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Research Article

Mutation Screening of *MED27* in a Large Dystonia Cohort

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Objectives. Recently, biallelic variants in *MED27* have been identified to correlate with complex dystonia. However, no replicative study has been conducted in larger dystonia cohorts. In this study, we aimed to systematically evaluate the genetic associations of *MED27* with dystonia in a large dystonia cohort. *Materials and Methods*. We analyzed rare variants (minor allele frequency < 0.01) of *MED27* in a large Chinese dystonia cohort with whole exome sequencing. The overrepresentation of rare variants in patients was examined with Fisher's exact test at allele and gene levels. *Results*. A total of 688 patients with dystonia were included in the study, including 483 isolated dystonia, 133 combined dystonia, and 72 complex dystonia. The average age at onset (SD) was 34.3 (19.1) years old. After applying filtering criteria, five rare variants, namely, p.R247H, p.P174A, p.P123A, p.L120F, and p.F56C, were identified in six individuals. All of them carried the variant in the heterozygous form, and no patients with compound heterozygous or homozygous alleles were identified. At allele level, no variant was associated with risk of dystonia. Gene-based burden analysis did not detect enrichment of rare variants of *MED27* in dystonia either. *Conclusion*. Variants of *MED27* were rare in Chinese dystonia patients, probably because that mutations in *MED27* are more associated with more complex neurodevelopmental disorders that can also include dystonia among the various neurological features. Further studies are needed to confirm the role of *MED27* in dystonia and other neurological disorders.

1. Introduction

Dystonia is a rare movement disorder characterized by abnormal involuntary movements or postures due to sustained or intermittent muscle contractions. It can be the isolated neurological manifestation (isolated dystonia), combined with other movements disorders such as myoclonus or Parkinsonism (combined dystonia), or combined with other neurological or systemic symptoms (complex dystonia) [1]. The aetiology of dystonia is complex and remained largely unknown. A number of genes have been linked to dystonia [2] since the first dystonia gene *GCH1* has been identified in 1994 [3]. However, still a large portion of dystonia patients have no known genetic causes, indicating that more genes are to be identified.

Recently, Meng et al. reported a novel neurodevelopmental syndrome manifested homogeneously as developmental delay, dystonia, and cerebellar hypoplasia caused by biallelic disease-causing variants in *MED27* gene [4]. *MED27* encodes MED27, a subunit of the transcriptional mediator complex [5]. A study found that MED27 plays an important role in neuronal development, and impairment of MED27 may have affected the glia location in the transgenic zebrafish model [6]. Dysregulation of neurodevelopmental mechanisms has been reported to be a key feature of dystonia pathophysiology [7–10]. Oligodendrocyte has been reported to play a role in the development and function of central nervous system motor circuits and the neurodevelopmental disease dystonia [11]. However, although the initial links have been built, replications from other cohorts are still necessary to further elucidate the role of *MED27* in dystonia.

In this context, we systematically analyzed the genetic associations of *MED27* in a dystonia cohort of Chinese ancestry, to elucidate the genetic involvement of *MED27* in dystonia and supplement current knowledge to dystonia-related genetic diversity.

2. Materials and Methods

2.1. Editorial Policies and Ethical Consideration. This study was approved by the Ethics Committee of West China Hospital of Sichuan University (2022-260). All participants have signed informed consent.

2.2. Participants. A total of 688 patients with dystonia were recruited from the Department of Neurology of West China Hospital of Sichuan University. The patients were diagnosed by neurologists specialized in movement disorders.

2.3. Genotyping. The whole exome sequencing was conducted routinely, and data were processed as described previously [12]. Briefly, genomic DNA was extracted from peripheral blood mononuclear cells using standard phenol-chloroform procedures. Whole exome sequencing was conducted routinely on the Illumina NovaSeq 6000 system following the manufacturer's instructions.

2.4. Variant Analysis. The rare variants which met the following criteria were analyzed: (1) minor allele frequency (MAF) was lower than 0.01 in the East Asian population from Genome Aggregation Database (gnomAD) [13] and Chinese population from ChinaMAP [14], which includes 10,588 random Chinese individuals with deep whole genome sequence; (2) variants were annotated as missense, splice donor, splice acceptor, start-lost, stop-gained, or frameshift substitution by ANNOVAR [15]; (3) the variant was either heterozygote or homozygote. Allelic association analysis was performed using standard Fisher's exact test with default parameters, and P value was adjusted by Bonferroni correction. The summary data of the East Asian population from gnomAD v2.1.1, and Chinese individuals from ChinaMap were used as controls in our study.

2.5. Gene-Based Burden Analysis. Gene-based rare variant burden analysis was conducted to evaluate the aggregate association of rare variants in *MED27* with dystonia on an allelic basis using Fisher's exact test with default parameters. We categorized variants into rare variants (MAF < 0.01) and ultrarare variants (MAF < 0.001). For each category, we tested the association for all rare variants and rare damaging variants, which were predicted as damaging or pathogenic by at least 5 of 10 in silico tools. The summary data of the East Asian from gnomAD v2.1.1 and Chinese individuals from ChinaMap were utilized as the control, and the same filtering criteria as the cases were applied.

3. Results

A total of 688 patients with dystonia were included in the analysis, including 483 isolated dystonia, 133 combined dystonia, and 72 complex dystonia. The average age at onset (SD) was 34.3 (19.1) years old among the included 688 patients with dystonia with a sex ratio of 0.67 (male/female: 277/411). Among the 72 patients with complex dystonia, 51 patients had concomitant cerebellar ataxia, 6 patients had concomitant intellectual disability, 8 patients had concomitant developmental delay, 3 patients had concomitant epilepsy, and 9 patients had concomitant pyramidal signs. We analyzed rare variants of *MED27* in these patients to explore the putative pathogenicity of *MED27* in dystonia.

After applying filtering criteria similar to the original study, five rare variants, namely, p.R247H., p.P174A, p.P123A, p.L120F, and p.F56C, were identified in six individuals (Table 1). Among the five variants, p.R247H, p.P174A, and p.F56C were predicted as pathogenic by at least five in silico prediction tools (Table 2), and p.R247H and p.L120F were absent in East Asian population from gnomAD and ChinaMAP. At variant level, no variants were associated with risk of dystonia (Table 1). Gene-based burden analysis did not detect enrichment of rare variants of *MED27* in dystonia either, though overall the patients carried more rare variants than the controls (Figure 1). We did not detect the reported 11 variants in the original study, and no patients with compound heterozygous or homozygous alleles were identified.

Among the six *MED27* variant carriers, two adolescent patients (the p.P123A carrier and one of the p.P174A carrier) who presented with generalized dystonia, Parkinsonism, and intellectual decline were detected to carry pathogenic variants in *ATP1A3* during the additional screening of the known dystonia genes. None of the known dystonia genes have been detected in the other four patients. All of the four patients presented with isolated dystonia (three with cervical dystonia and one with upper limbs' dystonia).

The p.R247H and p.L120F variants were absent in the Asian population, and the p.R247H variant was predicted as pathogenic by at least five in silico prediction tools. The patient who carried the p.R247H variant was a 52-year-old man who developed cervical dystonia at the age of 50. None of his family members had similar symptoms. His parents died of other diseases. His son, who was 26 years old when he came to our clinic, did not have similar symptoms or signs and did not carry the variant p.R247H. The patient who carried the p.L120F variant was a 50-year-old woman who developed cervical dystonia at the age of 49. Her parents were dead. None of her family members had similar symptoms.

The patient who carried the p.F56C variant was a 25-year-old man who developed upper limbs' dystonia at the age of 9. He has a positive family history. His father, who was 58 years old when he came to our clinic, presented with lower limb's dystonia. His old brother, a 32-year-old man, developed cervical dystonia at the age of 5. His father and old brother also carried the p.F56C variant in *MED27*. All of them had poor response to levodopa (Figure 2). No other dystonia causative gene was detected in the patient and his family members. The p.F56C variant was predicted as pathogenic by at least five in silico prediction tools. Therefore, the p.F56C variant cosegregated with dystonia in the family and might be the causative gene of this family.

4. Discussion

Biallelic variants in *MED27* have been linked to dystonia recently. Meng et al. identified 11 pathogenic variants in *MED27* in 16 individuals from 11 families presented with complex dystonia, including 7 missense variants

Table 1: Allelic Fisher's exact test for identified rare variants in *MED27*.

Genomic position	rsID	Annotation	hgvs_c	hgvs_p	Case MAF ($N = 688$)	gnomAD as control ($N = 9,977$)			ChinaMAP as control ($N = 10,588$)		
						Control MAF	*P*	OR (95% CI)	Control MAF	*P*	OR (95% CI)
9 : 134736013	rs756095470	Missense	c.G740A	p.R247H	0.0007 (1/1376)	0.00E + 0 (0/17806)	0.072	Inf (0.33-Inf)	0.00E + 0 (0/21176)	0.061	Inf (0.39-Inf)
9 : 134814821	rs199522535	Missense	c.C520G	p.P174A	0.0015 (2/1376)	2.12E − 3 (42/19826)	1.000	0.69 (0.08-2.64)	6.14E − 4 (13/21176)	0.232	2.37 (0.26-10.48)
9 : 134949111	rs1833989963	Missense	c.C367G	p.P123A	0.0007 (1/1376)	0.00E + 0 (0/19954)	0.065	Inf (0.37-Inf)	2.36E − 4 (5/21176)	0.315	3.08 (0.07-27.55)
9 : 134949120	rs539278505	Missense	c.C358T	p.L120F	0.0007 (1/1376)	0.00E + 0 (0/8782)	0.135	Inf (0.16-Inf)	0.00E + 0 (0/21176)	0.061	Inf (0.39-Inf)
9 : 134955065	rs537761179	Missense	c.T167G	p.F56C	0.0007 (1/1376)	5.44E − 5 (1/18392)	0.134	13.37 (0.17-1040.17)	1.89E − 4 (4/21176)	0.270	3.85 (0.08-38.93)

Genomic position was based on GRCh37. Summary data of East Asian from gnomAD or mbiobank was utilized as controls; variants not recorded in public databases were considered as undetected in all individuals; MAF: minor allele frequency; variants with MAF <0.01 were considered rare; *P* and OR values were obtained using Fisher's exact test implemented in R 3.6.2 with default parameters.

Table 2: In silico pathogenicity predictions for rare variants in *MED27* identified in patients with dystonia.

hgvs_c	hgvs_p	GERP++	Variant-effect predictions software (scores)									
			SIFT	Polyphen2 HDIV	Polyphen2 HVAR	LRT	Mutation taster	Mutation assessor	FATHMM	MetaSVM	MetaLR	CADD
c.G740A	p.R247H	5.3	0.023 D	0.999 D	0.984 D	0 D	1 D	2.24 M	n.a. n.a.	-0.131 T	0.43 T	35 D
c.C520G	p.P174A	5.33	0.459 T	0.939 P	0.556 P	0 D	1 D	1.555 L	n.a. n.a.	-0.755 T	0.228 T	12.59 D
c.C367G	p.P123A	-0.251	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.	1 N	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.	2.305 T
c.C358T	p.L120F	-1.69	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.	1 N	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.	4.718 T
c.T167G	p.F56C	5.07	0.002 D	0.999 D	0.987 D	0 D	1 D	1.735 L	n.a. n.a.	-0.273 T	0.371 T	28.2 D

T: tolerated; D: damaging or disease-causing; P: probably pathogenic; N: neutral; M: medium; L: low; B: benign; n.a.: not available. GERP: genomic evolutionary rate profiling; SIFT: sorting intolerant from tolerant; PolyPhen2 HDIV: polymorphism phenotyping version 2 human diversity; PolyPhen2 HVAR: polymorphism phenotyping version 2 human variation; LRT: likelihood ratio test; FATHMM: functional analysis through hidden Markov models; SVM: support vector machine; LR: logistic regression; CADD: combined annotation dependent depletion. Pathogenicity prediction was obtained using ANNOVAR.

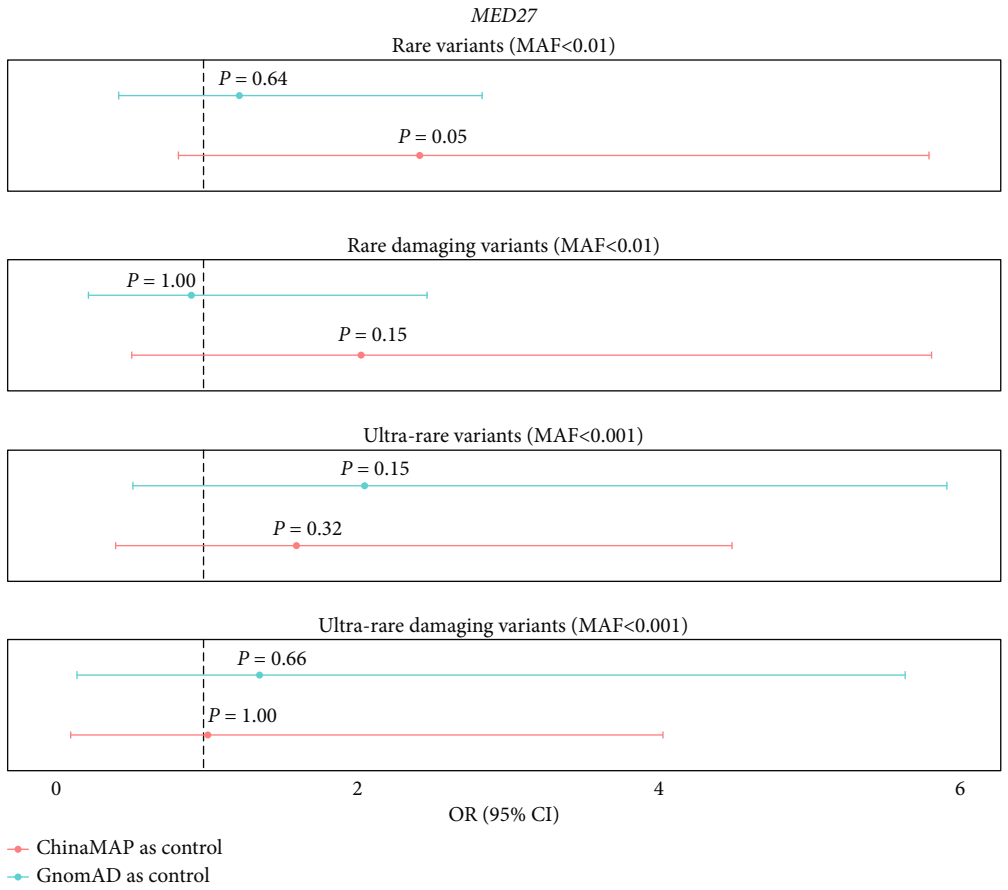

Figure 1: Enrichment analysis of rare variants in *MED27* in patients with dystonia. All rare variants and rare damaging variants with MAF < 1% and MAF < 1‰ were analyzed separately. *P* values, OR, and 95% confidence intervals (CI) were calculated using Fisher's exact test.

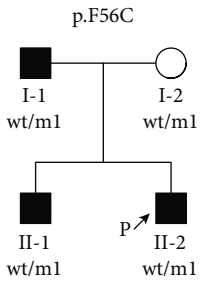

FIGURE 2: Pedigree of the family with rare *MED27* variants cosegregated. Symbols are defined as follows: black symbols: individuals affected by dystonia; circles: women; squares: men; arrows: probands; m1: p.F56C; m2: c.1296-1G>T; wt: wild-type.

(c.188T>G, c.695C>T, c.725T>C, c.776C>T, c.839C>T, c.871G>A, and c.878C>T), 3 frameshift variants (c.298_302del, c.392-393del, and c,565-566del), and 1 splice-site variant (c.682-2A>G) [4]. Meanwhile, homozygous variants of c.839C>T were also detected in another two individuals from one family presented with complex neurodevelopmental disorder with severe dystonia, although they also carried homozygous variants in *SLC6A7* and *MPPE1* [16]. However, no replication study from large cohort has been conducted to confirm the role of *MED27* in dystonia. In the current study, after comprehensive screening rare variants in *MED27* in a Chinese dystonia cohort, five rare variants were identified in six individuals. However, all of them carried the variant in the heterozygous form, and no patients with compound heterozygous or homozygous alleles were identified. In addition, although overall the patients carried more rare variants than the controls, no significant enrichment of *MED27* variants was observed in dystonia at gene level.

One of the possible reason for which we did not detect biallelic variants in *MED27* in our cohort is that the dystonia patients in our cohort had older onset age than the original study, and most (483/688, 70.2%) of the patients in our cohort presented with isolated dystonia. Otherwise, the patients reported by the original study presented mostly with dystonia associated with intellectual disability, cerebellar hypoplasia, cataracts, and other features of impaired neurodevelopment. The symptoms of the patients carrying heterozygous variant in *MED27* detected in the current study were much milder than the individuals carrying biallelic variants of *MED27* as the original study reported [4]. A study found that both homozygous and heterozygous mutation of *MED27* can be lethal at early embryonic stage in chicken [17]. Therefore, the identified heterozygous mutations might play a role in causing milder symptoms compared to biallelic mutations in our patients here due to a potential haploinsufficiency of *MED27*. The accordance of the coseparation in the p.F56C carrier's family also supported this speculation.

MED27 encodes a subunit of the transcriptional mediator, namely, MED27 [5]. A study reported that loss of function of *MED27* causes disruption of dopaminergic amacrine cells in zebrafish [6], suggesting an important role of *MED27* in neuronal development. The authors also found that impaired MED27 function may have affected the glia location in the transgenic zebrafish model [6]. Impairment of neurodevelopmental has been reported to be a key mechanism of dystonia [7–10]. Glial cells, especially the oligodendrocyte, have also been found to be important in the development of dystonia [11]. Most dystonia causative genes are expressed in both neuronal and glial cells and have an established role in myelination, such as *THAP1* [18], *YY1* [19], *TUBB4A* [20], and *SLC2A1* [21]. In addition, microstructural white matter changes have been detected in idiopathic dystonia [22], which also indicated an involvement of glial cells in dystonia.

5. Conclusion

In conclusion, the results of the current study indicated that variants of *MED27* were rare in Chinese dystonia patients, probably because that mutations in *MED27* are more associated with more complex neurodevelopmental disorders that can also include dystonia among the various neurological features. Further studies with larger sample size are still needed to confirm the role of *MED27* in dystonia and other neurological disorders.

Data Availability

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the Regulation of the People's Republic of China on the Administration of Human Genetic Resources.

Ethical Approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of West China Hospital of Sichuan University (2022-260).

Consent

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors report no competing interests.

Authors' Contributions

JL and CL contributed in the conception of the research project. JL, CL, and HS contributed in the organization of the research project. JL, CL, YH, LZ, RO, QW, KL, YX, and QJ contributed in the execution of the research project. JL and CL contributed in the design and execution of the statistical analysis. HS contributed in the review and critique of the statistical analysis. JL contributed in the writing of the first draft of the manuscript. CL contributed in the review and critique of the manuscript. JYL and CYL contributed equally to this work.

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References

[1] B. Balint, N. Mencacci, E. Valente et al., "Dystonia," *Nature Reviews Disease Primers*, vol. 4, no. 1, p. 25, 2018.

[2] H. Jinnah and Y. V. Sun, "Dystonia genes and their biological pathways," *Neurobiology of Disease*, vol. 129, pp. 159–168, 2019.

[3] H. Ichinose, T. Ohye, E. Takahashi et al., "Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene," *Nature Genetics*, vol. 8, no. 3, pp. 236–242, 1994.

[4] L. Meng, P. Isohanni, Y. Shao et al., "MED27 variants cause developmental delay, dystonia, and cerebellar hypoplasia," *Annals of Neurology*, vol. 89, no. 4, pp. 828–833, 2021.

[5] D. J. Taatjes, M. T. Marr, and R. Tjian, "Regulatory diversity among metazoan co-activator complexes," *Nature Reviews. Molecular Cell Biology*, vol. 5, no. 5, pp. 403–410, 2004.

[6] K. Dürr, J. Holzschuh, A. Filippi et al., "Differential roles of transcriptional mediator complex subunits Crsp34/Med27, Crsp150/Med14 and Trap100/Med24 during zebrafish retinal development," *Genetics*, vol. 174, no. 2, pp. 693–705, 2006.

[7] J. Li, S. Kim, S. S. Pappas, and W. T. Dauer, "CNS critical periods: implications for dystonia and other neurodevelopmental disorders," *JCI insight*, vol. 6, no. 4, 2021.

[8] J. Li, D. S. Levin, A. J. Kim, S. S. Pappas, and W. T. Dauer, "TorsinA restoration in a mouse model identifies a critical therapeutic window for DYT1 dystonia," *The Journal of Clinical Investigation*, vol. 131, no. 6, 2021.

[9] F. Y. Ismail, A. Fatemi, and M. V. Johnston, "Cerebral plasticity: windows of opportunity in the developing brain," *European Journal of Paediatric Neurology*, vol. 21, no. 1, pp. 23–48, 2017.

[10] M. Zech, R. Jech, S. Boesch et al., "Monogenic variants in dystonia: an exome-wide sequencing study," *The Lancet Neurology*, vol. 19, no. 11, pp. 908–918, 2020.

[11] D. Yellajoshyula, S. Pappas, and W. T. Dauer, "Oligodendrocyte and extracellular matrix contributions to central nervous system motor function: implications for dystonia," *Movement Disorders*, vol. 37, no. 3, pp. 456–463, 2022.

[12] C. Li, R. Ou, Y. Chen et al., "Mutation analysis of *DNAJC* family for early-onset Parkinson's disease in a Chinese cohort," *Movement Disorders*, vol. 35, no. 11, pp. 2068–2076, 2020.

[13] K. Karczewski, L. Francioli, G. Tiao et al., "The mutational constraint spectrum quantified from variation in 141,456 humans," *Nucleic Acids Research*, vol. 581, no. 7809, pp. 434–443, 2020.

[14] Y. Cao, L. Li, M. Xu et al., "The ChinaMAP analytics of deep whole genome sequences in 10,588 individuals," *Cell Research*, vol. 30, no. 9, pp. 717–731, 2020.

[15] K. Wang, M. Li, and H. Hakonarson, "ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data," *Nucleic Acids Research*, vol. 38, no. 16, article e164, 2010.

[16] K. M. Reid, R. Spaull, S. Salian et al., "*MED27*, *SLC6A7*, and *MPPE1* Variants in a Complex Neurodevelopmental Disorder with Severe Dystonia," *Movement Disorders*, vol. 37, no. 10, pp. 2139–2146, 2022.

[17] K. Tsujino, Y. Okuzaki, N. Hibino et al., "Identification of transgene integration site and anatomical properties of fluorescence intensity in a EGFP transgenic chicken line," *Development, Growth & Differentiation*, vol. 61, no. 7-8, pp. 393–401, 2019.

[18] D. Yellajoshyula, C. C. Liang, S. S. Pappas et al., "The DYT6 dystonia protein THAP1 regulates myelination within the oligodendrocyte lineage," *Developmental Cell*, vol. 42, no. 1, pp. 52–67.e4, 2017.

[19] Y. He, J. Dupree, J. Wang et al., "The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation," *Neuron*, vol. 55, no. 2, pp. 217–230, 2007.

[20] S. Sase, A. A. Almad, C. A. Boecker et al., "TUBB4A mutations result in both glial and neuronal degeneration in an H-ABC leukodystrophy mouse model," *eLife*, vol. 9, 2020.

[21] A. S. Saab, I. D. Tzvetavona, A. Trevisiol et al., "Oligodendroglial NMDA receptors regulate glucose import and axonal energy metabolism," *Neuron*, vol. 91, no. 1, pp. 119–132, 2016.

[22] S. Lehéricy, M. A. Tijssen, M. Vidailhet, R. Kaji, and S. Meunier, "The anatomical basis of dystonia: current view using neuroimaging," *Movement Disorders*, vol. 28, no. 7, pp. 944–957, 2013.