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RESEARCH ARTICLE

Rare variant analysis of essential tremor-associated genes in early-onset Parkinson's disease

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Abstract

Objective: Parkinson's disease (PD) and essential tremor (ET) are the two most common movement disorders. A significant overlap in clinical features, epidemiology, imaging, and pathology suggests that PD and ET may also share common genetic risk factors. Previous studies have only assessed a limited number of ET-associated genes in PD patients and vice versa. Consequently, the genetic association between PD and ET remains incompletely characterized. In this study, we systematically investigated a potential association between rare coding variants in ET-associated genes and PD, in a relatively large Chinese population cohort. **Methods**: To investigate the genetic association between ET and PD, we performed the sequence kernel association testing (SKAT-O) to explore the variant burden of 33 ET-associated genes, using whole-exome sequencing (WES) data from 1494 early-onset PD (EOPD) patients and 1357 control subjects from mainland China. **Results**: We report that rare loss-of-function and damaging missense variants of *TNEM4* are suggestively associated with EOPD ($P = 0.026$), damaging missense variants of *TNEM4* alone are also suggestively associated with EOPD ($P = 0.032$). No other rare damaging variants in ET-related genes were significantly associated with EOPD. **Interpretation**: This is the first systematic analysis of ET-associated genes in EOPD. The suggestive association between *TNEM4* and EOPD provides new evidence for a genetic link between ET and PD.

Introduction

Parkinson's disease (PD) and essential tremor (ET) represent the two most common movement disorders. The estimated prevalence of PD is approximately 0.3% in the general population, although increases to 1% and 3.5% in people aged over 60 and 85 years old, respectively.[1] Similarly, approximately 1% of the general population and 5% of individuals over 65 years old suffer from ET.[2]

There is a longstanding discussion surrounding a possible relationship between these two diseases. Firstly, there are several overlapping clinical features including resting and postural tremor, bradykinesia, rigidity, gait and balance impairment that are present in both conditions. Olfactory deficits, cognitive dysfunction and rapid eye movement, sleep behavior disorder, and other nonmotor symptoms can also occur in these two diseases.[3,4] Furthermore, some ET patients have shown neurodegenerative changes in their brain such as Lewy bodies (LBs); the key pathological hallmark found in brains of PD patients.[4,5] PD can also co-occur in select patients that present with earlier manifestations of ET.[6,7] Approximately 25% of the ET-PD co-occurring patients have a family history of ET.[3] Finally, ET patients have also been found to carry an increased risk of developing PD in certain populations.[8]

Although the precise etiology of these two diseases is unclear, they may share certain genetic risk factors. Notably, several variants in ET-related genes, such as *LINGO1*, *LINGO2*, *SLC1A2*, *H1S1BP3*, *FUS*, and *HTRA2*, have been evaluated in patients with PD.[4,9–11] A variety of variants in PD-related genes have been suggested to play a role in ET susceptibility, including *SNCA*, *LRRK2*, *DNAJC13*, *(NACP)-Rep1*, *MTHFR*, *HMOX1*, *HMOX2*, *MAPT*, and *TREM2*.[4,12,13] Additionally, mutations or variants in *NOS3*, *KCNS2*, *HAPLN4*, *USP46*, and several others are associated with ET, but have not been explored in PD patients.[3]

There is still a lack of extensive and comprehensive analysis of the relationship between ET-associated genes and PD using next-generation sequencing techniques, especially the rare variants in coding regions. To date, the majority of published studies have only evaluated the role of some specific ET-associated loci and genes in the PD patients with limited depth. To explore the possible genetic link between PD and ET, we performed a comprehensive analysis to study the relationship of rare variants of ET-associated genes in coding regions and PD from whole-exome sequencing (WES) data in a relatively large early-onset PD (EOPD) cohort from mainland China.

Materials and Methods

Subjects

EOPD patients (age at onset (AAO) ≤50 years) were recruited by the Department of Neurology of Xiangya Hospital, Central South University, from October 2006 to January 2019 and other cooperating centers of Parkinson's Disease & Movement Disorders Multicenter Database and Collaborative Network in China (PD-MDCNC, http://pd-mdcnc.com:3111/). All the patients were subjected to the standard clinical neurological examination by at least two neurologists and diagnosed with PD according to the UK PD Brain Bank Criteria or Movement Disorders Society (MDS) clinical diagnostic criteria for PD.[14,15] Controls collected from community did not have any neurological or psychiatric system diseases. Subjects with pathogenic/likely pathogenic variants of high- or very high-confidence PD disease-causing genes or established Mendelian Parkinson's disease were excluded from analysis.[16] This protocol was approved by the Ethics Committee of Central South University, and written informed consent was obtained from all the subjects.

Sequences, genotyping, and quality control

As described in our previous study,[16] genomic DNA was isolated from each participants' peripheral blood leucocytes and whole exome DNA was captured by the SureSelect Human All Exon Kit V6 (Agilent). Paired-end sequencing was generated using the Ilumina X10 platform with an average coverage of 123x. Reads were aligned against the reference genome (UCSC hg19) with Burrows-Wheeler Aligner.[17] Picard tool was used to sort and index alignment results and remove duplicate reads. Genome Analysis Toolkit (GATK) was used for base quality-score recalibration, local realignment around possible insertions/deletions (indels), calling and filtering variants.[18] Variants were further annotated with ANNOVAR[19] and VarCards based on RefSeq (UCSC hg19) for gene regions, amino acid alterations, functional effects and allele frequencies in the East Asian (EAS) population (genomAD database, ExAC database).[20] ReVe was used for in silico pathogenicity prediction (threshold as 0.7). PLINK1.90 was used for quality control for individuals and variants. Individuals with ambiguous sex (conflicting assignment in PLINK), deviating heterozygosity/genotype calls ((±3 standard deviations [SDs]), low genotype call rates (missing rate > 10%) or cryptic relatedness (identity by descent > 0.15) were excluded. Variants with low-quality genotypes (Phred-scaled genotype quality score [GQ] below 20, allele depth [AD] below 5, reads depth [DP] below 10 for SNP; Phred-scaled genotype quality score [GQ] below 30, allele depth [AD] below 10, reads depth [DP] below 20 for Indel), low call rates (missing rate > 10%), or departure from Hardy–Weinberg equilibrium ($P < 0.0001$) were removed. Independent high-quality variants were used for the principal component analysis on population stratification and main principal component variables for each sample were obtained, while outliers (suggesting non-Chinese ancestry) were excluded in further analysis.

Gene and variant selection

Although ET is well recognized as a familial condition, pathogenic causal variants have not yet been identified. A detailed literature search was performed manually to find ET-associated genes featured in the papers published in PubMed on April 23, 2020, using the key words "essential tremor", "ET", "gene", "variant", and "mutation". All the ET-associated genes selected in our study should be performed in at least one genetic study including linkage studies, WES or WGS studies performed in ET families, and studies investigated sporadic occurrence of diseases using genome-wide genotyping of single nucleotide polymorphisms (SNPs) and candidate gene studies.

All the variants from the target gene regions were extracted, yielding totally – coding variants. All variants are analyzed in a category manner based on the annotation with ANNOVAR: (1) all variants, (2) missense variants, (3) damaging missense, (4) loss of function, and (5) damaging missense and loss of function. Variants annotated as stop gain/loss, frameshift, or splicing mutations were categorized as loss of function. Variants predicted to be damaging by ReVe (>0.7) were selected to be likely damaging missense variants. Within these categories, the variants from target regions were extracted and divided into rare variants based on minor allele frequencies (MAF) in covered samples of cohort WES at a threshold of 0.01.

Statistical analysis

The aggregate burden of rare damaging variants of ET-associated genes between EOPD and controls was estimated using the sequence kernel association test–optimal (SKAT-O) implemented in the package SKAT in R.[21] SKAT-O test for associations by aggregating genetic information across defined genomic regions. Gender, age, and the first five principal components of ancestry were used as covariates for adjustment and the empirical *P* value is generated. The estimated number of independent tests was 33 and the corresponding Bonferroni-corrected significance threshold of *P* was 0.0015. Results with *P*-value less than 0.05, while not surviving the Bonferroni correction, were considered as "suggestive." We first performed SKAT-O analysis on the variants of complete ET gene sets and then on the variants for each gene.

Results

Altogether 33 loci and genes were selected as ET-associated from genetic studies and were analyzed in our study (Table 1). Despite the ETM3 locus on chromosome 6p23 and a locus on chromosome 5 having been previously linked to ET in previous studies, no pathogenic variants were identified in these candidate regions and were not included in our analysis.[22,23] In our study, a total of 1494 EOPD patients (AAO $\leq$ 50) including sporadic EOPD and EOPD probands with a positive family history of PD and 1375 age and sex-matched controls were collected to perform WES.

To investigate the genetic overlap between ET and PD, we implemented SKAT-O to assess the contribution of total ET gene set and each single gene within EOPD cohorts separately. In ET gene set analyses, we detected 1660 rare variants in the exons and exon–intron boundaries of the 33 genes with the rare frequency MAF < 1%. No association was detected between the ET gene set and EOPD cohort within those categories (Table 2). In ET singe-gene analysis, we initially observed a significant association of *TENM4* in EOPD cohort when considering damaging missense and loss-of-function allele ($P = 0.026$) or damaging missense alone ($P = 0.032$), but following the adjustment for multiple comparisons, the signals were attenuated and no longer significant ($P > 0.0015$) (Table 3). As shown in Table 3, rare variants of *TENM4* detected in our cohorts included 141 missense variants, 71 damaging missense variants, 1 loss-of-function variant, and 72 damaging missense and loss-of-function variants, resulting in a total of 209 rare variants.

Interestingly, missense variants in the MC1R gene were found to carry a positive association with EOPD ($P = 0.00031$), however, there are no significant difference when considering the rare damaging variants. None of the rare damaging variants of other ET-associated genes were found to be significantly associated with EOPD.

Discussion

In the past few decades, whole-exome sequencing has been used to investigate the entire exonic region of the human genome. A particular advantage of this approach is that defined rare genetic variants, which are associated with a given phenotype, can be readily identified. In our study, we performed WES screening in a relatively large cohort of patients with sporadic and familial EOPD among ethnic Chinese from mainland China and explored the gene-burden analysis on rare variants of 33 ET-associated genes using SKAT-O. Remarkably, only *TENM4* gene had a suggestive association with EOPD, and none of the other rare damaging variants of ET-associated genes were significantly associated with EOPD.

Pathogenic variants in *TENM4* were first identified in a Spanish ET cohort using WES in 2015.[30] Several genetic studies have looked at the association between *TENM4* with ET in different populations. However, one study performed in a cohort of Canadian ET cases did not support a positive association between *TENM4* and ET. In a Chinese ET cohort, the *TENM4* p.A1442T mutation was not described in any ET patients but rather two asymptomatic healthy individuals.[47] In 2020, only three previously reported synonymous SNPs of *TENM4* were found in an ET cohort from eastern China.[48] To date, only one study that has tested the genetic and allelic frequencies of *TENM4* p.T1367N in a PD cohort, but no alterative genotypes were observed in this point variant.[49] *TENM4*, a member of the teneurin gene family, is highly expressed in the nervous system.[50] *TENM4* has been shown to play a key role in the regulation of oligodendrocyte differentiation and axon myelination. Myelination is dramatically reduced in small-diameter axons and oligodendrocyte differentiation is inhibited in *TENM4* knockout mice.[50,51] Alterations to myelin have been described in PD and ET patients form pathological and neuroimaging studies.[52,53] Our results suggest *TENM4* mutations may affect the process of myelination regulation and axon guidance, which

Table 1. Summary of loci and genes associated with ET selected in our study.

Gene	Genomic location	Year of identification	Identification methods	References
ETM1(DRD3)	chr3q13	1997, 2006	Genome-wide scan + Candidate-gene approach	[24,25]
ETM2(HS1BP3)	chr2p24.1	1997, 2005	Genome-wide scan + Candidate-gene approach	[26,27]
FUS(ETM4)	chr16p11.2	2012	WES	[28]
HTRA2	chr2p13.1	2014	WES	[29]
TENM4(ETM5)	chr11q14.1	2015	WES and targeted resequencing	[30]
SCN4A	chr17q23.3	2015	WES	[31]
SORT1	chr1p13.3	2015	WES	[32]
NOS3	chr7q36.1	2016	WES	[33]
KCNS2	chr8q22.2	2016	WES	[33]
HAPLN4	chr19p13.11	2016	WES	[33]
USP46	chr4q12	2016	WES	[33]
SCN11A	chr3p22.2	2017	WES	[34]
CACNA1G	chr17q21.33	2019	WGS	[35]
SLIT3	chr5q34-q35.1	2019	WGS	[35]
KARS1	chr16q23.1	2019	WGS	[35]
KIF5A	chr12q13.3	2019	WGS	[35]
NTRK1	chr1q23.1	2019	WGS	[35]
MTHFR	chr1p36.22	2004	SNP Genotyping	[36]
LINGO1	chr15q24.3	2009	GWAS	[37]
LINGO2	chr9p21.2-p21.1	2010	SNP Genotyping	[38]
MAPT	chr17q21.31	2011	SNP Genotyping	[13]
SLC1A2	chr11p13	2012	GWAS	[37]
HMOX1	chr22q12.3	2015	SNP Genotyping	[39]
HMOX2	chr16p13.3	2015	SNP Genotyping	[39]
TREM2	chr6p21.1	2015	SNP Genotyping	[40]
STK32B	chr4p16.2	2016	GWAS	[41]
PPARGC1A	chr4p15.2	2016	GWAS	[41]
CTNNA3	chr10q21.3	2016	GWAS	[41]
ALAD	chr9q32	2017	SNP Genotyping	[42]
RIT2	chr18q12.3	2017	SNP Genotyping	[43]
MC1R	chr16q24.3	2018	SNP Genotyping	[44]
VDR	chr12q13.11	2018	SNP Genotyping	[45]
IL1B	chr2q14.1	2019	SNP Genotyping	[46]

Abbreviations: SNP, single nucleotide polymorphisms; WES, whole-exome sequencing; WGS, whole-exome sequencing; GWAS, genome-wide association study.

might be a significant contributors to the genetic burden of PD and ET. However, the suggestive correlation of *TENM4* and PD still needs to be further replicated in additional cohorts.

Perhaps surprisingly, we did not detect an association with other ET-associated genes with EOPD. Although genetic factors play an important role in the development of ET, only a limited number of genetic loci and possible pathogenic variants have been thus far identified.[12,54–56] The inconsistent and debatable clinical diagnostic criteria may further account for some of the difficulties in defining the genetic relationship between these two diseases partially. Furthermore, ET-PD patients have an older age at onset of parkinsonism relative to PD patients. The mean latency from onset of ET to the first signs of PD is 14-15 years, suggesting that aging, along with genetic and environmental risk factors, may play an important role in the etiology of ET-PD.[4] Therefore, in future studies, we

Table 2. Burden analysis of ET-associated gene set rare variants in EOPD cohort. (MAF < 0.01).

				MAF < 1%	
Cohort	Case (n)	Control (n)	Variants group	*n*	*P*
EOPD cohort	1494	1357	All	1660	0.593
			Missense	1003	0.802
			Dmis	329	0.578
			LoF	40	1
			LoF + Dmis	369	0.554

Variants were classified into All variants (All), Missense variants (Missense), damaging missense (Dmis), loss-of-function (LoF), and loss-of-function + damaging missense (LoF + Dmis) with the rare frequency thresholds MAF < 1%.

n = total number; *P* value was calculated by SKAT-O (Sequence Kernel Association Test – Optimal).

Abbreviation: EOPD, early-onset Parkinson's disease; MAF, Minor allele frequency.

Table 3. Burden analysis of ET-associated genes rare variants in EOPD cohort (MAF < 0.01).

	All		Missense		LoF		Dmis		LoF + Dmis	
Genes	n	*P*	*n*	*P*	*n*	*P*	*n*	*P*	*n*	*P*
MC1R	47	0.002	30	**0**	–	–	–	–	–	–
PPARGC1A	35	0.278	24	0.027	–	–	6	0.520	6	0.520
MTHFR	57	0.140	33	0.042	3	0.641	15	1	18	1
NTRK1	75	0.094	43	0.105	3	1	14	0.426	17	0.449
SCN4A	144	0.610	77	0.153	1	0.789	37	0.443	38	0.470
LINGO1	31	0.164	12	0.161	–	–	2	1	2	1
FUS	27	0.110	7	0.231	–	–	4	0.183	4	0.183
TENM4	209	0.131	141	0.253	1	0.491	71	**0.032**	72	**0.026**
HMOX2	24	0.258	15	0.282	2	1	3	0.553	5	0.708
SORT1	38	0.403	28	0.287	–	–	5	0.795	5	0.795
USP46	9	0.542	3	0.352	–	–	1	0.997	1	0.997
HMOX1	28	0.544	21	0.419	1	0.389	6	0.458	7	0.298
MAPT	45	0.655	27	0.430	2	1	–	–	2	1
SLC1A2	17	0.541	12	0.453	1	0.937	2	1	3	1
VDR	35	1	15	0.570	1	0.783	8	0.342	9	0.422
NOS3	78	0.385	46	0.575	0	1	11	0.393	11	0.393
HS1BP3	32	0.820	19	0.653	1	0.866	3	1	4	1
HTRA2	15	0.553	9	0.671	1	0.861	1	0.995	2	1
ALAD	29	0.102	21	0.677	–	–	7	0.761	7	0.761
SLIT3	110	0.583	78	0.703	–	–	30	0.470	30	0.470
KCNS2	31	0.486	17	0.731	1	0.762	8	0.300	9	0.346
STK32B	38	0.548	23	0.768	1	0.569	8	0.659	9	0.553
KIF5A	41	0.812	17	0.774	2	1	7	0.813	9	0.813
HAPLN4	22	0.396	11	0.829	2	0.857	2	0.712	4	0.797
KARS	48	0.352	26	0.842	1	1	11	0.699	12	0.699
DRD3	25	0.794	19	0.895	–	–	3	0.545	3	0.545
SCN11A	97	0.780	65	0.899	6	0.847	18	0.524	24	0.473
LINGO2	30	0.639	18	1	–	–	7	1	7	1
CACNA1G	141	0.168	78	1	1	0.919	22	0.555	23	0.559
CTNNA3	61	0.922	40	1	5	1	14	0.636	19	0.690
TREM2	23	1	20	1	1	0.807	2	0.404	3	0.613
IL1B	10	1	5	1	–	–	–	–	–	–
RIT2	8	0.786	3	1	3	1	1	0.987	4	1

Variants were classified into All variants (All), Missense variants (Missense), damaging missense (Dmis), loss-of-function (LoF), and loss-of-function + damaging missense (LoF + Dmis) with the rare frequency thresholds MAF < 1%.
n = total number; *P* value was calculated by SKAT-O (Sequence Kernel Association Test – Optimal).
The positive results are presented in bold text.

plan to investigate the potential genetic interplay between ET-associated genes and late-onset PD.

In summary, our study demonstrates *TENM4* is suggestively associated with EOPD-providing new evidence for the genetic link between ET and PD. To our knowledge, this is the first study to systematically analyze the association between rare variants of ET-associated genes in coding region and EOPD using a next-generation sequencing method in a Chinese population. A greater understanding of the genetic association between these two diseases will facilitate the interrogation of new disease pathways and better insights into identifying the ET causative genes. Additional genetic studies are required to replicate this result and may help to illuminate the potential genetic link between PD and ET.

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Conflict of Interest

The authors declare no conflict of interest.

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