\text{adj}}=0.05/41 = 0.0012 \)
| Rare variants | Rare damaging variants |
|---------------|------------------------|
| RSPRY1 < 0.001 | 0.380 0.114 0.048 0.114 0.114 0.198 0.042 0.042 0.042 0.042 |
| < 0.0001      | 0.272 0.010 0.010 0.010 0.010 0.458 0.066 0.066 0.066 0.066 |
| STUB1 < 0.001 | 0.452 0.250 0.606 0.250 0.250 0.572 0.440 0.534 0.440 0.440 |
| < 0.0001      | 0.438 0.064 0.064 0.064 0.064 0.644 0.638 0.130 0.638 0.638 |
| TRAF7 < 0.001 | 0.660 0.660 0.260 0.660 0.660 0.678 0.510 0.208 0.510 0.510 |
| < 0.0001      | 0.132 0.002 0.002 0.002 0.002 0.142 0.000 0.000 0.000 0.000 |
| TRAIP < 0.001 | 0.446 0.190 0.068 0.190 0.190 0.230 0.088 0.042 0.088 0.088 |
| < 0.0001      | 0.230 0.002 0.002 0.002 0.002 0.290 0.002 0.002 0.002 0.002 |
| TRIM2 < 0.001 | 0.578 0.578 0.190 0.578 0.578 0.584 0.584 0.182 0.584 0.584 |
| < 0.0001      | 0.384 0.006 0.006 0.006 0.006 0.400 0.002 0.002 0.002 0.002 |
| TRIM32 < 0.001 | 0.096 0.012 0.010 0.012 0.012 0.106 0.008 0.008 0.008 0.008 |
| < 0.0001      | 0.134 0.002 0.002 0.002 0.002 0.280 0.000 0.000 0.000 0.000 |
| TRIM36 < 0.001 | 0.668 0.534 0.242 0.534 0.534 0.420 0.264 0.102 0.264 0.264 |
| < 0.0001      | 0.110 0.006 0.006 0.006 0.006 0.046 0.002 0.002 0.002 0.002 |
| TRIM37 < 0.001 | 0.558 0.458 0.162 0.458 0.458 0.648 0.544 0.208 0.544 0.544 |
| < 0.0001      | 0.186 0.052 0.022 0.052 0.052 0.224 0.090 0.044 0.090 0.090 |
| TRIP12 < 0.001 | 0.540 0.540 0.196 0.540 0.540 0.542 0.542 0.188 0.542 0.542 |
| < 0.0001      | 0.168 0.012 0.012 0.012 0.012 0.170 0.012 0.012 0.012 0.012 |
| UBE3A < 0.001 | 0.462 0.248 0.592 0.248 0.248 0.572 0.440 0.534 0.440 0.440 |
| < 0.0001      | 0.634 0.634 0.314 0.634 0.634 0.634 0.634 0.314 0.634 0.634 |

\[
P_{\text{adj}} = 0.05/41 = 0.0012
\]
| Rare variants | Rare damaging variants |
|---------------|------------------------|
| **UBE3B**     | 0.040 0.064 0.960 0.064 0.064 0.120 0.158 0.862 0.158 0.158 |
| < 0.0001      | 1.000 0.768 0.300 0.768 0.768 0.794 0.590 0.246 0.590 0.590 |
| **UBR1**      | 0.590 0.396 0.200 0.396 0.396 0.556 0.456 0.168 0.456 0.456 |
| < 0.0001      | 0.120 0.004 0.004 0.004 0.004 0.058 0.000 0.000 0.000 0.000 |

\[ P_{\text{adj}} = 0.05/41 = 0.0012 \]

**Discussion**

The exact pathogenesis mechanism of PD remains unsolved, while certain molecular pathways have been implicated in the pathogenesis of PD, including dysfunction of the ubiquitin-proteasome system and lysosomal system, vesicular trafficking, neuroinflammation and mitochondrial dysfunction[17]. The current study reveals an important link between the E3 ubiquitin ligase genes responsible for EOPD. Specifically, among the 44 E3 ubiquitin ligase genes that causing disorders involved in the nervous system, we found evidence for an enrichment of rare variants and rare damaging variants in EOPD.

Moreover, our association analyses and rare variants burden analysis consistently implicated several PD susceptibility genes. On the one hand, our study expanded novel associations between several E3 ubiquitin ligase genes and PD, including **HERC1, KMT2D, RAPSN, RLIM, RNF168** and **RNF216**, while these genes have been found to be involved in the nervous system. For example, **HERC1** was found to provoke loss of cerebellar Purkinje cells and was required for normal axonal myelination in the peripheral nervous system[18]. **KMT2D**, encoding a histone methyltransferase that promotes transcriptional activation, can cause a congenital, multisystem disability disorder called Kabuki syndrome[19]. **RAPSN** was the causative gene for congenital myasthenic gravis and was involved in the survival for motor neurons[20]. Moreover, the human ring finger (RNF) family has been also implicated in neurological disorders, such as **RNF213** for Moyamoya disease[21]. On the other hand, our study confirmed the known associations between several genes and PD. For example, **IRF2BPL** belongs to a family of transcriptional regulators and has been found to be associated with neurodevelopmental and neurological disorders including dystonia, ataxia, and spasticity[22, 23]. **LRSAM1** was originally found to be the causative gene of Charcot-Marie-Tooth disease, axonal, type 2P (CMT2P) [24], while a previous study has described that 3 members of a CMT2P pedigree developed dopa-responsive parkinsonism but without any mutation in the known PD causative genes, which indicated that mutations in **LRSAM1** might link CMT to PD[25]. Nonetheless, functional studies are needed to ascertain the role of novel susceptible genes in PD.

Caution may be warranted in interpreting the results of rare variants burden analysis. First, in our burden analysis, the damaging variants included in the burden analysis were based on prediction tools to help exclude rare but benign missense variants; however, the pathogenicity needs to be further confirmed based on more experimental evidence. Secondly, controls from gnomAD database rather than age-, sex- and ethnic-matched controls might compromise the effect of burden analysis.
Conclusion

In conclusion, our findings highlight the importance of UPS mechanisms in the pathogenesis of PD. Moreover, our study also expanded the susceptibility gene spectrum for PD.

List Of Abbreviations

| Full name                                                                 | Abbreviation |
|---------------------------------------------------------------------------|--------------|
| ubiquitination proteasome system                                          | UPS          |
| Parkinson's disease                                                       | PD           |
| early onset PD                                                            | EOPD         |
| minor allele frequency                                                    | MAF          |
| protein-truncating variants                                               | PTVs         |
| Combined Annotation Dependent Depletion                                  | CADD         |
| Comprehensive Approach to Analyzing Rare Genetic Variants                | CARV         |
| Sum of Squared Score                                                     | SSU          |
| Sum Test                                                                  | SUM          |
| Cumulative Minor Allele Test                                             | CMAT         |
| Bayesian Score Test                                                       | BST          |
| HECT and RLD domain containing E3 ubiquitin protein ligase family member 1 | HERC1        |
| interferon regulatory factor 2-binding protein-like gene                  | IRF2BPL      |
| histone-lysine N-methyltransferase 2D gene                                | KMT2D        |
| leucine rich repeat and sterile alpha motif containing 1 gene            | LRSAM1       |
| 43 kDa receptor-associated protein of the synapse isoform 2 gene          | RAPSN        |
| Ring finger protein, LIM domain interacting gene                          | RLIM         |
| ring finger protein                                                       | RNF216       |
| Charcot-Marie-Tooth disease, axonal, type 2P                              | CMT2P        |

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants. The study was approved by the ethics committee of West China hospital, Sichuan University.

Consent for publication

Not applicable
**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interest**

All the authors declared no financial disclosure.

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**Author's contribution**

XJG concepted and designed the work, analyzed and interpreted the data and drafted the manuscript; YBH and YPC analyzed and interpreted the data; RWO, BC, QQW, LYZ, WS, BZ, YW acquired the data; CYL revised the manuscript; HFS concepted and designed the work, revised the manuscript and provided the funding support. All authors read and approved the final manuscript.

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Analysis of combined frequency of rare and rare damaging variants in ubiquitin E3 ligase genes in Chinese patients of EOPD. p value, odds ratio and 95% confidence interval were analyzed with Chi square test or Fisher exact test. A. Rare variants in the whole E3 ubiquitin ligase genes were enriched in EOPD patients. B. Genes with rare variants with MAF<0.001 enriched in EOPD patients. C. Genes with extra rare variants with MAF<0.0001 enriched in EOPD patients. D. Genes with rare damaging variants with MAF<0.0001 enriched in EOPD patients. E. Genes with extra rare damaging variants with MAF<0.0001 enriched in EOPD patients.
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