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Insights in movement disorders: 2021

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Published in
Frontiers in Neurology

ISSN 1664-8714
ISBN 978-2-83251-097-1
DOI 10.3389/978-2-83251-097-1

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Insights in movement disorders: 2021

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Citation

Albanese, A., Contarino, M. F., eds. (2023). *Insights in movement disorders: 2021*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-097-1

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frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 03 May 2022
doi: 10.3389/fneur.2022.837023

Efficacy and Safety of Zolpidem for Focal Dystonia After Neurosurgical Treatments: A Retrospective Cohort Study

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OPEN ACCESS

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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *16 December 2021*
Accepted: *12 April 2022*
Published: *03 May 2022*

Citation:
Horisawa S, Kohara K, Ebise H, Nishitani M, Kawamata T and Taira T (2022) Efficacy and Safety of Zolpidem for Focal Dystonia After Neurosurgical Treatments: A Retrospective Cohort Study. Front. Neurol. 13:837023. doi: 10.3389/fneur.2022.837023

Although there are several reports of the significant efficacy of zolpidem for treating dystonia, zolpidem is still considered an anecdotal treatment. Here, we evaluated the efficacy and safety of zolpidem for treating residual dystonia in patients who previously received various neurosurgical treatments majorly including deep brain stimulation and radiofrequency ablation. We retrospectively reviewed medical records from January 2021 to September 2021 to identify patients with dystonia who had been prescribed zolpidem after undergoing neurosurgery. Twenty patients were enrolled in this study, including those with blepharospasm (two), tongue dystonia (four), mouth dystonia (one), spasmodic dysphonia (two), cervical dystonia (six), focal hand dystonia (three), hemidystonia (two), blepharospasm with cervical dystonia (one), and mouth dystonia with cervical dystonia (one). Single doses of zolpidem ranged between 2.5 and 10 mg, while daily dosages ranged from 10 to 30 mg. The zolpidem dose prescribed was 5–10 mg, with single and daily doses of 7 ± 2.9 and 14.5 ± 6.0 mg, respectively. With zolpidem administration, the participants' Burke-Fahn-Marsden Dystonia Rating Scale-Movement Scale score significantly improved from 8.1 ± 6.7 to 3.7 ± 2.5 (50.6% improvement, $p < 0.0001$). Improvements in arm dystonia, blepharospasm, and spasmodic dysphonia were observed using the Arm Dystonia Disability Scale, Jankovic Rating Scale, and Voice Handicap Index, respectively. No improvements were observed in cervical dystonia on the Toronto Western Spasmodic Torticollis Rating Scale. Drowsiness, including three cases each of mild and moderate drowsiness, was the most frequent adverse effect (30%), which persisted for 2–3 h. Transient amnesia and rapid eye movement sleep behavior disorder occurred in two patients and one patient, respectively. Although our findings suggest that zolpidem can be a valuable treatment option for patients with residual dystonia after neurosurgical treatments, the beneficial effects for cervical dystonia were limited.

Keywords: dystonia, efficacy, neurosurgery, safety, zolpidem

INTRODUCTION

Treatments for dystonia include pharmacological therapies, botulinum toxin injections, and surgery (1). Botulinum toxin injection and surgical treatment are widely accepted treatments for medically refractory dystonia (2, 3). Pharmacological therapy with trihexyphenidyl, clonazepam, baclofen, and dopamine-related medications is generally the first-line treatment for dystonia (2).

Zolpidem is an imidazopyridine, non-benzodiazepine hypnotic agent that has been widely prescribed for the treatment of insomnia. Zolpidem was reported to have therapeutic effects on Parkinson's disease, which was confirmed by several studies including a double-blinded, placebo-controlled study. Additionally, several reports have shown significant efficacy of zolpidem for dystonia (4–13). However, zolpidem remains an anecdotal treatment due to the lack of randomized and controlled studies, and the mechanism of action of zolpidem in relieving dystonia remains unclear.

We have performed several neurosurgical treatments for dystonia, including selective peripheral denervation, deep brain stimulation (DBS), and ablative surgeries using radiofrequency, gamma knife, and focused ultrasound (14–19). All surgical candidates in those reports were refractory to conventional oral medications including trihexyphenidyl, clonazepam, and baclofen. Because botulinum toxin injections are covered by health insurance for the treatment of cervical dystonia and blepharospasm, many dystonia patients other than cervical dystonia and blepharospasm in Japan cannot afford it. We prescribed 5–10 mg of zolpidem at a time to postoperative neurosurgical patients with dystonia. In this study, we report the efficacy and safety of zolpidem for treating residual dystonia in patients who previously received neurosurgical treatment.

MATERIALS AND METHODS

Patient Population

We retrospectively reviewed medical records from January 2021 to September 2021 to identify patients with dystonia who were prescribed zolpidem. Zolpidem was prescribed only to patients who were not satisfied with surgical outcomes and wanted further improvements. We included patients with dystonia who had received zolpidem for dystonia treatment. The exclusion criteria were missing data regarding subjective evaluation using scales or side effects and concomitant use of additional medications or botulinum toxin injections after starting zolpidem use.

Medication

Zolpidem was prescribed at a single dose of 5 mg daily for the first 3 days. Thereafter, the dose was increased to a single dose of 10 mg daily tolerated. In case of difficulty in continuation of zolpidem due to its side effects, such as drowsiness, the dose was decreased to a single dose of 2.5 mg per time. Patients were allowed to take zolpidem several times per day if the daily dose was <10 mg.

Evaluation

The patients' demographic and clinical characteristics, including the distribution and etiology of dystonia, failed treatments, including surgery for dystonia prior to zolpidem prescription, zolpidem dose, the effective duration of treatment, and adverse events were evaluated. Patients receiving DBS were evaluated with stimulating-on conditions.

All patients were evaluated 1 month after starting zolpidem use at the outpatient clinic. Previous studies reported that onset of effects and peak effects after zolpidem administration were 15–45 min and 1–2 h, respectively (5, 7–10, 12). Therefore, evaluation was performed before the initiation of zolpidem and at the time of maximum drug concentration (1–2 h after oral administration). Rating scales were used for evaluation. All the patients were administered the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS)-Movement Scale (BFMDRS-MS; range: 0–120; higher scores indicate greater severity). Patients with blepharospasm were administered the Jankovic Rating Scale (JRS; range: 0–8; higher scores indicate greater severity), and those with spasmodic dysphonia were administered the Voice Handicap Index (VHI; range: 0–120; higher scores indicate greater voice-related handicap). Patients with cervical dystonia were administered the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS; range: 0–85; higher scores indicate greater severity, disability, and pain), whereas those with focal hand dystonia were administered the Arm Dystonia Disability Scale (ADDS; range: 0–100%; lower scores indicate greater disability).

Statistical Analysis

Statistical analysis was performed using the JMP statistical package, version 15.0.0 (SAS Institute, Cary, NC). The data were considered non-parametric; therefore, the Wilcoxon signed-rank test was used to compare the pre- and post-treatment BFMDRS-MS scores. Statistical significance was set at $p < 0.05$.

Ethical Considerations

The data for this study were retrospectively collected and analyzed. The Ethics Committee of our institution approved this study, and considering the observational nature of the study, the requirement for the provision of consent by patients was waived. Written informed consent for the publication of the videos was obtained.

RESULTS

Twenty-seven patients were prescribed zolpidem for dystonia treatment. Seven patients were excluded, three due to missing data regarding subjective evaluation scales and four due to concomitant use of additional medications or botulinum toxin injections after starting zolpidem use. The patients' characteristics are shown in **Table 1**. Twenty patients were enrolled in this study, including those with blepharospasm (two), tongue dystonia (four), mouth dystonia (one), spasmodic dysphonia (two), cervical dystonia (six), focal hand dystonia (three), hemidystonia (one), focal hand and foot dystonia (one), blepharospasm with cervical dystonia (one), and mouth

TABLE 1 | Patient characteristics and clinical outcomes.

Case	Distribution of dystonia	Etiology	Sex	Age at onset (years)	Disease duration (years)	Failed treatments prior to surgery	Neurosurgical treatments	Interval between last surgery and zolpidem administration	Zolpidem	BFMDRS-MS score			
									Single dose/Daily dose	Pre surgery	Pre medication	Post medication	% improvement
1	Blepharospasm	Primary	Female	52	11	BTX, Tri, Clo	GPi-DBS*, GPi-RF	45	2.5/10 mg	8	8	4	50
2	Blepharospasm	Primary	Female	63	6	BTX	GPi-DBS	13	10/20 mg	12	8	4	50
3	Spasmodic dysphonia	Stroke	Male	23	6	BTX, Tri, Clo	GPi-DBS*	20	10/20 mg	4	4	2	50
4	Spasmodic dysphonia	Primary	Male	44	5	BTX, Tri	GPi-DBS*, GPi-RF	50	5/10 mg	6	3	2	33.3
5	Tongue dystonia	Primary	Female	59	10	BTX	FF-DBS	6	5/10 mg	4	2	0	100
6	Tongue dystonia	Primary	Male	29	7	BTX	FF-DBS, RF	6	10/20 mg	4	2	0.5	75
7	Tongue dystonia	Primary	Female	44	1	BTX, Tri	PTT-RF	3	5/10 mg	9	1	0.5	50
8	Tongue dystonia	Primary	Male	33	2	Tri	FF-DBS, PTT- RF	3	5/10 mg	4	1	0.5	50
9	Mouth dystonia	Primary	Male	73	2	BTX, Tri, Clo	GPi-DBS	12	2.5/10 mg	6	6	4	33.3
10	Blepharospasm/ Cervical dystonia†	Primary	Male	47	4	Tri	PTT-GK	3	5/10 mg	12	12	10	16.7
11	Mouth dystonia/ Cervical dystonia†	Tardive	Female	52	12	BTX, Tri, Bac	GPi-DBS	60	5/10 mg	12	10	8	20
12	Cervical dystonia‡	Primary	Male	52	13	Tri, Clo	GPi-DBS	70	5/10 mg	8	4	2	50
13	Cervical dystonia‡	Traumatic	Male	45	2	Tri, Clo, Bac	FF-DBS	6	10/10 mg	6	4.5	4.5	0
14	Cervical dystonia†	Tardive	Female	51	2	BTX, Tri, Clo	PTT-RF	3	5/10 mg	6	4.5	4.5	0
15	Cervical dystonia‡	Primary	Male	47	9	Tri, Clo	GPi-DBS, GPi-RF, SPD	12	5/10 mg	8	6	6	0
16	Focal hand dystonia	Stoke	Female	5	46	BTX, Tri	GPi-DBS*, Vo-RF	6	10/20 mg	16	16	6	62.5
17	Hemidystonia	Stroke	Male	28	2	BTX, Tri, Clo	DN-DBS**	6	10/30 mg	24	24	5	79.2
18	Focal hand dystonia	Stroke	Male	48	8	Tri, Clo	PTT-RF	12	10/20 mg	16	16	4	75
19	Hemidystonia	Primary	Male	27	9	BTX, Tri	DN-DBS, Vim-DBS**, Vo-RF,	16	10/20 mg	36	20	5	75
20	Focal hand dystonia	Hereditary (DYT-1)	Male	9	19	BTX, Tri	Vo-DBS, GPi-RF	10	10/20 mg	20	12	6	50

BFMDRS-MS, Burke-Fahn-Marsden Dystonia Rating Scale-Movement Scale; BTX, botulinum toxin injections; Tri, trihexyphenidyl; Clo, clonazepam; Bac, baclofen; GPi, Globus pallidus internus; FF, Forel's field; PTT, Pallidothalamic tract; DN, Dentate nucleus; Vo, Ventro-oral nucleus; DBS, deep brain stimulation; RF, radiofrequency ablation; GK, gamma knife ablation; SPD, selective peripheral denervation.

†*Phasic cervical dystonia.*

‡*Tonic cervical dystonia.*

**DBS was removed for insufficient efficacy.*

***DBS was removed for infection.*

dystonia with cervical dystonia (one). The etiologies of dystonia were idiopathic in 13 patients, post-stroke in four patients, post-traumatic in one patient, drug-induced (tardive) in one patient, and hereditary (DYT-1 dystonia) in one patient. Failed treatments prior to surgery included botulinum toxin injections (15 patients), trihexyphenidyl (1–6 mg/day, 18 patients), clonazepam (1.5 mg/day, 10 patients), and baclofen (15 mg/day, two patients). The neurosurgical treatments performed in the patients included deep brain stimulation (16 patients), radiofrequency ablation (12 patients), gamma knife ablation (one patient), and selective peripheral denervation (one patient). The pre- and post-operative BMFDRS-MS scores were 11.1 ± 8.1 and 8.2 ± 6.6, respectively (25.8% improvement).

The mean single and daily doses of zolpidem were 7 ± 2.9 and 14.5 ± 6.0 mg, respectively. The daily dose of zolpidem was spontaneously increased to 20–30 mg in eight patients to improve their quality of life. Among eight patients, five with focal hand dystonia, focal hand and foot dystonia, and hemidystonia (case 16–20) experienced pain due to severe dystonic postures. Both pain and dystonia were remarkably relieved by zolpidem administration. Case 2 was functionally blind due to severe blepharospasm (forced eyelid closure), and at least 10 mg of zolpidem twice a day had to be administered to sustain eye-opening. Case 3 with spasmodic dysphonia and Case 6 with tongue dystonia had difficulty with daily communication with others due to dystonia. A single dose of 10 mg of zolpidem twice a day was necessary for their daily lives.

With zolpidem administration, the BFMDRS-MS score significantly improved from 8.1 ± 6.7 to 3.7 ± 2.5 (50.6% improvement, $p < 0.0001$; **Figure 1**, **Table 2**). Three patients with cervical dystonia did not respond to zolpidem, while 13 patients (68.4%) showed 50 to 100% reduction in BFMDRS-MS score. Six patients with cervical dystonia responded poorly to zolpidem in our study. The mean total TWSTRS score was 30.5 ± 7.3 before administration and 28 ± 6.9 after zolpidem administration (5.8 ± 2.0 mg). Although severity and disability scores did not change after zolpidem administration, pain scores decreased from 8.3 ± 3.8 to 6.1 ± 2.7 with zolpidem administration. The mean BFMDRS-MS neck subscale score was 5 ± 0.9 before administration and 4.6 ± 1.6 after administration (8% improvement). Symptomatic improvements were confirmed in patients with focal hand dystonia, blepharospasm, and spasmodic dysphonia using the ADDS, JRS, and VHI, respectively; however, no improvements in TWSTRS score were noted in patients with cervical dystonia (**Figure 2**). The representative movies illustrating spasmodic dysphonia (case 4), tongue dystonia (case 5), and focal hand dystonia (case 16) are shown in **Supplementary Videos 1–3**.

The duration of beneficial effects of zolpidem on dystonias ranged from 3 to 4 h, and the time-to-effect ranged from 15 to 30 min, which corresponded to previous study findings (5, 7–10, 12). Drowsiness, including three cases each of mild and moderate drowsiness, was the most frequent adverse effect (30%), which persisted for 2–3 h. Transient amnesia and rapid eye movement sleep behavior disorder occurred in two patients and one patient, respectively. Four patients with hand dystonia, excluding those with DYT-1 dystonia, had a fixed dystonic posture (clenched fist). Five milligrams of zolpidem did not improve hand dystonia in these four patients; however, 10 mg significantly improved their symptoms. Two of these patients increased the single dose of zolpidem to 15–20 mg once on their own and showed significant dose-dependent improvement. However, sleepiness increased with increasing doses; therefore, zolpidem was discontinued. Two patients with cervical dystonia who did not achieve any benefit ceased taking zolpidem voluntarily. One patient with blepharospasm and one patient with mouth dystonia showed significant improvement without any complications with 2.5 mg of zolpidem. Eighteen patients thought that zolpidem improved their daily life by relieving dystonia. Two patients with cervical dystonia discontinued zolpidem because of poor response and drowsiness.

DISCUSSION

In this study, zolpidem use significantly improved residual dystonic symptoms after neurosurgical treatment. Thirteen patients (68.4%) showed more than 50% improvement, as measured using the BFMDRS-MS. Drowsiness was the most common adverse event (30%), and no serious adverse events were observed.

The most notable finding of this study was that zolpidem appears to be effective for treating residual dystonia after neurosurgical treatment. Neurosurgical treatments for dystonia are usually considered after failed conservative treatment, including oral medications such as trihexyphenidyl, clonazepam, baclofen, and botulinum toxin injections (1, 2, 20). Unfortunately, the effectiveness of neurosurgical treatment for dystonia varies between patients, and 10 to 25% of patients with cervical, segmental, or generalized dystonia do not respond to selective peripheral denervation or pallidal deep brain stimulation (21–23). Zolpidem can be a treatment option for patients who fail to respond to neurosurgery and conventional conservative treatments.

There have been three studies that evaluated the effects of zolpidem for dystonia improvement using the BFMDRS (4, 10, 12). Among the various phenotypes of dystonia, hand dystonia, evaluated using the ADDS and BFMDRS-MS, showed the most significant improvement, from 26.6% ± 18.7 and 15.2 ± 1.8 at baseline to 61.7% ± 9.9 and 4.8 ± 1.1 after zolpidem intake, respectively. Miyazaki et al. reported improvements in BFMDRS-MS hand scores from 2.9 ± 2.0 before zolpidem administration to 2.0 ± 0.9 after administration of 8.8 ± 5.1 mg zolpidem in eight patients with hand dystonia (10). Five out of eight patients did not respond to zolpidem administration. The effect of zolpidem on dystonia may increase in a dose-dependent manner (10). Pre-treatment BFMDRS values of 58 and 22.5 have been reported in case reports of DYT-6 dystonia and generalized torsion dystonia, respectively. These values improved to 33.5 and 27 with 10 mg and 25.5 and 19 with 20 mg of zolpidem, respectively (12). Our study showed that a single dose of 10 mg zolpidem appeared to be effective in relieving hand dystonia. The relatively lower efficacy of zolpidem for hand dystonia in their study (5) (31.0% improvement in BFMDRS-MS score) compared with our study

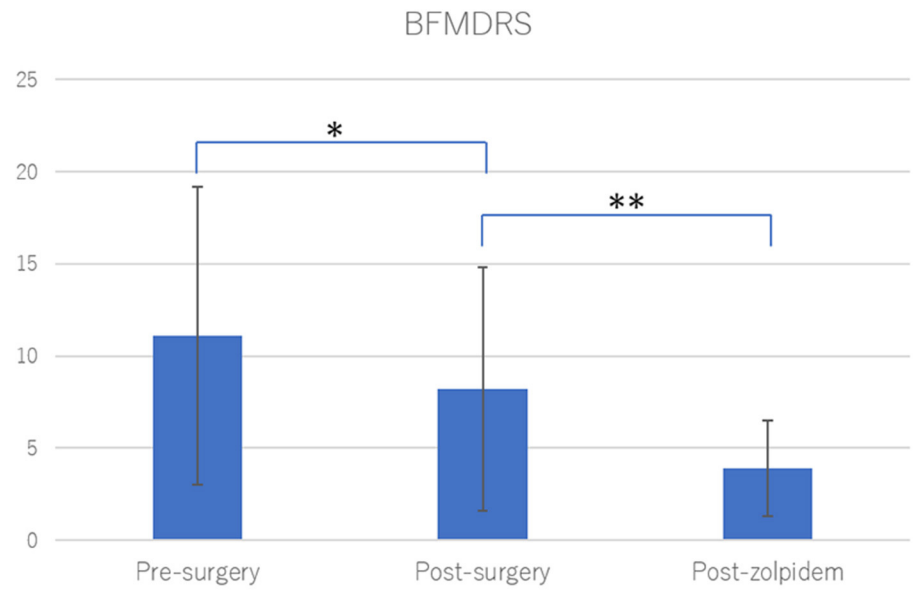

FIGURE 1 | Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) after neurosurgical treatments and zolpidem. Neurosurgical treatments significantly improved dystonia among the study participants (29.9% improvement, $^{*}p = 0.0002$). Zolpidem administration significantly improved residual dystonic symptoms after neurosurgical treatments (50.6% improvement, $^{**}p < 0.0001$). Statistical significance was evaluated by Wilcoxon signed rank test.

TABLE 2 | BFMDRS scores off and on medication.

BFMDRS-MS	Number of patients	Off medication	On medication	
Total	20	8.2 ± 6.6	3.9 ± 2.6	$^{*}p < 0.0001$
Eyes	3	7.3 ± 1.2	4	
Mouth	6	2.7 ± 2.0	1.3 ± 1.5	
Speech/swallowing	2	3.5 ± 0.7	2	
Neck	6	5.2 ± 0.9	4.8 ± 1.6	
Arm	5	15.2 ± 1.8	4.8 ± 1.1	
Leg	2	6 ± 2.8	1	

BFMDRS-MS: Burke-Fahn-Marsden Dystonia Rating Scale-Movement Scale.
**The Wilcoxon signed-rank test was used to compare the BFMDRS-MS scores off and on medication.*

(68.4% improvement in BFMDRS-MS score) may be due to a lower single dose of zolpidem.

In this study, cases with cervical dystonia responded relatively poorly to zolpidem compared to other regions of dystonia, which nearly corresponded to that in a previous study of seven patients with cervical dystonia who showed a poor response to 10 mg of zolpidem (10). The reason behind the poor response of cervical dystonia to zolpidem is unknown; hence, further studies to determine whether higher doses of zolpidem (≥15 mg) can achieve a better response in patients with cervical dystonia are needed. However, four patients considered that zolpidem obviously improved neck pain measured by the TWSTRS pain scale. Five patients with focal hand or hemidystonia who required a daily dose of 20–30 mg of zolpidem reported zolpidem as a highly effective pain relief medication. Careful evaluation is required for not only objective movement evaluation but also subjective evaluation such as pain and quality of life evaluation.

The mechanism underlying the improvement of dystonia by zolpidem remains unknown. The basal ganglia and thalamus have a high density of GABA-A receptors, which is the binding site of zolpidem (24, 25). In the basal ganglia, the ventral pallidum, substantia nigra pars reticulata, and subthalamic nucleus have the highest density of zolpidem-binding GABA-A receptors, suggesting that zolpidem may help restore the influence of basal ganglia output on the thalamus and motor cortex (26, 27). Badillo et al. suggested that the effects of zolpidem result from the facilitation of inhibitory pathways in the basal ganglia-thalamo-cortical circuit, which leads to the improvement of dystonia (13). However, zolpidem also has therapeutic effects on motor symptoms of Parkinson's disease, which is a hypokinetic movement disorder (28–30). A double-blinded, placebo-controlled study demonstrated that a single oral dose (10 mg) of zolpidem reduced 30.2% of Unified Parkinson's Disease Rating Scale in 10 patients

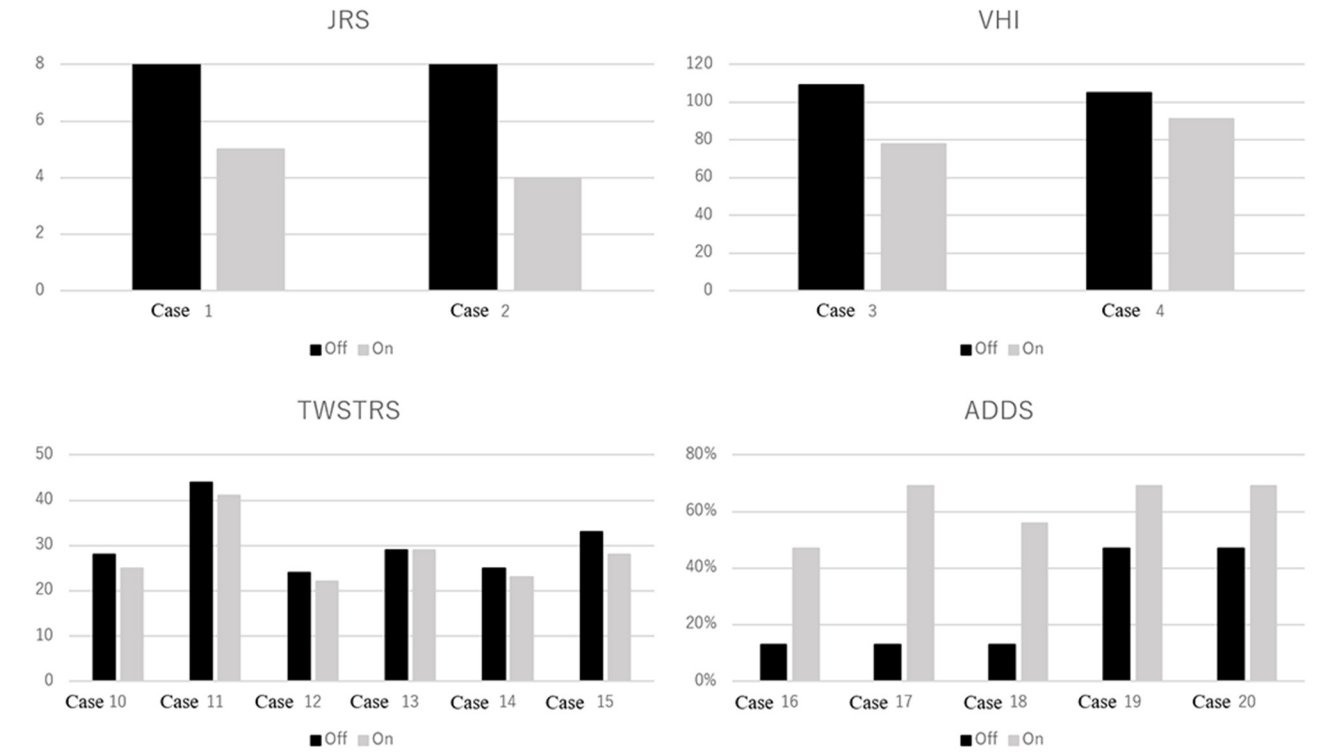

FIGURE 2 | Focal dystonia rating scale scores with and without medication. JRS, Jankovic Rating Scale. Range: 0–8. Higher scores indicate greater blepharospasm severity. VHI, Voice Handicap Index. Range: 0–120. Higher scores indicate greater voice-related handicap in patients with spasmodic dysphonia. TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale. Range: 0–85. Higher scores indicate greater severity, disability, and pain in patients with cervical dystonia. ADDS, Arm Dystonia Disability Scale. Range: 0–100%. Lower scores indicate greater disability in patients with focal hand dystonia.

with Parkinson's disease 1 h after the administration (28). The confirmed effects of zolpidem in this study included improvement of rigidity, akinesia, bradykinesia, posture, gait, and facial expression, indicating that zolpidem could serve as a pharmacological equivalent of posteroventral pallidotomy (28). We hypothesize that the underlying mechanism of zolpidem in Parkinson's disease involves the selective inhibition of GABAergic inhibitory neurons in the abnormally overactivated globus pallidus internus (GPi) and substantia nigra pars reticulata, both of which have a high density of zolpidem-binding sites (28). Supposing our hypothesis regarding this mechanism is correct, the suggested mechanism of zolpidem in Parkinson's disease cannot explain the effects of zolpidem on dystonia, which causes reduced output from GPi, leading to increased thalamic and cortical activities (31). Similar paradoxical findings have been suggested in terms of the underlying mechanism that GPi-DBS and pallidotomy (GPi ablation) have similar therapeutic effects on both PD (hypokinetic movement disorder) and dystonia (hyperkinetic movement disorder) (32). So far, available evidence cannot explain in detail the mechanism of zolpidem in dystonia and Parkinson's disease.

This study has some limitations. This is an open-label study and does not have a randomized and controlled design. All patients enrolled in this study received neurosurgical treatment; therefore, the improvements in dystonia in this study did not reflect the true effects of zolpidem on dystonia, which implies that further improvements may have been observed if zolpidem was prescribed before surgery or the lack of response may have been caused by the improved dystonia through surgery. Additionally, the effects of zolpidem on the affected body parts were not statistically evaluated due to the small sample size.

In conclusion, this study suggests that zolpidem can be a valuable treatment option for patients with dystonia. Nevertheless, a randomized controlled trial with larger sample size is needed to elucidate the efficacy and safety of zolpidem for dystonia.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tokyo

Women's Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SH: conception and design of the study, acquisition and analysis of data, and drafting a significant portion of the manuscript and figures. KK: conception and design of the study and acquisition and analysis of data. HE and MN: acquisition and analysis of data. TK: conception and design of the study. TT: conception and design of the study and acquisition and analysis of data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by JSPS KAKENHI Grant Number JP21K09113.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.837023/full#supplementary-material

Supplementary Video 1 | Condition of case 4 (spasmodic dystonia) with and without zolpidem administration. Recording of voice from the cell phone after administering 10 mg of zolpidem (speech condition with zolpidem).

Supplementary Video 2 | Condition of case 5 (tongue dystonia) with and without zolpidem administration.

Supplementary Video 3 | Condition of case 16 (post-stroke focal hand dystonia) with and without zolpidem administration.

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APPENDIX

Further Reading

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frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 18 May 2022
doi: 10.3389/fneur.2022.882628

Intestinal Barrier Dysfunction in the Absence of Systemic Inflammation Fails to Exacerbate Motor Dysfunction and Brain Pathology in a Mouse Model of Parkinson's Disease

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OPEN ACCESS

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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: 24 February 2022
Accepted: 14 April 2022
Published: 18 May 2022

Citation:
Jackson A, Engen PA, Forsyth CB, Shaikh M, Naqib A, Wilber S, Frausto DM, Raeisi S, Green SJ, Bradaric BD, Persons AL, Voigt RM and Keshavarzian A (2022) Intestinal Barrier Dysfunction in the Absence of Systemic Inflammation Fails to Exacerbate Motor Dysfunction and Brain Pathology in a Mouse Model of Parkinson's Disease. Front. Neurol. 13:882628. doi: 10.3389/fneur.2022.882628

Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disease associated with aging. PD patients have systemic and neuroinflammation which is hypothesized to contribute to neurodegeneration. Recent studies highlight the importance of the gut-brain axis in PD pathogenesis and suggest that gut-derived inflammation can trigger and/or promote neuroinflammation and neurodegeneration in PD. However, it is not clear whether microbiota dysbiosis, intestinal barrier dysfunction, or intestinal inflammation (common features in PD patients) are primary drivers of disrupted gut-brain axis in PD that promote neuroinflammation and neurodegeneration.

Objective: To determine the role of microbiota dysbiosis, intestinal barrier dysfunction, and colonic inflammation in neuroinflammation and neurodegeneration in a genetic rodent model of PD [α-synuclein overexpressing (ASO) mice].

Methods: To distinguish the role of intestinal barrier dysfunction separate from inflammation, low dose (1%) dextran sodium sulfate (DSS) was administered in cycles for 52 days to ASO and control mice. The outcomes assessed included intestinal barrier integrity, intestinal inflammation, stool microbiome community, systemic inflammation, motor function, microglial activation, and dopaminergic neurons.

Results: Low dose DSS treatment caused intestinal barrier dysfunction (sugar test, histological analysis), intestinal microbiota dysbiosis, mild intestinal inflammation (colon shortening, elevated MPO), but it did not increase systemic inflammation (serum cytokines). However, DSS did not exacerbate motor dysfunction, neuroinflammation (microglial activation), or dopaminergic neuron loss in ASO mice.

Conclusion: Disruption of the intestinal barrier without overt intestinal inflammation is not associated with worsening of PD-like behavior and pathology in ASO mice.

Keywords: Parkinson's disease, intestinal hyperpermeability, dextran sodium sulfate (DSS), microbiome, gut-brain axis

INTRODUCTION

Studies from our group and others support a role for the microbiome and intestinal tract (gut) in Parkinson's disease (PD) (1, 2). This model is known as the "gut-brain axis" (GBA) which is a bi-directional communication axis involving the intestinal microbiome, the intestinal barrier, intestinal inflammation, and the intestinal/systemic/brain immune systems (among other components) (3, 4). The gut-brain axis contributes to normal function and pathology of the central nervous system (4, 5). PD patients have an abnormal gut-brain axis (6–10).

PD patients have intestinal barrier dysfunction (6–10). Under normal conditions, the pro-inflammatory contents of the intestine are retained within the lumen of the intestine by the intestinal barrier which is comprised of both physical (mucus, tight junction proteins) and chemical (anti-microbial peptides) components. The barrier can become dysfunctional permitting the entrance of pathogenic bacteria and bacterial components including lipopolysaccharide (LPS) into the intestinal mucosa and the systemic circulation, prompting mucosal and systemic inflammation (1, 6, 11, 12), which may promote neuroinflammation, a key feature of PD.

In 2015, it was reported that patients with PD have intestinal microbiota dysbiosis (13, 14) and more than 20 studies since then have similarly demonstrated that the intestinal microbiome in PD patients is distinct from age matched subjects without PD (6–10). Although there is no unique PD microbiota signature, studies show that the dysbiosis in PD is characterized by an increased relative abundance of "putative" pro-inflammatory bacteria especially LPS-containing, Gram-negative bacteria and reductions in the relative abundance of putative anti-inflammatory bacteria [e.g., short chain fatty acid (SCFA)-producing bacteria] (1, 3, 15, 16). The pro-inflammatory microbiota can cause intestinal barrier dysfunction and disruption of the intestinal barrier can impact the microbiota leading to a positive feedback loop.

Intestinal barrier dysfunction and microbiota dysbiosis appear to be biologically meaningful. Studies demonstrate that the abundance of pro-inflammatory, LPS-containing, Gram-negative bacteria in PD subjects correlates with motor impairment in PD patients (1, 14, 17). Additionally, LPS is associated with more severe neuroinflammation in animal models of PD (8, 15, 18), and administration of LPS to mice is used as a model for neurodegeneration and PD (19–21). Taken together, studies suggest that the gut microbiota and microbiota-derived, pro-inflammatory molecules like LPS may contribute to PD pathogenesis.

One consequence of intestinal barrier dysfunction and microbiota dysbiosis is intestinal inflammation (1). Indeed, intestinal (e.g., stool calprotectin) and systemic (IL-1β, IL-6, and TNF-α) inflammation are reported in patients with PD and animal models of PD (8, 9, 22–26). Furthermore, inflammatory bowel disease (IBD), characterized by intestinal barrier dysfunction, pro-inflammatory changes in the intestinal microbiome, and chronic intestinal and systemic inflammation (27), is a risk factor for PD (27–29). This suggests that the inflammatory consequences of intestinal barrier dysfunction and intestinal microbiota dysbiosis are important in PD.

Intestinal microbiota dysbiosis, intestinal barrier dysfunction, and inflammation typically occur together in both animal models of PD and PD patients, therefore it is not clear which one these three elements is a primary driver of neuroinflammation and neurodegeneration in PD or whether all three "pro-inflammatory" factors are required. This study determined whether disruption of intestinal barrier/dysbiosis without significant intestinal inflammation was sufficient to worsen neuroinflammation and neurodegeneration in an animal model of PD.

METHODS AND MATERIALS

Mice

Transgenic mice overexpressing human wild type α-synuclein under the Thy1 promoter were used for this study, known as ASO or "Line 61" mice (16, 30). Mice hemizygous for Thy1-α-synuclein overexpression were maintained on a mixed C57BL/6-DBA/2 background by breeding female BDF1 background, Thy1-α-synuclein animals hemizygous for the Thy1-α-synuclein transgene on the X-chromosome with wild-type male BDF1 (Charles River, Wilmington, USA) to generate the male ASO and control littermates (without the transgene). Breeding pairs were replenished every 6 months with transgenic females and newly generated BDF1 males. The genotype of ASO and control mice was verified with PCR (16). The transgene is inserted in the X chromosome, which undergoes random chromosomal silencing, so only male mice are used experimentally (30).

Mice were maintained on a 12 h light/dark cycle with free access to water and food and were singly housed. All animal husbandry and experiments were approved by the Rush University Institutional Animal Care and Use Committee (IACUC).

Dextran Sodium Sulfate Administration

DSS has a direct toxic effect on intestinal epithelial cells leading to disruption of the intestinal barrier (31–33). DSS (molecular weight 36,000–50,000, MP Biomedicals, Santa Ana, CA) was given to mice in filtered drinking water and was replenished every other day. DSS was administered beginning when mice were 14 weeks of age and was given over three cycles. A DSS cycle is defined by 7 days on DSS followed by a 14-day recovery period

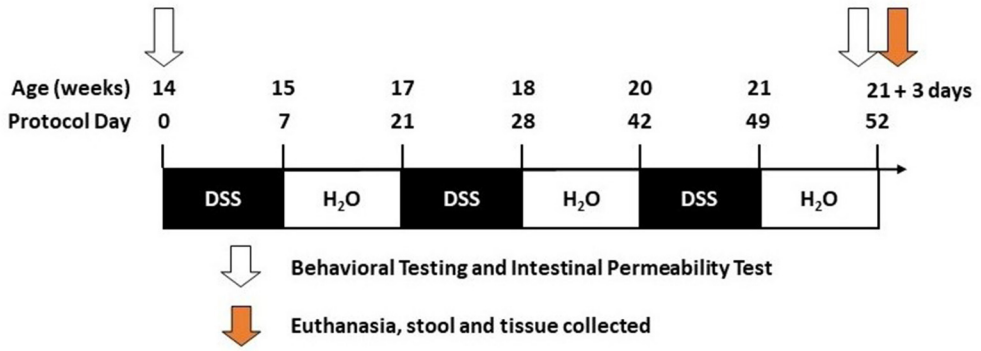

FIGURE 1 | Timeline. The diagram illustrates the dextran sodium sulfate (DSS) treatment over given over three cycles during the 52 days of the study (see Methods).

with no DSS (21-day cycle; **Figure 1**). Vehicle-treated mice (i.e., H_2O) were given only drinking water (i.e., without DSS).

Tissue Collection and Processing

Tissue was collected 3 days after the last DSS cycle (**Figure 1**). Necropsy was performed under anesthesia as approved by Rush IACUC#18–052. Mice were deeply anesthetized (90 mg/kg ketamine, 10 mg/kg xylazine in a 0.9% saline diluent). Blood was collected by cardiac puncture and stored on ice until serum isolation/collection. After blood collection, mice were perfused with cold PBS. The abdomen was clamped using hemostatic forceps to perfuse the upper body. The brain was collected and immersion-fixed in 4% paraformaldehyde, intestinal tissue was measured for length (end of the cecum to the anus), and colon samples were collected and stored either in optimal cutting temperature (OCT) media (4583, Tissue-Tek), 4% paraformaldehyde, or flash frozen in liquid nitrogen.

Intestinal Barrier Integrity

The oral sugar test was used to assess region-specific differences in intestinal barrier integrity (34–36). We have previously published that sucralose, and especially the Sucralose/Lactulose ratio is primarily a marker of the colonic permeability, including in PD patients (35). Lactulose and mannitol are markers of small intestinal permeability with an elevated Lactulose/Mannitol ratio indicating the small intestinal barrier hyperpermeability (36).

In vivo intestinal barrier integrity was assessed at baseline (14 weeks of age) and the end of the last DSS cycle (22 weeks of age) as previously described (8, 35). Briefly, mice were fasted for 8 h prior to the test. A 200 ul solution containing lactulose (3.2 mg), sucrose (0.45 mg), sucralose (0.45 mg), and mannitol (0.9 mg) was administered *via* gavage, after which 2 ml of 0.9% saline was administered subcutaneously to promote urine output. Mice were placed in metabolic cages and urine was collected for 5 h and the total volume recorded. Intestinal permeability was calculated by measuring urinary sugar concentration with gas chromatography which is expressed as percent excretion of the oral dose of sugar (8, 36).

Immunofluorescent Staining

Gastrointestinal Tissue

The integrity of the intestinal barrier is maintained by a series of inter-locking proteins between intestinal epithelial cells known as the Apical Junctional Complex (AJC) (37, 38). This AJC is composed of tight (zonula adherens 1, ZO-1) and adherens (E-cadherin) junctions which were examined in this study (38, 39). OCT-embedded intestinal tissue (ZO-1) was cut into 5 μm sections, and then fixed using acetone at −20° for 20 min. Paraffin-embedded intestinal tissue (E-Cadherin) was cut into 5 μm sections, which were de-paraffinized and rehydrated using serial ethanol dilutions (100, 95, and 70%) (40). Heat-induced antigen retrieval was completed by submerging tissue in an EDTA buffer for 4 min using a pressure cooker. Slides were blocked with 10% donkey serum (Jackson ImmunoResearch, 017-000-12) overnight, followed by overnight incubation with antibody (ZO-1: 1:500 Invitrogen #61-7300; E-Cadherin: 1:500 Cell Signaling #14472). Secondary antibody diluted at 1:250 (Alexa Fluor 555, #4409) was applied for 45 min, followed by washing. Sections were then DAPI-stained and mounted using Fluoromount Aqueous Mounting Medium (Sigma-Aldrich, #F4680). Immunofluorescence images were acquired using a Zeiss Axio Observer 7 at 20x magnification, two images per sample (40).

Brain Tissue

Ionized calcium binding adaptor molecule-1 (Iba-1) is a microglia/macrophage-specific calcium-binding protein that is a widely validated marker for microglia identification and microglial morphology characterization (41). Tyrosine hydroxylase (TH) is the rate-limiting enzyme of catecholamine biosynthesis and a robust marker of dopaminergic neurons (40, 42). Loss of TH staining in the striatum is a hallmark for loss of dopaminergic terminals that is characteristic of neurodegeneration in PD (43). Brain tissue was cut at 30 μm thickness using a cryostat (CM3050, Leica) and was stored in cryoprotectant until analysis (40). In brief, sections were washed with dilution media for 60 min. An antigen retrieval step was performed using a citric acid buffer solution (6.0 pH) for 20 min. Then, an endogenous sodium peroxidase

block was performed using a sodium periodate solution for 20 min. Following peroxidase blocking, sections were washed multiple times in dilution media and incubated in serum blocking solution for an hour (2% BSA and 3% serum targeting host of the secondary antibody). Sections were incubated in primary antibody (Iba-1: 1:1000, Wako 019–19741; TH: 1:10,000, Immunostar 22941) overnight at room temperature. The next day, sections were washed and processed with biotinylated secondary antibodies (1:200, Vector Laboratories BA1000, BA2000). Immunoperoxidase sections were treated with a standard ABC HRP Biotin/Avidin Complex Kit (Vector Laboratories). Incubation was performed before developing a color reaction in the presence of DAB chromogen and hydrogen peroxide. Once completed, immunoperoxidase stained sections were mounted on glass slides, cover-slipped using Cytoseal TM 60 mounting medium (8310-16) and analyzed.

Western Blot Analysis

Isolation of Nuclear and Cytoplasmic Extracts and Analysis

The cytoplasmic and membrane extraction was prepared using an NE-PER Nuclear Cytoplasmic Extraction Reagent kit (Pierce, Rockford, IL, USA) as previously described (36). Briefly, tissue was washed twice with cold PBS and centrifuged at 500 × *g* for 5 min. The pellet was suspended in 200 μl of cytoplasmic extraction reagent I by vortexing. The suspension was incubated on ice for 10 min followed by the addition of 11 μl of cytoplasmic extraction reagent II, vortexed for 5 s, incubated on ice for 1 min and centrifuged for 5 min at 16,000 × *g*. The supernatant fraction (cytoplasmic extract) was transferred to a pre-chilled tube. The insoluble pellet fraction, which contains crude nuclei, was resuspended in 25 μl of nuclear extraction reagent by vortexing during 15 s and incubated on ice for 10 min, then centrifuged for 10 min at 16,000 × *g*. The remaining insoluble pellet, containing membrane fragments, was suspended in 100 μl of tris-triton buffer. Samples were incubated on ice for 20 min and then centrifuged (16,000 × *g*, 10 min). The supernatant was collected and stored at −80°C.

Western Blot

Equal amounts of the protein concentrations were quantified and normalized to the β-actin band. Homogenized colon samples (30 μg) were boiled at 95°C for 5 min with 2x Laemmli sample buffer (Bio-Rad Laboratories, Hercules, CA). Samples were electrophoresed on 7.5% tris-HCl gels and transferred to a nitrocellulose membrane (GE Healthcare Limited, Buckinghamshire, UK). Non-specific binding sites were blocked for 1 h at room temperature {E-cadherin and ZO-1: 5% bovine serum albumin (BSA); β-actin: 2.5% BSA and 2.5% non-fat dry milk [all in tris-buffered saline / Tween-20 (TBS-T)]}. Membranes were incubated overnight at 4°C with primary antibody [E-cadherin: 1:1,000, Cell signaling 14472; ZO-1: 1:1,000, Invitrogen 61-7300; β-actin: 1:5,000, Sigma A2066 (all in TBS-T)]. Membranes were incubated in HRP-conjugated anti-rabbit secondary antibody (1:2,000) for 1 h at room temperature. Chemiluminescent substrate (ECL, GE Healthcare) was applied to the membrane for protein visualization using autoradiography film (HyBlot CL, Denville Scientific, Metuchen, NJ). Films and were scanned and optical density determined using ImageJ software (NIH, Bethesda, MD) (36, 44).

Intestinal Inflammation

Myeloperoxidase (MPO)

MPO is a reliable and well-established marker of intestinal inflammation (45–47). Colon tissue was homogenized and MPO was quantified using the MPO enzyme-linked immunosorbent assay (ELISA) kit (Hycult Biotechnology, Uden, The Netherlands) according to the manufacturer's instructions (47). Briefly, 10 mg of colon tissue was homogenized in 200 μl lysis buffer. Then, sample aliquots were applied onto microtiter well-precoated with capture antibody. After washing, biotinylated tracer antibody was added to each well. After incubation, the color development with tetramethylbenzidine was performed and the color reaction was stopped by the addition of oxalic acid. Absorbance at 450 nm was measured with a spectrophotometer. MPO concentration of each sample was calculated from a standard curve (serial dilution).

Calprotectin

Calprotectin is produced by neutrophils in the intestine and is a reliable and well-accepted method to assess intestinal inflammation (48). PD patients also have increased levels of calprotectin (9, 25, 49). Cecal content of the mice was collected during tissue collection was stored at −80°C until used for this assay. Calprotectin ELISA was performed using S100A8/S100A9 Elisa kit (ref K6936) from Immunodiagnostik (Immunodiagnostik, Bensheim, Germany) following the manufacturer's protocol. The concentration of calprotectin was calculated from measured OD 450 nm values by the Gene5 program (Biotek, Winooski, VT) (50).

Hematoxylin and Eosin Histology

Formalin-fixed colon was stained with hematoxylin & eosin (H&E). Blinded assessment of samples was conducted by a gastrointestinal pathologist (SS). Histological analyses, including inflammatory cell infiltrate, epithelial changes, and the mucosal architecture, were scored according to an established criterion (51). Mild colonic inflammation is operationally defined in this study as an increase in MPO levels and decrease in colon length, without elevated fecal calprotectin values. We chose to use elevated stool calprotectin as part of our definition of severe intestinal inflammation because, according to the American College of Gastroenterology, fecal calprotectin levels are a sensitive and specific marker of intestinal inflammation. Indeed, evaluation of stool calprotectin level has become routine for many clinicians who are managing patients with intestinal inflammatory diseases, such as ulcerative colitis (52). Relevant to our study, stool calprotectin is routinely used to define intestinal inflammation in patients with inflammatory bowel disease (53–55) and in patients with Parkinson's disease (49, 56, 57).

Microbial Translocation and Systemic Inflammation

LPS-Binding Protein (LBP)

LBP is a type 1 acute-phase protein that binds to LPS to facilitate an immune response that our group and others have shown is altered in PD patients with intestinal permeability (8, 58, 59). Serum collected at the time of cardiac puncture was used to measure systemic LBP levels using an LBP ELISA kit (HK205; Hycult Biotech) as previously described (8).

Cytokines

Serum cytokine levels were assessed with Meso Scale 10-plex V-PLEX Proinflammatory Panel 1 Mouse Kit (Cat. # K15048D, Meso Scale Diagnostics, Rockville, MD) as previously described (60).

Motor Function

Motor performance and coordination were assessed at 14 and 22 weeks of age including adhesive removal, beam traversal, and hindlimb clasping reflex.

Adhesive Removal

This test evaluates somatosensory and motor function. A one-quarter inch round adhesive (Avery, Glendale, CA) was placed on the nasal bridge between the nostrils and the forehead of the mouse, and the time to make contact and remove the adhesive was recorded. All testing was performed in the home cage. If the mouse did not remove the adhesive within 60's, the trial was ended. Time to make contact/remove the adhesive was recorded over three trials (16).

Beam Transversal

This test assesses motor coordination and balance. A 1 m plexiglass beam (Stark's Plastics, Forest Park, OH) was used. The beam was constructed of four segments of 0.25 m in length with each segment having a progressively thinner width: 3.5, 2.5, 1.5, and 0.5 cm. The widest segment acted as the loading platform for the animals and the narrowest end was placed into the home cage. Mice had 2 days of training prior to testing. On the 1st day of training, mice received one trial with the home cage positioned close to the loading platform and the mice were guided forward along the narrowing beam. Mice received two more trials with limited or no assistance to encourage forward movement on the beam. On the 2nd day of training, mice had three trials to transverse the beam and generally did not require assistance in forward movement. On the 3rd day, mice were tested over three trials for time to transverse from the loading platform to the home cage. Timing began when mice placed their forelimbs onto the 2.5 cm segment and ended when one forelimb reached the home cage. Maximum test time (cut-off time) was 60 s, and the mice were videotaped. Videos were viewed in slow motion to count errors made by each mouse. An error was counted when, during forward movement, at least 50% of a limb (forelimb or hindlimb) slipped off the beam. Slips were not counted if the mouse was not making forward movement or when the mouse's head was oriented to the left or right of the beam. Percentage of misstep errors were calculated for control and ASO mice across all three trials and averaged (16).

Hindlimb Clasping Reflex

This reflex indicates uncoordinated movement and precedes the symptomatic onset of hindlimb paralysis. Mice were gently lifted upward by the mid-section of the tail and observed over ∼5–10 s (16, 61). Mice were assigned a score of 0–3 based on the extent to which the hindlimbs clasped inward. A score of 0, indicating no clasping, was given to mice that freely moved both their limbs and extended them outward. A score of 1 was assigned to mice which clasped one hindlimb inward for the duration of the restraint or if both legs exhibited partial inward clasping. A score of 2 was given if both legs clasped inward for most of the observation, but still exhibited some flexibility. A score of 3 was assigned if mice displayed complete paralysis of hindlimbs that immediately clasped inward and exhibited no signs of flexibility.

Stool Sample Collection and Microbiota Analyses

Mice stool pellets were collected over a 24 h period before tissue collection and stored at −80°C until analysis. Total genomic DNA was extracted from the mice feces using the FastDNA SPIN Kit from the manufacturer's protocol (FastDNA Spin Kit for Soil, MP Biomedicals, Solon, OH), and verified with fluorometric quantitation (Qubit 3.0, Life Technologies, Grand Island, NY, USA). To reduce batch effects, all samples were extracted using the same DNA extraction kit at the same time, and library preparation for all samples was conducted in 96-well plates simultaneously. Primers 515F/806R (515F: GTGTGYCAGCMGCCGCGGTAA; 806R: CCGGACTACNVGGGTWTCTAAT) modified from the Earth Microbiome Project primers, and targeting the V4 variable region of microbial 16S ribosomal RNA (rRNA) genes, were used for PCR, and prepared for high-throughput amplicon sequencing using a two-stage PCR method, as previously described (62). Sequencing was performed using an Illumina MiniSeq, with a V2 kit and paired-end 150 base reads at the Genomics and Microbiome Core Facility (GMCF) at Rush University Medical Center.

16S rRNA V4 Sequencing Analysis

Raw sequences were merged using the software package PEAR (Paired-End read merger) algorithm (v0.9.11) (Dalhousie University, Halifax, Nova Scotia, Canada) (63). Merged sequences shorter than 240 bases were removed. Merged sequences were then processed (including denoising) using the DADA2 algorithm within the QIIME2 (v 2020.8.0) workflow (64, 65). The amplicon sequence variants (ASVs) generated were used for all downstream analyses. Taxonomy was assigned to each ASV using the naïve Bayes classifier employing the SILVA 138 99% OTUs reference database (66, 67). A total of 1,156,631 sequencing clusters were generated, with an average of 20,654 clusters per sample (median = 27,444; min = 0; max = 41,767). One reagent contaminant ASV (*Pseudomonas*) was identified and removed using decontam package based on the prevalence of the ASV in the reagent negative blank controls ($n = 5$), using

default parameters (68). Unassigned, eukaryote, chloroplast, and mitochondrial ASVs were removed from datasets prior to statistical analyses (69). Raw sequence data were deposited in the NCBI Sequence Read Archive under BioProject PRJNA781983.

Statistical Analysis

Experimental and Behavioral Statistical Analyses

These data are reported as mean + standard error of the mean (SEM), unless otherwise stated. Differences among means were analyzed using GraphPad Prism (v9.3.1) software (GraphPad Software, La Jolla, CA). We removed outlier points by eliminating any points that were two standard deviations above and below the mean of each respective group. Two-way analysis of variance (ANOVA) was performed to evaluate the significant differences with genotype (control vs. ASO) or treatment (vehicle vs. DSS). Multiple group comparisons were performed using Tukey's *post-hoc* comparison. Pearson correlation analysis was performed to evaluate associations between intestinal permeability and brain-related outcomes. Significance was considered at the value $p < 0.05$ (16, 40).

Microbiota Statistical Analysis

Analyses of alpha- and beta-diversity were used to compare fecal microbial community structure. All analyses were performed on feature (ASV) counts. Alpha-diversity metrics (i.e., Shannon index, Simpson's index, Observed features, and Pielou's Evenness) were calculated on rarefied datasets (19,000 sequences/sample). Differences in alpha diversity were assessed for significance using the Mann-Whitney *U*-test (MWU) with Benjamini–Hochberg false-discovery rate (FDR) correction for multiple comparisons ($q < 0.05$). Analyses were performed using the software package GraphPad Prism (v9.3, GraphPad Software LLC San Diego California). Permutation Multivariate Analysis of Variance (PERMANOVA) with 9,999 permutations was used to assess global differences in microbial community structure between treatments (70). Adjustment for multiple testing was conducted using the Benjamini–Hochberg FDR correction. Visualization of data was performed using principal coordinates analysis (PCoA) based on a Bray–Curtis dissimilarity distance matrix within the software package QIIME2 (65). Differential abundance analyses of individual taxa between groups were performed using the software package DESeq2, generating an FDR *q*-value (71, 72). DESeq2 has been shown to be appropriate for differential abundance comparisons in studies with small sample size groups (<20) or unbalanced design (73). Individual taxa percent mean relative abundances (>1%) and standard deviations (SD) calculated and depicted as stacked histograms. To identify taxa that most strongly explained between group differences, a Linear discriminant analysis Effect Size (LEfSe) analysis was performed (74). LEfSe uses the non-parametric factorial Kruskal-Wallis sum-rank test to detect individual taxa that differ between treatments and animal genotype. Taxa that are significant by Kruskal-Wallis are subsequently investigated using a set of pairwise tests among subclasses using the (unpaired) Wilcoxon rank-sum test. As a last step, LEfSe uses Linear Discriminant Analysis to estimate the effect size of each differentially abundant taxa. Differentially abundant taxa that were statistically significant using an alpha of (0.05) and exceeded an LDA log score of at least (±2) were graphically represented.

RESULTS

Effects of DSS on Intestinal Barrier Function

A dose response was conducted to identify an optimal dose of DSS that would be used for all subsequent experiments. Mice were given a range of DSS from 0.5 to 2% (given daily over three cycles) to determine the lowest dose that caused intestinal barrier dysfunction [sucralose/lactulose ratio (S/L)] without overt intestinal inflammation. Results demonstrated that the 0.5% dose of DSS did not induce intestinal barrier dysfunction and 2% induced overt intestinal inflammation whereas 1% DSS increased the S/L ratio without an increase in intestinal inflammation (i.e., calprotectin) data not shown. Therefore, 1% DSS was used for all experiments in this study.

DSS disrupted intestinal barrier integrity in the colon (i.e., large intestine) but not in the small intestine (**Figures 2A,B**). Specifically, DSS administration significantly increased the S/L ratio (**Figure 2A**: two-way ANOVA: genotype $p = 0.13$, treatment $p = 0.02$, interaction $p = 0.60$) without affecting urinary mannitol (data not shown), lactulose (data not shown), nor the lactulose/mannitol ratio (**Figure 2B**: two-way ANOVA: genotype $p = 0.08$, treatment $p = 0.11$, interaction $p = 0.66$).

Results from the sugar test indicated that DSS-induced intestinal barrier dysfunction primarily occurred in the colon, therefore the AJC proteins E-cadherin and ZO-1 were assessed in colon tissue. DSS administration reduced E-cadherin staining (**Figure 2C**) and caused a significant shift from E-cadherin from the membrane to the cytosolic fraction as indicated by the decrease in the membrane/cytoplasmic ratio which was observed in both control and ASO mice (**Figure 2D**: two-way ANOVA: genotype $p = 0.56$, treatment $p = 0.01$, interaction $p = 0.26$). Similarly, ZO-1 staining was reduced by DSS treatment (**Figure 2E**). The membrane/cytoplasmic ratio of ZO-1 was significantly reduced by DSS in both control and ASO mice (**Figure 2F**: two-way ANOVA: genotype $p = 0.34$, treatment $p = 0.02$, interaction $p = 0.34$).

Taken together the sugar test and the AJC protein data support that DSS induced intestinal barrier dysfunction in the colon (treatment effect), but these effects could not be distinguished based on genotype (i.e., control and ASO mice respond similarly to DSS).

Effects of DSS on Intestinal Inflammation

DSS caused mild colonic inflammation in both control and ASO mice (**Figure 3**). Specifically, DSS-treated control and ASO mice had shorter colon than vehicle treated mice which is consistent with intestinal inflammation (**Figure 3A**: two-way ANOVA: genotype $p = 0.12$, treatment $p = 0.02$, interaction $p = 0.77$). There was a concurrent increase in tissue MPO, marker of tissue inflammation in a subset of both control and ASO mice (**Figure 3B**: two-way ANOVA: genotype $p = 0.91$, treatment $p < 0.00$, interaction $p = 0.92$). The increase in MPO was significant

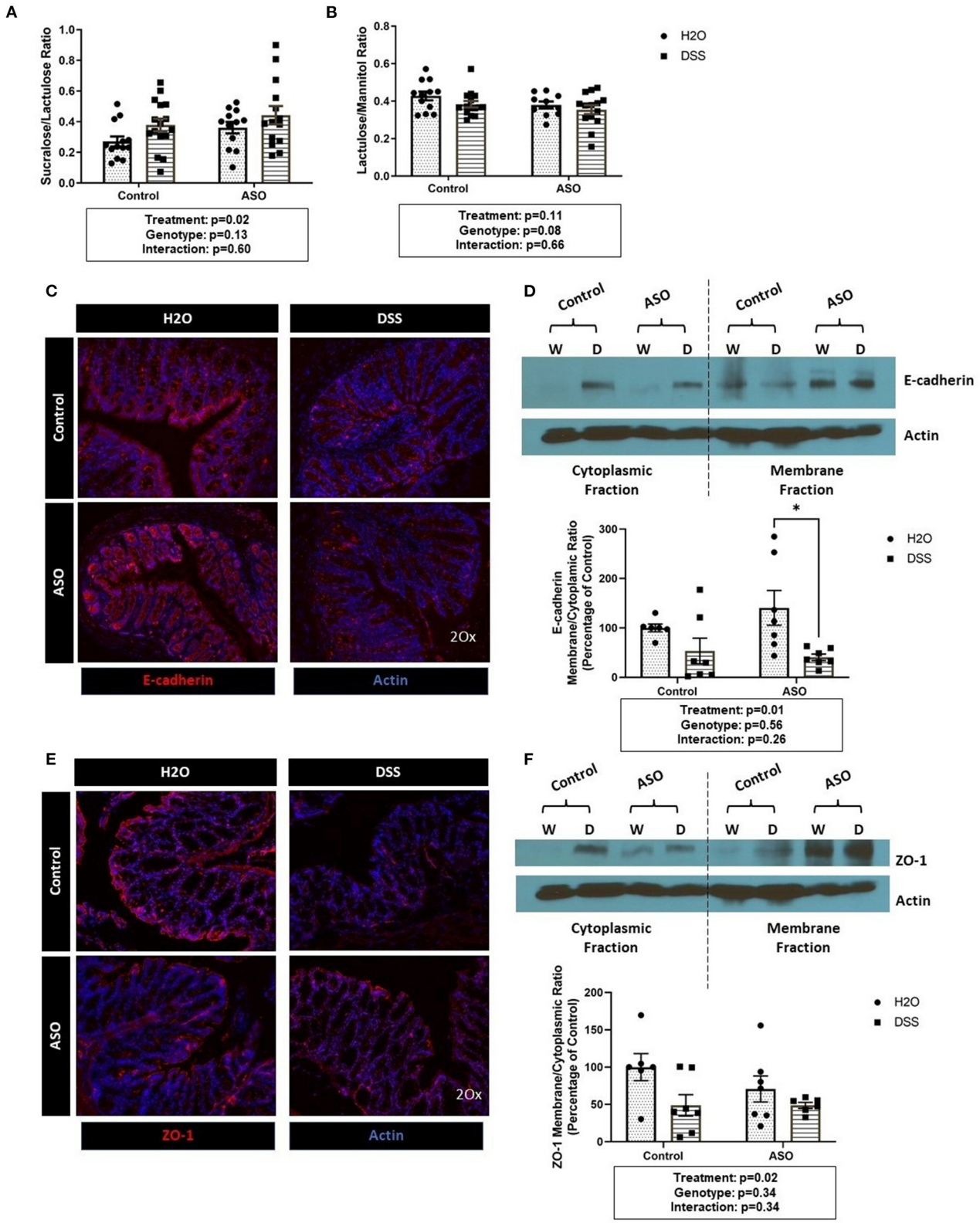

FIGURE 2 | Impact of DSS on the intestinal barrier. **(A)** Mice treated with DSS exhibited intestinal barrier dysfunction, specifically in the colon, as assessed the by the sucralose/lactulose ratio. **(B)** Small intestinal permeability was similar in all groups regardless of genotype and treatment, based on the lactulose/mannitol ratio. **(C)** Immunofluorescent staining of E-cadherin (red) in colonic tissue (DAPI, blue) showing reduced staining in DSS treated tissue. **(D)** The membrane/cytoplasm ratio of

(Continued)

FIGURE 2 | E-cadherin showed that DSS-treated mice have less E-cadherin in the cytosol compared to H_2O-treated mice. **(E)** Immunofluorescent staining of ZO-1 (red) in colonic tissue (DAPI, blue) shows reduced staining in DSS treated tissue. **(F)** There was a significant reduction of ZO-1 in the membrane/cytoplasm ratio in mice treated with DSS. Outliers were omitted prior to analysis (>2 standard deviations from the mean). Two-way ANOVA was conducted and values for different factors are indicated in the graphs followed by *post-hoc* Tukey which is indicated on each graph when appropriate $^*p < 0.05$. ASO, α-synuclein overexpressing; W/H_2O, water; DSS, dextran sodium sulfate.

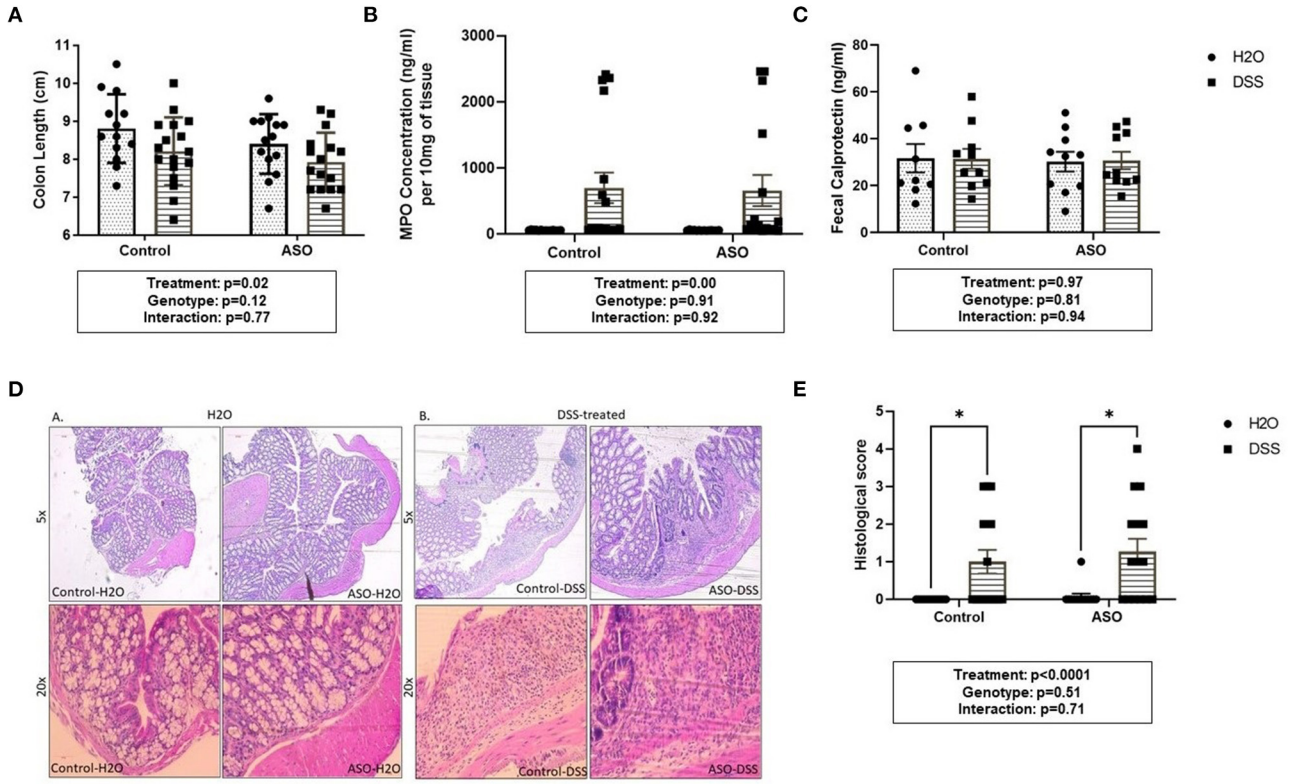

FIGURE 3 | Measures of colon inflammation in DSS-treated control and ASO mice. We used three measures of colonic inflammation: colonic shortening, colonic MPO, and fecal calprotectin (see Methods). **(A)** DSS-treated mice had significantly shorter colons than mice on water, regardless of genotype. **(B)** Mice treated with DSS also had significantly higher colonic MPO levels compared to mice on water. **(C)** Fecal calprotectin levels were similar for WT and ASO mice, regardless of treatment. **(D)** H&E staining showed diffuse inflammatory infiltration in the mucosa and submucosa in DSS-treated mice, regardless of genotype. **(E)** DSS treatment was associated with increased histological score compared to H_2O-treated mice, regardless of genotype. Outliers were omitted prior to analysis (>2 standard deviations from the mean). Two-way ANOVA was conducted and values for different factors are indicated in the graphs followed by *post-hoc* Tukey which is indicated on each graph when appropriate $^*p < 0.05$. ASO, α-synuclein overexpressing; H_2O, water; DSS, dextran sodium sulfate; MPO, Myeloperoxidase.

but was driven by a few mice, with most mice not demonstrating an increase in MPO. Fecal calprotectin was not significantly increased by DSS (**Figure 3C**: two-way ANOVA: genotype $p = 0.81$, treatment $p = 0.97$, interaction $p = 0.94$). To support the tissue inflammation data, colonic tissue was stained with H&E and scored for intestinal inflammation by a pathologist. H&E staining showed diffuse inflammatory infiltration in the mucosa and submucosa in DSS-treated mice, regardless of genotype (**Figures 3D,E**). These data collectively showed that DSS administration induced colon shortening length and increase in MPO in a subset of mice but had no effect on fecal calprotectin which can be interpreted as mild colonic inflammation. Additionally, DSS had similar inflammatory effects on control and ASO mice.

Effects of DSS on the Intestinal Microbiota

Microbial communities were examined for an overall treatment effect, regardless of genotype. No significant differences in alpha-diversity indices were observed (MWU: **Supplementary Table 1**), however between group differences in beta diversity were noted ($q < 0.00$, PCoA: **Figure 4A**; PERMANOVA: **Table 1**). Compared to H_2O-fed mice, DSS-treated mice demonstrated a significant increase in differential abundance for the putative pro-inflammatory genus *Bacteroides*, along with a loss of putative beneficial SCFA-producing genera that included *Lachnospiraceae* (A2; UCG-001; and Uncultured), *Bifidobacterium*, *Roseburia*, *Dorea*, *Marvinbryantia*, *Eubacterium xylanophilum*, and *Blautia* ($q < 0.05$, **Supplementary Tables 2, 3**). LEfSe analysis showed

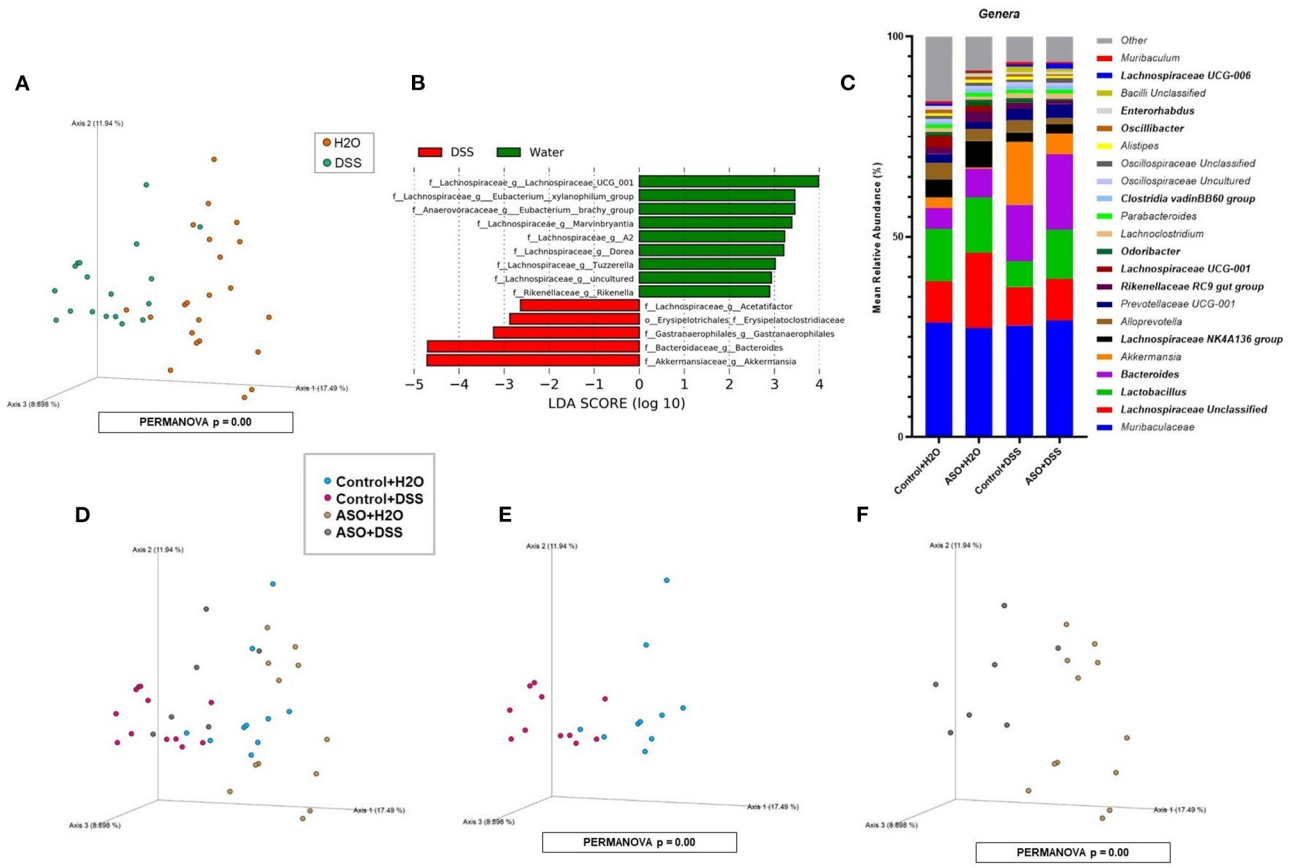

FIGURE 4 | Characterization of fecal microbiota in mice treatment and genotype groups using 16S rRNA sequencing. **(A)** Mice samples clustered separately between treatments, regardless of genotype. **(B)** LEfSe identified bacterial genera clades that are differentially abundant with treatment, irrespective of genotype. Clade colors: H_2O (green) and DSS (red). Clades in this graph were statistically significant ($p < 0.05$) and exceed an LDA log score of at least (± 2). **(C)** Bar charts of the mean relative abundance of fecal microbial communities at the taxonomic level of genus for mice genotypes with treatments. The mean relative abundance of taxa with >1% average relative abundance are shown. **(D–F)** Fecal microbial communities clustered separately between mice genotypes with treatments. PCoA plots were built on Bray-Curtis dissimilarity metrics. H_2O, water; DSS, dextran sodium sulfate; ASO, α-synuclein overexpressing.

that H_2O-fed mice were associated with multiple putative SCFA-producing bacteria, whereas DSS-treated mice were associated with putative pro-inflammatory bacteria genera *Akkermansia* and *Bacteroides* (**Figure 4B**). Irrespective of genotype, DSS administration resulted in a robust dysbiotic pro-inflammatory microbial profile characterized by loss of putative SCFA-producing bacteria with a concurrent enrichment in putative pro-inflammatory bacteria.

Next, microbial communities were examined for treatment effects within each genotype (i.e., control and ASO). Two-way ANOVA indicated no significant genotype, treatment, or interaction effects for alpha diversity examined at the feature level (data not shown). However, between group differences in beta-diversity in stool microbial community structures were noted (PERMANOVA: **Table 1**; **Figures 4C–F**). Microbial communities across all groups were different and dominated by bacteria from the genera *Muribaculaceae*, *Lachnospiraceae* Unclassified, *Lactobacillus,* and *Bacteroides* (>50% of all sequences; **Figure 4C**; **Supplementary Table 4**). DSS administration to control mice significantly increased ($q < 0.05$) the abundance of putative pro-inflammatory genus *Bacteroides* and significantly decreased putative beneficial SCFA-producing genera *Lachnospiraceae* (A2 and UCG-001), *Dorea*, *Eubacterium xylanophilum*, and *Lactobacillus* (DeSeq2: **Table 2**). This dysbiotic microbial profile was similarly noted in ASO mice given DSS which increased putative pro-inflammatory genus *Bacteroides*, with a significant decrease ($q < 0.05$) in the abundance of putative beneficial SCFA-producing genera, including *Lachnospiraceae* (A2; UCG-001; Uncultured; and Unclassified), *Dorea*, *Eubacterium xylanophilum*, *Marvinbryantia*, *Anaerotruncus, Dorea,* and *Blautia* (DeSeq2: **Table 2**). Overall, DSS induced microbiota dysbiosis in both control and ASO mice; however, the ASO mice given DSS showed a greater loss of beneficial SCFA-producing bacteria, than control mice given DSS.

Effect of DSS on Motor Function

Time to remove an adhesive from the nasal bridge was significantly impacted by genotype, although DSS-induced

TABLE 1 | Significant differences in intestinal microbial community structures were observed between mice groups in beta diversity analyses conducted on microbial features.

Mice comparisons	Feature taxonomic level				
	Sample size	Permutations	Pseudo-*F*	*p*-value	*q*-value
H_2O vs. DSS	40	9,999	5.53	*0.0001*	**0.0001**
CONTROL+H_2O vs. CONTROL+DSS	21	9,999	3.28	*0.0001*	**0.0003**
ASO+H_2O vs. ASO+DSS	19	9,999	3.37	*0.0004*	**0.0008**
CONTROL+H_2O vs. ASO+H_2O	22	9,999	1.93	*0.0156*	**0.0187**
CONTROL+DSS vs. ASO+DSS	18	9,999	1.80	*0.0255*	**0.0255**

PERMANOVA results are based on a Bray-Curtis distance matrix. Significance was determined using 9,999 permutations and corrected for multiple testing using the Benjamini-Hochberg adjusted p-values (q < 0.05 indicated by bold; p < 0.05 indicated by italics).
H_2O, water; DSS, dextran sodium sulfate; ASO, alpha-synuclein overexpressing.
Mice sizes: H_2O (n = 22); DSS (n = 18); CONTROL+H_2O (n = 10); CONTROL+DSS (n = 11); ASO+H_2O (n = 12); ASO+DSS (n = 7).

intestinal barrier dysfunction did not alter this behavior (**Figure 5A**: two-way ANOVA: genotype $p < 0.00$, treatment $p = 0.51$, interaction $p = 0.80$). There were no significant differences in time to cross the beam (**Figure 5B**: two-way ANOVA: genotype $p = 0.60$, treatment $p = 0.85$, interaction $p = 0.18$). However, evaluating the number of errors (i.e., stepping off the beam) revealed a significant effect of genotype wherein ASO mice had significantly greater missteps than control mice, but this was unaltered by DSS-induced intestinal barrier dysfunction (**Figure 5C**: two-way ANOVA: genotype $p < 0.00$, treatment $p = 0.59$, interaction $p = 0.08$). Finally, Chi-square analysis of the hindlimb clasping score indicated that more ASO mice had impaired motor function compared to control mice (**Figure 5D**: Chi-square $p < 0.00$). Taken together, a genotype-specific effect was found in three of the behavioral outcomes including adhesive removal, missteps in beam crossing, and hindlimb clasping reflex score. However, DSS administration did not impact motor function.

Effect of DSS on Brain-Specific PD-Like Outcomes

There was a significant impact of genotype on Iba-1 with levels being lower in ASO mice compared to control mice; however, DSS administration did not impact Iba-1 (**Figure 6A**: two-way ANOVA: genotype $p = 0.02$, treatment $p = 0.69$, interaction $p = 0.58$). However, perhaps more important than evaluating the presence of microglia (i.e., Iba-1 optical density) is microglia morphology. Non-activated microglia morphologically are ramified in shape. Once microglia are activated (e.g., in response to damaged cells, bacterial products), they retract their processes and take on an ameboid morphology with includes an increase in cell body size (40, 41, 75). Assessing cell body cell size revealed a genotype-specific significant difference between control and ASO mice with greater activated Iba-1 positive microglia in ASO mice; however, there was no impact of DSS administration on microglial morphology (**Figure 6B**: two-way ANOVA: genotype $p = 0.04$, treatment $p = 0.89$, interaction $p = 0.14$). ASO mice had significantly lower levels of TH staining compared to controls, however DSS treatment did not impact TH staining (**Figure 6C**: two-way ANOVA: genotype $p = 0.01$, treatment $p = 0.61$, interaction $p = 0.77$). We have included representative images of Iba-1 (**Figures 6D,E**) and TH (**Figure 6F**) that was used for analysis. These data demonstrate the ASO mice have fewer microglia than controls, a distinct microglia phenotype compared to controls, and fewer dopaminergic terminals than control mice. However, there is no evidence that these PD-like brain outcomes were impacted by DSS.

Effect of DSS on Bacterial Translocation and Systemic Inflammation

DSS administration significantly increased serum LBP in ASO mice, an effect that was not observed in control mice (**Figure 7A**: two-way ANOVA: genotype $p = 0.55$, treatment $p = 0.14$, interaction $p = 0.02$). This suggests that the host immune response to barrier dysfunction is different in control and ASO mice. Despite the increase in LBP, none of the pro-inflammatory cytokines evaluated in the serum were increased by DSS administration (nor were they impacted by genotype) including IL-1β, TNF-α, or IL-6 (**Figures 7B–D**). Paradoxically, DSS-induced intestinal barrier dysfunction increased serum IL-10 in ASO mice (**Figure 7E**: two-way ANOVA: genotype $p = 0.19$, treatment $p = 0.03$, interaction $p = 0.02$). IL-10 is generally considered an anti-inflammatory cytokine, and this may (at least partially) represent a compensatory mechanism that may have prevented DSS-induced barrier dysfunction from promoting PD-like behavior and brain pathology. Taken together, these data indicate that ASO mice given DSS have higher levels of LBP than control mice and ASO mice given water which may reflect the increase in pro-inflammatory LPS-containing bacteria in this group. Despite this increase there was not an increase in pro-inflammatory cytokines, but the anti-inflammatory cytokine IL-10 was increased.

Relationship Between Intestinal Outcomes and Motor Function / Brain Pathology

Despite being inbred and genetically similar, outcomes reflect heterogenous outcomes in terms of intestinal

TABLE 2 | Genus taxonomic level differential abundance DeSeq2 analysis between control or ASO mice treated with water and dextran sodium sulfate.

(Phylum) *Genus*	Base mean	Log2 FC	*p*-value	*q*-value
Control+DSS over Control+H_2O				
(Firmicutes) *Erysipelatoclostridiaceae* Unclassified	10.56	6.50	*8.78E-05*	**0.002**
(Cyanobacteria) *Gastranaerophilales*	57.99	1.72	*0.009*	0.096
(Bacteroidota) *Bacteroides*	2,400.21	1.34	*0.002*	**0.026**
(Firmicutes) *Clostridia vadinBB60 group*	171.38	0.87	*0.031*	0.229
(Actinobacteriota) *Enterorhabdus*	152.25	−1.12	*0.004*	**0.048**
(Proteobacteria) *Parasutterella*	33.20	−1.80	*0.010*	0.096
(Firmicutes) *[Eubacterium] brachy group*	5.43	−1.88	*0.031*	0.229
(Desulfobacterota) *Desulfovibrio*	88.80	−2.27	*4.52E-04*	**0.007**
(Firmicutes) *Lactobacillus*	4,531.14	−2.77	*2.44E-04*	**0.005**
(Actinobacteriota) *Atopobiaceae* unclassified	5.44	−4.17	*0.017*	0.150
(Firmicutes) *[Eubacterium] xylanophilum group*	68.83	−4.36	*0.003*	**0.041**
(Firmicutes) *Lachnospiraceae* UCG-001	246.12	−8.33	*4.45E-07*	**1.54E-05**
(Firmicutes) *Lachnospiraceae A2*	49.43	−9.70	*3.68E-09*	**1.92E-07**
(Firmicutes) *Dorea*	35.45	−9.95	*1.17E-10*	**1.22E-08**
ASO+DSS over ASO+H_2O				
(Firmicutes) *Tyzzerella*	0.54	4.12	*0.047*	0.200
(Firmicutes) *Erysipelotrichaceae*	2.21	3.39	*0.011*	0.073
(Firmicutes) *Lachnospiraceae UCG-006*	109.28	2.55	*9.50E-05*	**1.80E-03**
(Bacteroidota) *Bacteroides*	2,400.21	1.22	*0.010*	0.073
(Firmicutes) *Colidextribacter*	92.93	−0.82	*0.045*	0.204
(Firmicutes) *Lachnospiraceae* Unclassified	2,717.42	−1.02	*0.005*	**0.045**
(Firmicutes) *Oscillibacter*	155.29	−1.03	*0.024*	0.127
(Bacteroidota) *Rikenellaceae RC9 gut group*	442.34	−1.77	*0.002*	**0.026**
(Firmicutes) *Lachnospiraceae NK4A136 group*	930.44	−1.84	*0.029*	0.142
(Firmicutes) *Ruminococcaceae* Unclassified	35.57	−1.87	*0.001*	**0.016**
(Bacteroidota) *Odoribacter*	226.57	−2.11	*0.024*	0.127
(Desulfobacterota) *Bilophila*	21.61	−2.41	*0.015*	0.099
(Campilobacterota) *Helicobacter*	28.16	−2.91	*0.03*	0.142
(Firmicutes) *Blautia*	55.79	−3.84	*3.29E-05*	**7.82E-04**
(Firmicutes) *Dorea*	16.54	−4.07	*0.003*	**0.033**
(Firmicutes) *Anaerotruncus*	6.98	−4.18	*0.003*	**0.033**
(Firmicutes) *Erysipelotrichaceae* Unclassified	4.00	−4.29	*0.022*	0.127
(Firmicutes) *Marvinbryantia*	64.65	−5.38	*0.001*	**0.014**
(Firmicutes) *Lachnospiraceae* Uncultured	13.63	−5.69	*3.65E-06*	**1.73E-04**
(Firmicutes) *Lachnospiraceae UCG-001*	246.12	−6.54	*1.30E-04*	**0.002**
(Firmicutes) *Lachnospiraceae A2*	49.43	−7.36	*5.85E-06*	**1.85E-04**
(Firmicutes) *[Eubacterium] xylanophilum group*	68.83	−8.86	*9.04E-09*	**8.59E-07**

DeSeq2: Taxa shown have adjusted p-values (q < 0.05 indicated by bold; p < 0.05 indicated by italics). Base Mean is the mean of normalized samples. Log2FC, Log2 fold change of taxa in Control or ASO+DSS mice in comparison to Control or ASO+H_2O mice samples.
H_2O, water; DSS, dextran sodium sulfate; ASO, alpha-synuclein overexpressing.
Control+H_2O (n = 10); Control+DSS (n = 11); ASO+H_2O (n = 12); ASO+DSS (n = 7).

barrier dysfunction, intestinal and systemic inflammation. Thus, it is conceivable that those mice with the highest DSS-induced intestinal barrier dysfunction, intestinal inflammation, microbiota dysbiosis, and systemic inflammation may correspondingly show greater motor dysfunction and brain pathology. However, none of the intestinal outcomes nor systemic inflammation significantly correlated with motor function or brain outcomes (**Supplementary Table 5**).

DISCUSSION

There are numerous different pathways by which the gut could contribute to PD and in this study, we evaluated the contribution of intestinal hyperpermeability to the PD-like phenotype based on the following rationale: (1) data from our group has demonstrated intestinal hyperpermeability is observed in PD patients (8, 76, 77), (2) numerous conditions that may be risk factors for PD (e.g., diabetes, ulcerative colitis)

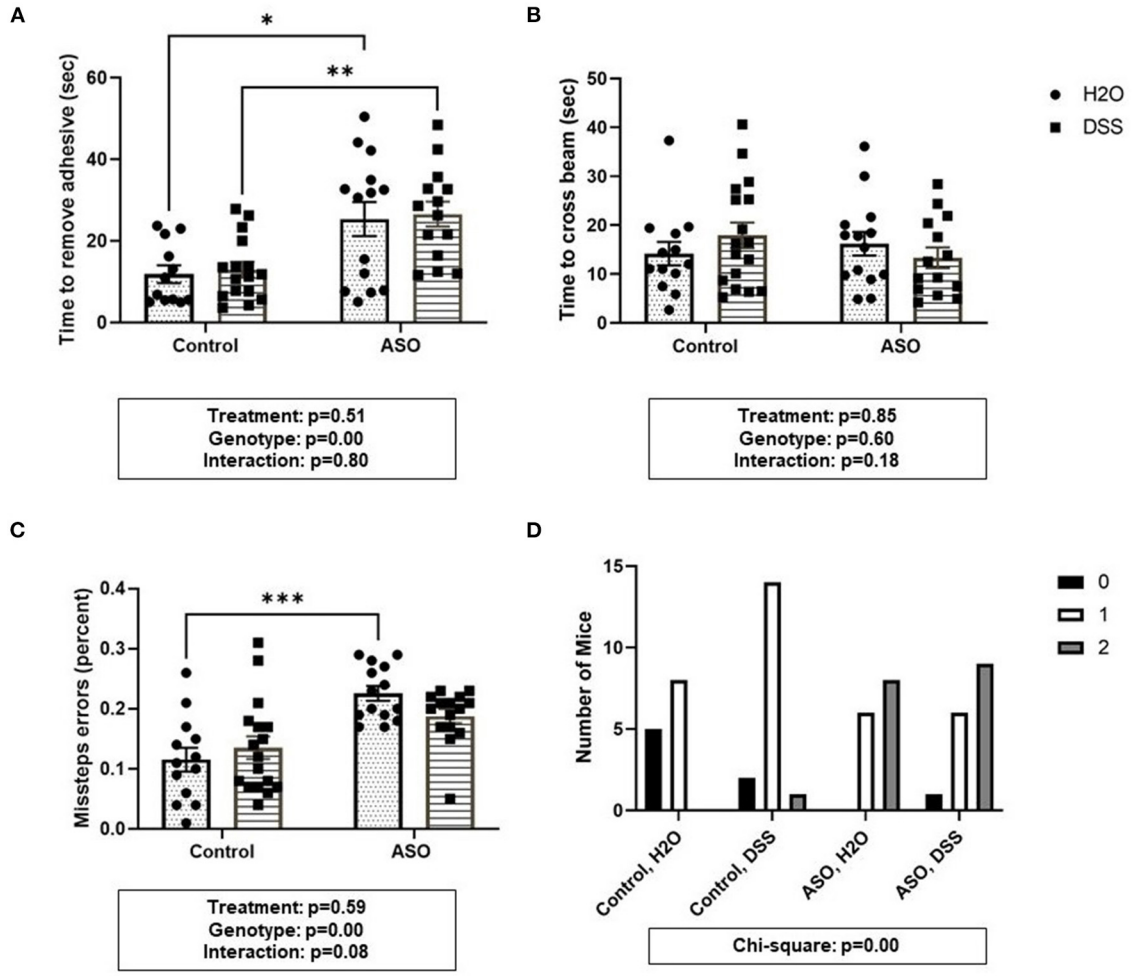

FIGURE 5 | Parkinsonian behavioral analysis in control and ASO mice. Motor function was evaluated using four behaviors. **(A)** Adhesive removal: ASO mice significantly took longer to remove the adhesive from the bridge of the nose than WT mice. **(B)** Beam traverse: There was no difference in the time to cross the beam between WT and ASO mice. **(C)** ASO mice had significantly more missteps off the beam than WT mice. **(D)** Hindlimb Clasp Reflex Score: ASO mice had significantly worse hindlimb clasp than WT littermates. Outliers were omitted prior to analysis (>2 standard deviations from the mean). Two-way ANOVA was conducted and values for different factors are indicated in the graphs followed by *post-hoc* Tukey which is indicated on each graph when appropriate $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. **(D)** Chi-square analysis. ASO, α-synuclein overexpressing; H_2O, water; DSS, dextran sodium sulfate.

are associated with intestinal hyperpermeability (78, 79), and (3) intestinal hyperpermeability is associated with increased systemic inflammation which may drive neuroinflammation in PD (80, 81). Thus, the rationale behind this study was to investigate if increased intestinal permeability could promote neuroinflammation and exacerbate the PD phenotype in ASO mice through a mechanism including intestinal and/or systemic inflammation.

Administration of DSS in drinking water is a well-established rodent model to induce intestinal barrier dysfunction, intestinal inflammation, and pro-inflammatory changes in the intestinal microbiota (82, 83). This study demonstrated that the low dose (1%) DSS was sufficient to cause intestinal (colonic) barrier dysfunction and intestinal microbiota dysbiosis, but only mild intestinal inflammation without systemic inflammation and that this was not sufficient to worsen PD-like brain pathology nor motor function in ASO mice.

This finding appears to be in contradiction to prior studies demonstrating that a high dose of DSS causes marked intestinal inflammation and exacerbates the PD-like phenotype in rodent toxin models of PD. One recent study combined administration of 2.5% DSS treatment with paraquat/LPS and found that DSS exacerbates LPS/paraquat effects on microglial activation (84). Houser et al. used 2% DSS and demonstrated showed worsening of PD-like brain pathology induced by MPTP (85). Higher doses of DSS (2–2.5%) are well-established to cause severe intestinal and systemic inflammation. The difference between these prior studies and this current report suggests that overt intestinal inflammation (high levels of stool calprotectin) and/or systemic inflammation may be required to promote the PD-like phenotype

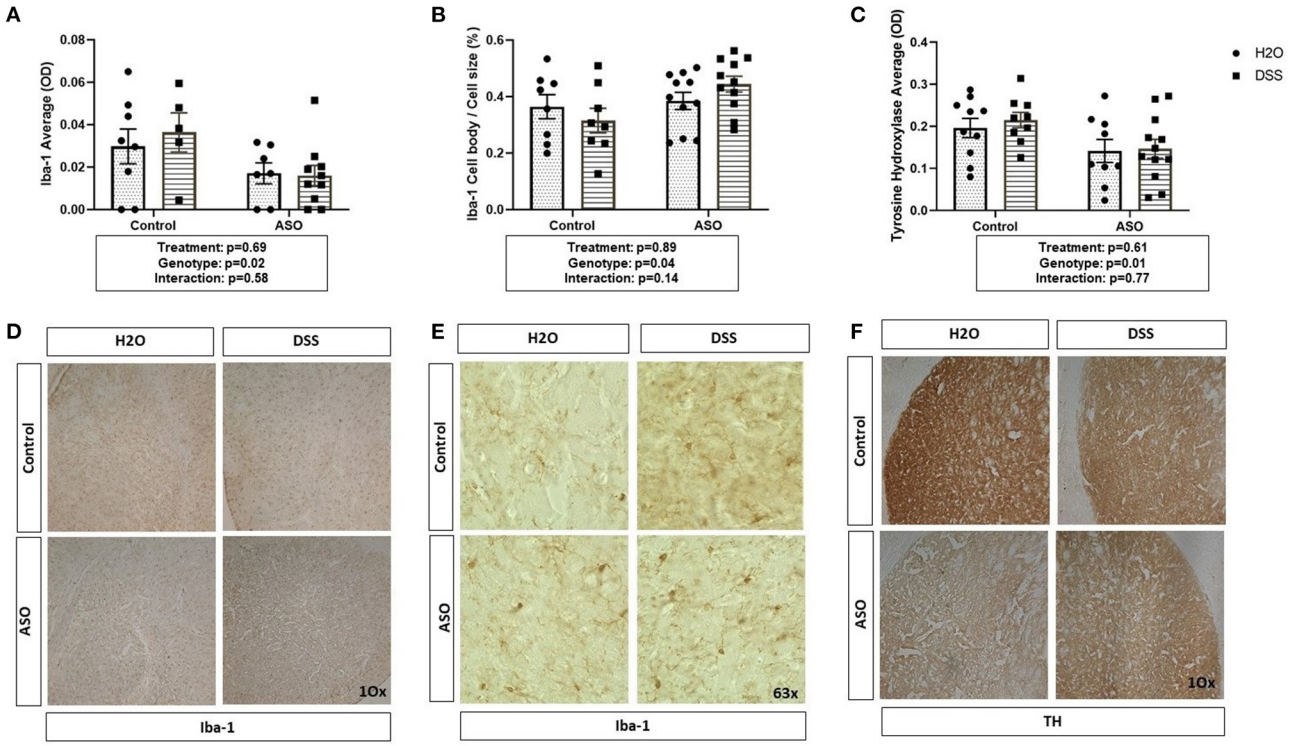

FIGURE 6 | Brain related PD outcome measures exhibit genotypic ASO but not DSS treatment effects. Mice were evaluated for brain pathology. **(A)** ASO mice had a significantly lower amount of microglia than controls. **(B)** ASO mice had significantly more activated microglia than WT. **(C)** ASO mice had a significantly less TH than control. **(D)** Representative images of Iba-1 positive microglia at 10x magnification. **(E)** Representative images of Iba-1 positive microglia at 63x magnification. **(F)** Representative images of TH positive dopaminergic terminals at 10x magnification Outliers were omitted prior to analysis (>2 standard deviations from the mean). **(A–C)** Two-way ANOVA was conducted and values for different factors are indicated in the graphs followed by *post-hoc* Tukey (no *post hoc* significance was identified). ASO, α-synuclein overexpressing; H_2O, water; DSS, dextran sodium sulfate; TH, Tyrosine hydrolase; Iba-1, Ionized calcium binding adaptor molecule 1.

in ASO mice, but such a conclusion will require additional investigation (e.g., DSS dose response). However, differences in microbiota communities between institutions [so "cage effects" (86)] may also partially account for differences between studies as the microbiota can dictate the response to intestinal disruptors such as alcohol, stress, and non-steroidal anti-inflammatory medications (87).

The concept that intestinal inflammation is a key feature in promoting PD is supported by observations in humans. Epidemiological studies demonstrate that inflammatory bowel disease (IBD) is a risk factor for PD (29, 79, 88, 89). IBD is characterized by chronic intestinal inflammation, pro-inflammatory dysbiosis and intestinal leak (27, 28, 90). Treatment of IBD patients with biologics (e.g., TNF antibody) that effectively control intestinal inflammation and induce remission in IBD patients, reduces risk of PD (despite most patients still having intestinal barrier dysfunction and microbiota dysbiosis) (91). This evidence supports that intestinal inflammation is a critical feature mediating the effects of the intestinal barrier and intestinal microbiota on PD.

A few findings observed in ASO mice require additional discussion. First, despite having similar levels of intestinal barrier dysfunction ASO mice had higher levels of LBP. This can be explained in one of two ways: (1) DSS treatment had different impact on microbiota function in ASO mice compared to control mice leading to release of more LPS in ASO mice or other metabolic impact that is not reflect in microbiota composition (92, 93) or (2) differences in LBP levels could reflect differences in hepatic immune response to intestinal barrier dysfunction. Second, the finding that serum IL-10 was increased in only DSS-treated ASO mice was unexpected because DSS caused intestinal barrier dysfunction in both ASO and control mice. But elevated serum IL-10 along with elevated serum LBP in DSS-treated ASO mice suggest that immune response to the inflammatory trigger in ASO mice is different than control mice. This possibility is supported by recent studies in patients with PD who have dysregulated and exaggerated immune/inflammatory signaling pathways (94). Future studies are required to directly test this hypothesis in ASO mice.

There are some study limitations worth noting. There is no ideal animal model for PD and each model recapitulates only some aspects of PD. In this study, a genetic model of misfolded α-synuclein was used but the effects of low dose DSS should be studied in other PD models such as transgenic mice that overexpress human α-synuclein with a PD-associated mutation (A53T) (95), *Parkin* knockout mice (96, 97), and the

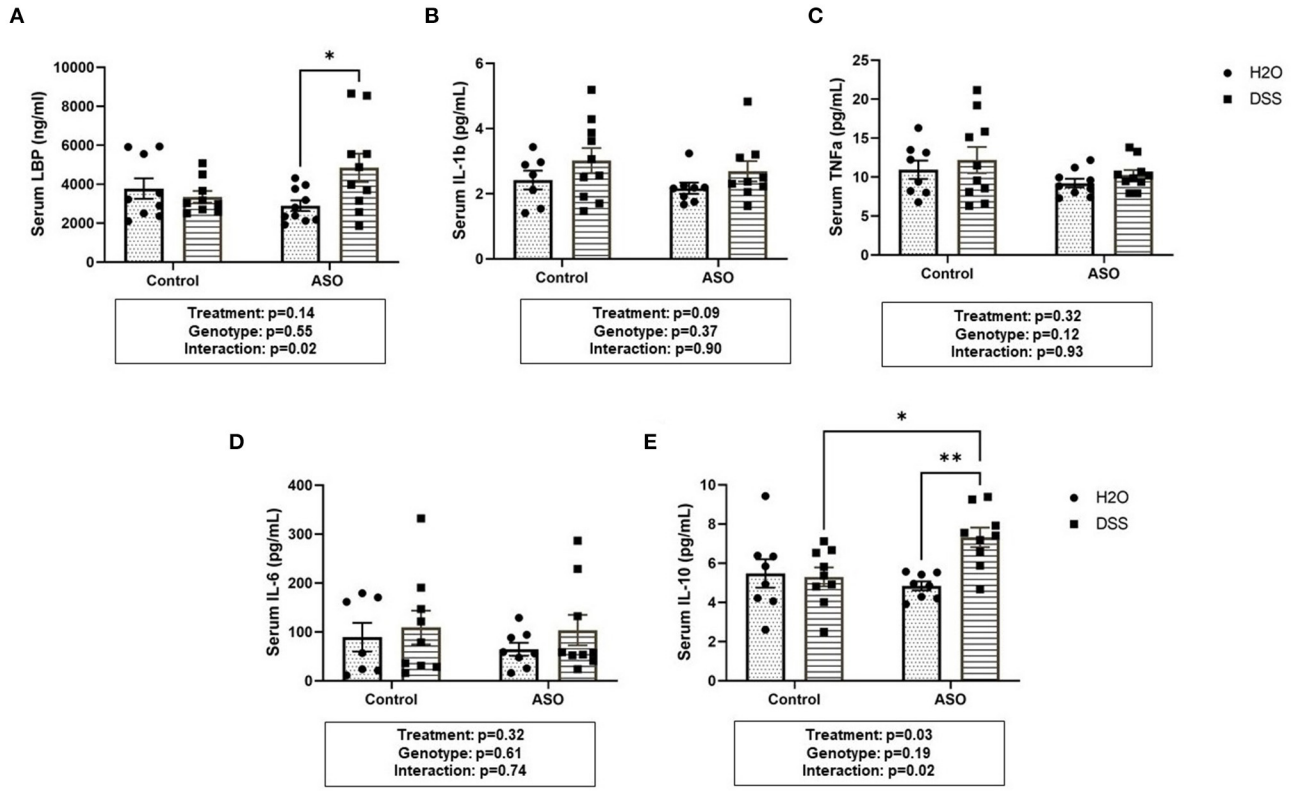

FIGURE 7 | Systemic Inflammation Markers. **(A)** Serum LBP was higher in ASO mice that received DSS although this failed to reach statistical significance. However, there was significance in the interaction between genotype and treatment ($p = 0.0240$). Data are presented as means (ng/ml). There was no significant elevation of systemic inflammatory cytokines in DSS treated control or ASO mice including **(B)** IL-1b, **(C)** TNFa, and **(D)** IL-6 the three key markers in systemic inflammation. **(E)** Only DSS treated ASO mice displayed significantly ($p = 0.0328$) increased serum IL-10. Outliers were omitted prior to analysis (>2 standard deviations from the mean). Two-way ANOVA was conducted and values for different factors are indicated in the graphs followed by *post-hoc* Tukey which is indicated on each graph when appropriate $^{*}p < 0.05$, $^{**}p < 0.01$. ASO, α-synuclein overexpressing; LBP, Lipopolysaccharide binding protein.

mitopark mouse model (98, 99) could all be considered for future studies. Additionally, this study administered DSS in three cycles to mimic chronic, intermittent barrier dysfunction; future studies could either use a higher dose of DSS (e.g., 2% DSS) or extend this treatment period to four to five cycles to determine if longer duration would be sufficient to trigger more severe intestinal barrier dysfunction / inflammation and promote the PD-like phenotype. Another consideration is how within group variability and between institution differences in microbiota may be modifying the response to low dose DSS. Although these mice are genetically identical there clearly is variability in the response to DSS. The low dose of DSS likely contributed to the variability (as opposed to a higher dose that would induce robust barrier dysfunction associated with severe intestinal inflammation). However, this variability also represents what happens in the population insomuch as individuals have a different response to the same "disruptor" examples include alcohol (100–102), stress (103, 104), NSAID (105, 106) so the variability could be viewed as a strength in that it models individual susceptibility.

To the best of our knowledge, no studies have investigated the role of intestinal permeability without severe intestinal inflammation in rodent models of PD to determine whether intestinal inflammation is a critical element of the gut-brain axis in the PD pathogenesis. This study provides a significant step forward in our understanding of the role of intestinal permeability, intestinal inflammation and the gut microbiome in the gut-brain axis and PD insomuch as intestinal and systemic inflammation appear to be key features mediating the impact of the intestine on the brain in (at least) the ASO PD model.

DATA AVAILABILITY STATEMENT

The raw sequence data supporting the findings have been deposited in the NCBI Sequence Read Archive under BioProject PRJNA781983. Further queries should be directed to the corresponding author(s).

ETHICS STATEMENT

The animal study was reviewed and approved by Rush University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

AK, CF, RV, and AJ: conceptualization and study design. AJ, MS, RV, PE, AN, and SG: data analysis. AJ, PE, MS, SW, DF, SR, BB, and AP: data collection. AK and RV: resources. AJ, AK, CF, RV, SG, PE, and AN: writing-original draft. AK, CF, and RV: supervision. All authors: writing-review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

AK would like to acknowledge philanthropy funding from Barbara and Larry Field, Ellen and Philip Glass, and Marcia and Silas Keehn as well as funding from the Department of Defense (W81XWH-17-1-0587 to AK), the National Institutes of Health including National Institute on Alcohol Abuse and Alcoholism (R24AA026801 to AK), and the National Institute of Aging (R01AG056653 to RV).

ACKNOWLEDGMENTS

AK would like to thank Larry Field, Glass, Marcia, and Silas Keehn, the Sklar Family, and Johnson for their philanthropic funding. AJ would like to show gratitude to Sarkis Mazmanian and Reem Abdel-Haq for explaining and troubleshooting the behavioral tests and Michael Ohene-Nyako for his help with measuring microglial morphology.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.882628/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 19 May 2022
doi: 10.3389/fneur.2022.890168

The "Black Straight-Line Sign" in the Putamen in Diffusion-Weighted Imaging: A Potential Diagnostic MRI Marker for Multiple System Atrophy

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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *05 March 2022*
Accepted: *28 April 2022*
Published: *19 May 2022*

Citation:
Zheng Y, Wang X, Zhao H, Jiang Y, Zhu Y, Chen J, Sun W, Wang Z and Sun Y (2022) The "Black Straight-Line Sign" in the Putamen in Diffusion-Weighted Imaging: A Potential Diagnostic MRI Marker for Multiple System Atrophy. Front. Neurol. 13:890168. doi: 10.3389/fneur.2022.890168

Background and Purpose: The diagnosis of multiple system atrophy (MSA) remains challenging in clinical practice. This study investigated the value of hypointense signals in the putamen ("black straight-line sign") in diffusion-weighted imaging (DWI) of brain MRI for distinguishing (MSA) from Parkinson's disease (PD).

Methods: We retrospectively enrolled 30 MSA patients, 30 PD patients, and 30 healthy controls who had undergone brain MRI between 2016 and 2020. Two readers independently assessed the signal intensity of the bilateral putamen on DWI. The putaminal hypointensity was scored using 4-point visual scales. Putaminal hypointensity and the presence of a "black straight-line sign" were statistically compared between MSA and PD or healthy controls.

Results: The mean scores of putaminal hypointensity in DWI in the MSA group were significantly higher than in both the PD ($U = 315.5$, $P = 0.034$) and healthy control groups ($U = 304.0$, $P = 0.022$). Uni- or bilateral putaminal hypointensity in DWI with a score ≥ 2 was identified in 53.3% (16/30), 16.7% (5/30), and 13.3% (4/30) of MSA, PD, and healthy controls, respectively, with significant differences between MSA and PD ($X^2 = 8.864$, $P = 0.003$) or healthy controls ($X^2 = 10.800$, $P = 0.001$). Notably, the "black straight-line sign" of the putamen was observed in 16/30 (sensitivity 53.3%) patients with MSA, while it was absent in PD and healthy controls (specificity 100%). There were no significant differences for the presence of "black straight-line sign" in the MSA-P and MSA-C groups ($X^2 = 0.433$, $P = 0.510$).

Conclusion: The "black straight-line sign" of the putamen in DWI of head MRIs has the potential to serve as a diagnostic marker for distinguishing MSA from PD.

Keywords: multiple system atrophy, diffusion-weighted imaging, MRI, putamen hypointensive signal, Parkinson's disease

INTRODUCTION

Multiple system atrophy (MSA) is a rare neurodegenerative disease with the pathological hallmark of oligodendrocyte inclusion bodies (GCI) composed of alpha-synuclein aggregation (1). Clinically, the main manifestations of MSA are autonomic dysfunction superimposed with motor disorders of varying extent, specifically either Parkinsonian type (MSA-P) with Parkinson's syndrome as the main manifestation and cerebellar type (MSA-C) with cerebellar ataxia as the main manifestation (1). The MSA diagnostic criteria were revised in 2008 by Gilman et al. (2); however, its diagnosis remained challenging for clinical neurologists. For example, when autonomic dysfunction is not obvious until the advanced stage or when it only manifests as isolated Parkinsonism or cerebellar ataxia (1, 3, 4). It is therefore difficult to distinguish MSA from other Parkinsonisms, such as idiopathic Parkinson's disease (PD), progressive supranuclear palsy, and progressive ataxia such as spinocerebellar ataxia (1, 4, 5). Despite being the focus of various studies, the biomarkers used for MSA diagnosis, such as detection of alpha-synuclein in serum or cerebrospinal fluid, still lack generalized application and their accuracy remains unconfirmed (6, 7). Imaging findings play an important role in the diagnosis of MSA. The classic "hot cross bun" sign, "hyperintense putaminal rim" sign, cerebellopontine atrophy, an abnormally high signal in the pontine peduncle, and other abnormalities that reflect neuronal cell death and gliosis on structural magnetic resonance images (MRI) are widely known, but they are not specific to MSA (8, 9). The application of functional MRI has a certain diagnostic value, but it is difficult to perform these complicated examinations and analyses in routine clinic conditions (10, 11). Recent research has revealed that a hypointense putaminal signal on susceptibility-weighted imaging (SWI) of MRI is of great significance in the diagnosis of MSA (12, 13). A signal hypointensity score over 2 [unilateral or bilateral, a score of 2 when the intensity was similar to the Vein of Galen and with a posterolateral gradient; and score of 3 when marked posterolateral to anteromedial hypointensity (13)] in the putaminal margin is specific to MSA. Currently, we also find that there are MSA patients who also have similar hypointense signals in the putamen on the diffusion-weighted imaging (DWI) sequences and the low signals at the edge of the putamen show a straight distribution—which we term the "black straight-line sign". The characteristics of the "black straight-line sign" and its diagnostic value in MSA remains unknown. The current study explored the characteristics of the putaminal "black straight-line sign" and its differential diagnosis between MSA, PD, and normal controls.

MATERIALS AND METHODS

This study was approved by the ethics committee of Peking University First Hospital in accordance with the Declaration of Helsinki. Each participant or their legal guardians signed a written informed consent before participating in the study.

Subjects and Patient Consents

This was a retrospective study undertaken in the department of neurology at the Peking University First Hospital. Thirty consecutive inpatients with MSA, 30 non-consecutive inpatients with PD, and 30 age-matched, normal, healthy controls were enrolled from 2016 to 2020. The MSA patients were diagnosed as "probable MSA" based on the second consensus clinical criteria (2). The MSA group included 19 MSA-P (predominant Parkinsonian features) and 11 MSA-C (predominant cerebellar features) patients. The PD patients were diagnosed using the Movement Disorder Society Criteria and all PD patients fulfilled with the clinically established PD (14). All MSA and PD patients were clinically assessed at the first visit and confirmed the diagnosis again during this study by an experienced neurologist (JC, YS, and ZW). All the healthy controls reported no major neurological or psychiatric diseases and none of the positive signs were detected during neurological examinations. The following demographic and clinical information of the MSA and PD subjects, including gender, age at evaluation, disease duration, and Hoehn and Yahr (H-Y) stages (15), were abstracted from medical records.

MRI Protocol and Image Evaluation

All participants underwent a 1.5 or 3.0 Tesla MRI scanning (MAGNETOM Aera 1.5T Siemens Healthcare, Erlangen, Germany. Ingenia 3.0T, Philips Medical Systems, Netherlands). The parameters of DWI of the 1.5 T MR were as follows: repetition time (TR) = 7,280 ms; echo time (TE) = 81 ms; slice number = 20; slice thickness = 6mm slice gap = 0.9mm; flip angle = 180; field of view (FOV) = 240 × 240 mm2; voxel size = 1.3 × 1.3 × 6 mm. The parameters of DWI of the 3.0 T MR were as follows: repetition time (TR) = 4,000 ms; echo time (TE) = 72 ms; slice number = 20; slice thickness = 6 mm slice gap = 1 mm; flip angle = 90; field of view (FOV) = 230 × 230 mm2; voxel size = 1.44 × 1.44× 6 mm; NSA = 1.

The signal intensity and the location of each putaminal abnormality on the DW images were evaluated separately by two readers with 14 and 8 years of neuroimaging MRI research experience (YS and MYZ) in a blind manner in which the demographic and disease information were concealed. If the score of the evaluation was inconsistent between the two readers in a given subject, the final grade for analysis was decided by a consensus between them. The evaluation process is detailed as follows:

First, we assessed the margin of the putamen on DW images, the evaluation of which was similar to SWI assessment (**Figure 1**) (12): Score of 0: the intensity was normal, no hypointense signal was observed; Score of 1: the intensity was similar to cerebrospinal fluid and without a posterolateral gradient; Score of 2: the intensity was similar to cerebrospinal fluid with a posterolateral gradient; Score of 3: the intensity was similar to cerebrospinal fluid with a marked gradient of posterolateral to anteromedial hypointensity. A hypointense signal score over 2 in the putamen margin was considered a criterion for an MSA diagnosis. If the scores of the two sides (right/left) differed, the higher score of the unilateral side and the mean score of both sides were calculated separately. The scores were graded previously to the imaging analysis. Second, we assessed the shape of the putaminal margin hypointensity in subjects with a score $\geq$2. If the low signal at the edge of the putamen showed a straight distribution (the "black straight-line sign"), it was considered a

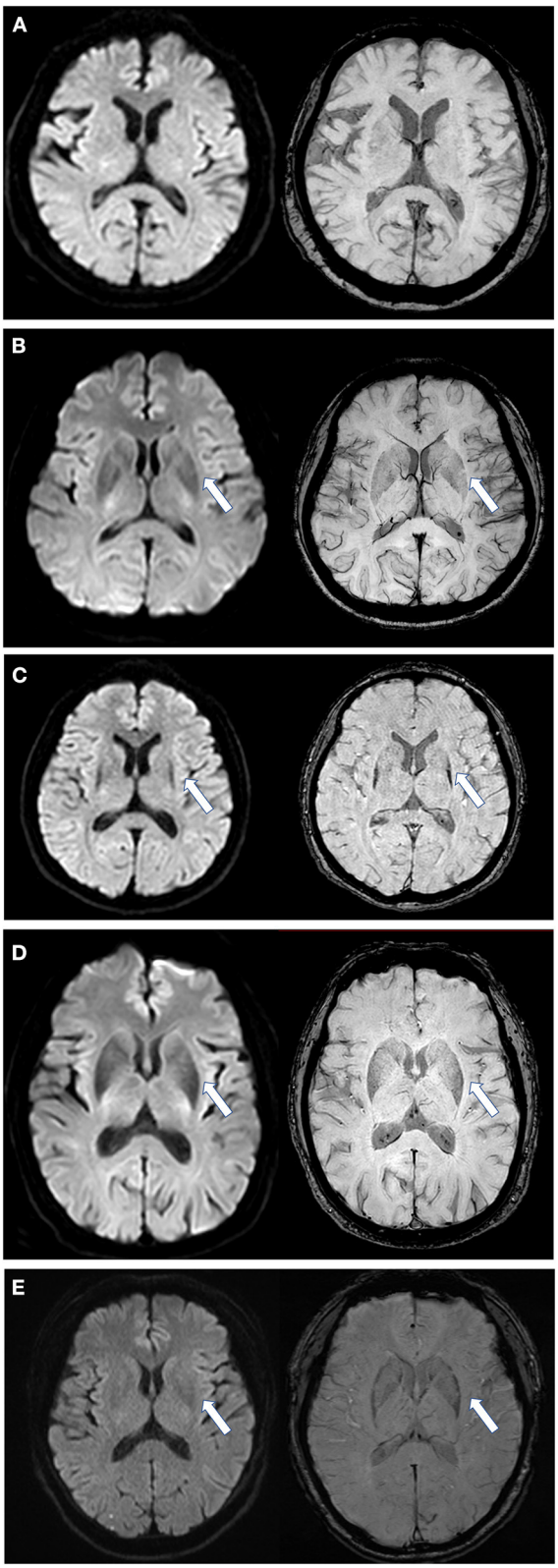

FIGURE 1 | Scores of putaminal hypointensity in DWI and the corresponding SWI sequence. Left, DWI sequence; right, SWI sequence. **(A)** DWI and SWI *(Continued)*

FIGURE 1 | score are all 0 as shown by an arrow; **(B)** DWI and SWI score are all equal to 1; **(C)** DWI and SWI scores are all 2 points, and in a straight distribution; **(D)** DWI and SWI scores are all 2, but with an arc shape and an unclear boundary; **(E)** from a PD patient, with a DWI score of 1 and SWI score of 2. DWI, diffusion-weighted imaging; SWI, susceptibility-weighted imaging. All illustrations are denoted as arrow.

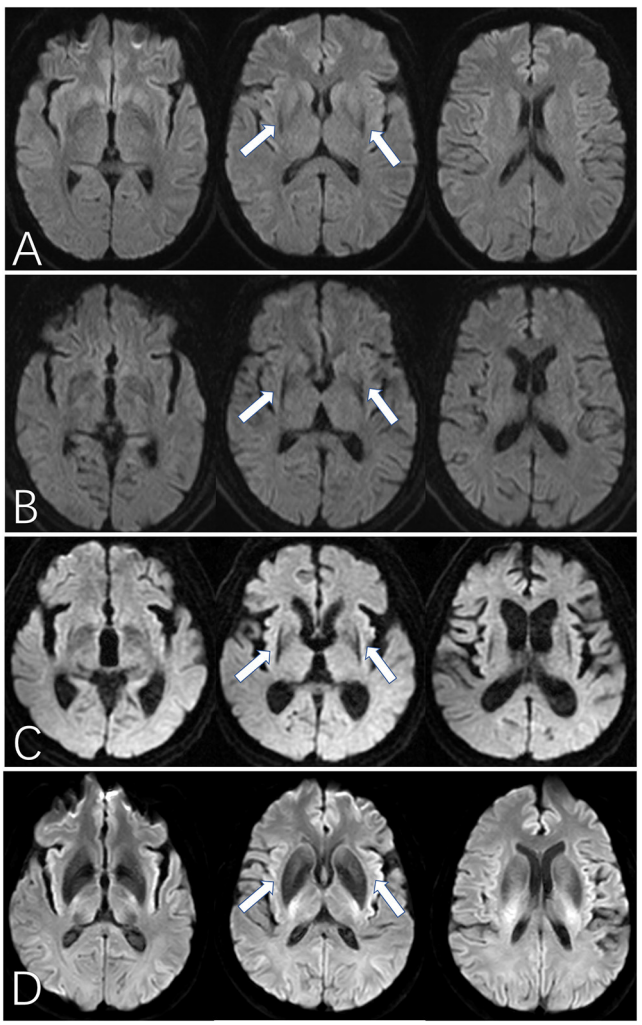

FIGURE 2 | The "black straight-line sign" on DWI for MSA patients. **(A–C)** show the "black straight-line sign" from mild to obvious, showing a linear, abnormally low signal with a clear border in the putamen (arrow). The most obvious section is at the fornix. The "black straight-line sign" can be asymmetric **(B)** or symmetric **(C)**. **(D)** shows the hypointense putamen signal distributed in an arc in a healthy control subject. MSA, multiple system atrophy. All illustrations are denoted as arrow.

criterion for diagnosing MSA. If the low signal at the edge of the putamen was distributed in an arc, it was considered as a non-MSA abnormal signal (**Figures 1**, **2**).

Third, other image parameters including SWI scores of putaminal hypointensity (12), hyperintense putaminal rim, hyperintensity of the pons ("hot cross bun" sign, including only

cruciform hyperintensity), brain stem atrophy, and cerebellar atrophy were also evaluated by the two readers (16, 17).

Statistical Analysis

Data were processed using SPSS 23.0 statistical software (SPSS Inc., Chicago, IL, USA). Continuous data were denoted as Mean ± SD, categorical data were denoted as Number (%). Shapiro-Wilk tests were used to assess the normality of continuous variables. A Kendall's tau-b grade correlation was used to evaluate inter-rater agreement on DWI. A Mann-Whitney U test was used to compare the grade of putaminal hypointensity on DWI between groups. Oneway analysis of variance (ANOVA), a chi-square test, and a Mann-Whitney U test were used to compare the differences for the clinical data and the "black straight-line sign" between groups. An independent sample *t*-test was used for age comparisons between two groups. Sensitivity (true positive/true positive + false negative) and specificity [true negative/(true negative + false positive)] parameters of the images were calculated. Results were considered significant at $p < 0.05$ (two-tailed).

RESULTS

Comparison of Demographic and Disease Information

There were no significant between-group differences in age, gender, disease duration and 1.5 or 3.0 Tesla MRI among the MSA, PD, and healthy control groups. The MSA group had a significantly higher Hoehn and Yahr stage ($U = 200.5$, $p < 0.001$) than the PD group (**Table 1**).

Comparison of Hypointense Putaminal Signals in DWI

The inter-rater reliability for putaminal hypointensity in DWI images was high (Kendall tau-b $R = 0.849$, $P < 0.001$). The putaminal hypointensity scores are summarized in **Table 2**. The scores (both mean and higher unilateral scores were significantly different among three groups with $F = 4.007$, $P = 0.022$ and $F = 4.316$, $P = 0.016$, respectively) of putaminal hypointensity in the MSA group were significantly higher than those in the PD and healthy control groups. There were no significant differences in putaminal hypointensity scores (both mean and higher unilateral scores) between the PD and healthy control groups. Uni- or bilateral putaminal hypointensity with a score ≥2 was significantly more common in MSA than in both PD and healthy controls and was identified in 53.3%(16/30), 16.7%(5/30), and 13.3%(4/30) of MSA, PD, and healthy controls, respectively. The mean and higher unilateral scores in 3.0T MRI were significantly higher than 1.5T MRI for the total population.($U = 581.000$, $P < 0.001$ and $U = 590.000$, $P < 0.001$, respectively).

When assessing the shape of the signal edge in cases with a score ≥2, we found that the rims of the low signal areas in the putamen of PD patients and healthy controls were always arcuate, while those of all the MSA patients were straight (**Figure 2**). The sensitivity of the "black straight-line sign" (uni- or bilateral putaminal hypointensity score ≥2 with a straight rim) in diagnosing MSA was 53.3% and the specificity was 100%. The diagnostic value of other image parameters including SWI feature of putaminal hypointensity, cerebellar atrophy, hyperintense putaminal rim, hyperintensity of the pons ("hot cross bun" sign) and brain stem atrophy were also displayed in (**Table 2**).

There were no significant differences in the proportion of cases with the "black straight-line sign" present between the MSA-P and MSA-C groups ($X^2 = 0.433$, $P = 0.510$). No significant differences in age ($t = -0.837$, $p = 0.410$), disease duration ($U = 84.500$, $p = 0.257$), Hoehn and Yahr stage ($U = 70.500$, $p = 0.085$), or 1.5T/3.0T MRI ($X^2 = 1.265$, $P = 0.261$) were observed between "black straight-line sign" positive and negative MSA patients. Except for SWI scores of putaminal hypointensity ≥2 ($X^2 = 6.000$, $P = 0.014$), none of the other image parameters (i.e., hyperintense putaminal rim, hyperintensity of the pons, and brain stem atrophy) showed a significant difference between "black straight-line sign" positive and negative groups.

DISCUSSION

To our knowledge, this is the first study to assess the diagnostic value of hypointense putaminal signals in DWI between MSA, PD, and normal controls. Our results demonstrate that a score higher than 2, especially in the presence of a "black straight-line sign", can differentiate MSA from PD and normal people. The "black straight-line sign" was specific to both MSA-P and MSA-C subgroups.

The hypointense signal in the posterior putamen was first noticed in the SWI sequence in MSA patients (12, 13, 18). Previous studies have found that this abnormal signal (unilateral or bilateral) with a score higher than 2 had high specificity in the diagnosis of MSA-P, although the sensitivity was relatively low (12, 13). We observed similar hypointense signal manifestations in DWI sequences among MSA patients in clinical practice, so we used a grading method similar to that used in SWI research in our study. Similar to the SWI studies, the current study found that the proportion of hypointense signals with a score >2 (unilateral or bilateral) in the posterior putamen in MSA was significantly higher than that in PD and normal healthy controls. However, we also found a high proportion of hypointense signals with scores over 2 in both PD and normal controls. After further assessment, we found that the morphological characteristics of this putaminal hypointensity in PD and normal controls were rather different from those in MSA. In MSA patients, the morphology of the hypointense signal was thin, straight, and bordered by the surrounding structure (i.e., black straight-line sign). On the contrary, in patients of PD and normal controls, the morphology of the hypointense signal was thick, with an arcuate shape consistent with the anatomical structure, and with a vague boundary with the surrounding normal structure. When the black straight sign was compared among the three groups, it was found that this sign only existed in patients with MSA, but not in PD and normal controls. Our results also show that although the sensitivity of the "black straight-line sign" is limited, its high specificity may be a novel imaging manifestation in the diagnosis of MSA.

TABLE 1 | Demographic characteristics of the subject groups.

	MSA ($n = 30$)	MSA-P ($n = 19$)	MSA-C ($n = 11$)	PD ($n = 30$)	HCs ($n = 30$)	MSA vs. PD	MSA vs. HCs
Age (y)	62.2 ± 8.6	62.4 ± 9.3	61.9 ± 7.6	65.9 ± 6.9	60.0 ± 9.3	$t = -1.854$, $p = 0.069$	$t = 0.965$, $P = 0.339$
Gender (Male)	12 (40%)	9 (45%)	3 (27%)	14 (46.7%)	13 (43%)	$X^2 = 0.271$, $P = 0.602$	$X^2 = 0.069$, $P = 0.793$
Disease duration (y)	3.1 ± 2.2	3.1 ± 2.0	3.2 ± 2.6	3.2 ± 2.8	NA	U = 422.0, $p = 0.676$	NA
Hoehn and Yahr	3.2 ± 1.1	3.1 ± 1.1	3.6 ± 1.1	2.1 ± 0.9	NA	U = 200.5, p<0.001	NA
3.0T MRI	14/30	10/19	4/11	12/30	16/30	$X^2 = 0.271$, $P = 0.602$	$X^2 = 0.267$ $P = 0.606$

MSA, multiple system atrophy, PD, Parkinson's disease, HCs, healthy controls.

TABLE 2 | DWI scores of putaminal hypointensity and other image parameters.

	MSA ($n = 30$)	MSA-P ($n = 19$)	MSA-C ($n = 11$)	PD ($n = 30$)	HCs ($n = 30$)	MSA vs. PD	MSA vs. HCs
DWI scores of putaminal hyperintensity (Left; Right)							
0 score	11; 11	6; 6	5; 5	16; 16	16; 16	-	
1 score	5; 4	2; 3	3; 1	9; 9	10; 11		
2 score	13; 15	10; 10	3;5	5; 5	3; 3		
3 score	1; 0	1; 0	0; 0	0; 0	1; 0		
Mean score	1.1 ± 0.9	1.3 ± 0.9	0.9 ± 0.9	0.6 ± 0.8	0.6 ± 0.7	U = 315.5, $P = 0.034$	U = 304.0, $P = 0.022$
Unilateral higher score	1.2 ± 1.0	1.3 ± 1.0	1.0 ± 1.0	0.6 ± 0.8	0.6 ± 0.8	U = 308.0, $P = 0.024$	U = 307.0, $P = 0.024$
≥2 score	16	11	5	5	4	$X^2 = 8.864$, $P = 0.003$	$X^2 = 10.800$, $P = 0.001$
Shape of the hypointensity with a score ≥2						**Sensitivity**	**Specificity**
Straight	16	11	5	0	0	-	
Arc	0	0	0	5	4		
Black straight-line sign	16/30	11/19	5/11	0/30	0/30	53.3%	100%
Other image parameters							
SWI scores of putaminal hypointensity ≥2	4/6	3/5	1/1	5/21	0/19	66.7%	87.5%
"hot cross bun" sign	7/30	2/19	5/11	0/30	0/30	23.3%	100%
Hyperintense putaminal rim on T2	8/30	6/19	2/11	2/30	1/30	26.7%	95.0%
Brain stem atrophy	7/30	2/19	5/11	0/30	0/30	23.3%	100%
Cerebellar atrophy	15/30	7/19	8/11	1/30	0/30	50.0%	98.3%

MSA, multiple system atrophy, PD, Parkinson's disease, HCs, healthy controls.

We further graded the "black straight-line sign" into 3 different degrees. As shown in **Figure 2**, three different layers of the basal ganglia are presented on the axial image. A mild "black straight-line sign" is featured as a light abnormality appearing only in the middle plane. A moderate "black straight-line sign" is an obvious low signal degree appearing at the bottom and the middle planes, with a gradual trend but without pronounced posterolateral to anteromedial differences. An obvious "black straight-line sign" features a pronounced low-signal degree displayed at all three planes, with an obvious gradient from posterolateral to anteromedial shapes. Nonetheless, we did not find any association between the occurrence or severity of the "black straight-line sign" and disease duration or H-Y stages of MSA. Therefore, the emergence of this "black straight-line sign" should be viewed only as a diagnostic marker and not a grading marker of disease severity.

Previous research on hypointense posterior putaminal signals in SWI has mainly focused on the subgroups of MSA-P, while its characteristics in MSA-C were rarely investigated (12, 13, 18). In our study of the "black straight-line sign", it appeared in a high proportion in both MSA-P and MSA-C, suggesting that this sign has limited significance in distinguishing the subtypes of MSA. This may reflect the fact that both MSA-P and MSA-C have similar neuropathological presentations despite the distributed scope of GCI being different in both subtypes (19). The "black straight-line sign" had comparable sensitivity and specificity in the diagnosis of MSA, which was comparable to the SWI feature of putaminal hypointensity ≥2 (with a sensitivity of 66.7%, and a specificity 87.5%) and cerebellar atrophy (with a sensitivity of 50.0%, and a specificity 98.3%) but better than the other image parameters, including the hyperintense putaminal rim (with a sensitivity of 26.7%, and a specificity 95.0%) and hyperintensity of the pons ("hot cross bun" sign) (with a sensitivity of 23.3% and a specificity 100%) in T2-weighted images, and brain stem atrophy (with a sensitivity of 23.3%, and a specificity of 100%). However, the fact that a hypointense posterior putaminal signal in SWI had a high false positivity (5/21,about 24%) in Parkinson's disease may limit its application. The combination of the "black

straight-line sign" and posterior putaminal hypointensity in SWI may further improve the accuracy of disease diagnosis.

The reason for the appearance of the "black straight-line sign" in DWI remains unknown. Pathological results indicated that the neuronal cell loss, gliosis, and ferritin and Fe (3+) was predominantly located in the posterolateral part of the putamen (20–22). Because SWI sequences are highly sensitive to the paramagnetic effects of iron deposition in the putamen, we speculate that the causality is similar to that of the hypointense signal in the posterior putamen in SWI. Since the DWI sequence is also imaged based on the T2 sequence, we suspect that the appearance of the "black straight-line signal" also reflects the deposition of ferrous or iron ions. In addition, neuropathological and SWI image examinations showed obvious putamen atrophy in MSA (22, 23), which may account for its characteristic shape in DWI (i.e., clearly enclosed by the surrounding structures). More research is needed to explore the reason for the "black straight-line sign" on DWI.

Both 1.5 and 3.0 Tesla MRI scannings were used in this study. Previous studies demonstrated that as the field strength increased the occurrence of hypointensity at the dorsolateral putaminal margin increased in MSA (24). In this study, we also found that subjects that in 3.0 Tesla group had higher scores of hypointensity in the margin of the putamen on DW images. However, the occurrence of "black straight-line sign" in DWI showed no different between 1.5 and 3.0 Tesla MRI groups. Our study has several limitations: firstly, few patients underwent DWI and SWI imaging simultaneously, which may lead to limited accuracy of sensitivity and specificity of posterior putamen low signal sign in SWI. So more research is needed to compare the diagnostic value of the "black straight-line sign" and posterior putamen low signal sign in SWI. Secondly, our study did not include patients with other types of Parkinsonism, including progressive supranuclear palsy, dementia with Lewy body disease which were also difficult to differentiate from MSA in the early stages of the disease. Thirdly, neurologists did not blind to MRI data when establishing the diagnosis of these patients. Although they did not refer to DWI characteristics in diagnosis, it still could be a potential source of bias. Fourthly, other image parameters including the vertical pons hyperintensity which has been reported to be more sensitive than "hot cross bun" sign and "swallow-tail" sign were not evaluated (16, 20). Finally, the absence of a definite postmortem diagnosis increases the likelihood of misdiagnosis in our patients. Patients in the PD group included in the study had a relatively short disease duration (3.2 ± 2.8 years), and it cannot be said with great certainty that some of these patients will not turn out to have MSA-P several years later. Additional studies that investigate the association between DWI and pathological relations are needed.

In conclusion, we evaluated hypointense posterolateral putaminal signal in head DWI—the "black straight-line sign". This sign had a favorable applicative value comparable to the hypointense putaminal posterolateral signal in SWI. The "black straight-line sign" may be added as a potential imaging marker for the diagnosis of MSA. It will be valuable for differentially diagnosing MSA, PD, and normal subjects in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board and Ethics Committee at Peking University First Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YZhe and YS contributed to the concept and drafting and revision of the manuscript. XW, YJ, JC, WS, and HZ contributed to the collection of images and clinical data. ZW and YZhu contributed to revision of the manuscript. All authors have read and approved the final manuscript.

FUNDING

The study was supported by Scientific Research Seed Fund of Peking University First Hospital (2018SF033). Funding bodies did not play a role in the collection, analysis, and interpretation of data. Funding bodies did not contribute to the writing of this manuscript.

ACKNOWLEDGMENTS

The authors would thank all the patients and their families for their support of this research.

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frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 02 June 2022
doi: 10.3389/fneur.2022.866502

A Randomized Clinical Trial to Evaluate the Effects of Safinamide on Apathetic Non-demented Patients With Parkinson's Disease

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OPEN ACCESS

Edited by:
Pedro J. Garcia-Ruiz, University Hospital Fundación Jiménez Díaz, Spain

Reviewed by:
Santiago Perez-Lloret, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
Juan Carlos Martinez Castrillo, Ramón y Cajal University Hospital, Spain

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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: 31 January 2022
Accepted: 02 May 2022
Published: 02 June 2022

Citation:
Kulisevsky J, Martínez-Horta S, Campolongo A, Pascual-Sedano B, Marín-Lahoz J, Bejr-kasem H, Aracil-Bolaños I, Horta-Barba A, Puig-Davi A and Pagonabarraga J (2022) A Randomized Clinical Trial to Evaluate the Effects of Safinamide on Apathetic Non-demented Patients With Parkinson's Disease. Front. Neurol. 13:866502. doi: 10.3389/fneur.2022.866502

Background: Apathy is highly prevalent and disabling in Parkinson's disease (PD). Pharmacological options for its management lack sufficient evidence.

Objective: We studied the effects of safinamide on apathy in PD.

Methods: Prospective, 24-week, two-site, randomized, double-blind, placebo-controlled, parallel-group exploratory study in non-demented PD on stable dopaminergic therapy randomized 1:1 to adjunct safinamide (50 mg/day for 2 weeks and 100 mg/day for 22 weeks) or placebo. The primary endpoint was the mean change from baseline to week 24 on the Apathy Scale (AS) total score. Secondary endpoints included changes in cognition, activities of daily living, motor scores, the impression of change, and safety and tolerability measures.

Results: In total, 30 participants (active treatment = 15; placebo = 15; 80% showing clinically significant apathetic symptoms according to the AS) were enrolled, and included in the intention-to-treat analysis. Change in AS (ANOVA) showed a trend to significance [$p = 0.059$] mediated by a more marked decrease in AS score with safinamide (-7.5 ± 6.9) than with placebo (-2.8 ± 5.7). *Post-hoc* analysis (paired *t*-test) showed a significant positive change in the AS score between 12-week and 24-week [$p = 0.001$] only in the active group. No significant or trend changes were found for any of the secondary outcome variables. Adverse events were few and only mild in both treatment groups.

Conclusions: Safinamide was safe and well-tolerated, but failed to provide evidence of improved apathy. The positive trend observed in the *post-hoc* analyses deserves to be studied in depth in larger studies.

Trial Registration: EudraCT 2017-003254-17.

Keywords: Parkinson's disease, apathy, safinamide, RCT-randomized controlled trial, clinical trial

INTRODUCTION

Apathy, is one of the more common and debilitating neuropsychiatric disturbances in Parkinson's disease (PD) (1, 2). It substantially contributes to reductions in quality of life, higher levels of care dependency, increased caregiver distress, and increased risk of developing dementia (2–5).

Apathy is manifested as a quantitative reduction of goal-directed activity in comparison to the person's previous level of functioning, which can be observed in behavioral, cognitive, emotional, or social dimensions (1, 6). Considering all the stages of PD, estimates of the prevalence of apathy range from 35 to 70%(1, 7).

While apathy highly overlaps along the course of the disease with symptoms of depression, anxiety, and cognitive impairment (8–11), it is a distinct neuropsychiatric syndrome (12) that can be properly identified using appropriate instruments (7, 13–16).

Apathy in PD is thought to mainly be due to the denervation of ascending dopaminergic pathways causing dysfunction of the prefrontal cortex-basal ganglia circuits (1, 6, 17) but other degenerated neurotransmitter systems can be compromised as well.

Among the behavioral complications of PD, apathy is likely the most underserved in terms of specific drug therapy. Very few high-quality randomized-control trials (RCTs) used apathy as an inclusion criterion (18). Two small-sized RCT in people with PD (PwP)–one with the dopamine agonist piribedil in PwP that turned apathetic after STN–DBS (19) and one with the anticholinesterase agent rivastigmine (20)—showed some positive results, the but evidence was not considered enough to qualify these compounds both as "efficacious" and useful' agents for the treatment of apathy in PD (18). Two studies using rotigotine for apathy in PwP were negative (21, 22), and one using 5-hydroxytryptophan observed positive effect on depression but not on apathy (23). Among non-RCT studies, rivastigmine failed to improve apathy in a 1-year open-label study in PD dementia (24), and positive effects in some non-motor symptoms, including apathy, were reported in open-label or *post-hoc* studies with rotigotine (25), pramipexole (17), istradefylline (26), and safinamide (27, 28).

Thus, there are no guidelines currently for managing apathy in PD (18, 29) and recommendations are limited to debatable expert opinion (1, 18, 29, 30). Hence, there is an urgent unmet need to adequately explore treatments to improve apathy in PD.

The dopaminergic system plays a core role in the regulation of goal-directed and motivating effortful behavior for reward, and its dysfunction has been proposed to play a crucial role in the etiology of apathy in PD (31). In this line, there is remarkable evidence regarding the involvement of the mesolimbic system and structures such as the nucleus accumbens—which play a central role in motivation—in the etiology of apathy in PD (1, 32, 33). However, dopaminergic replacement therapy generally has a partial or no effect on apathy in PD (19). Therefore, considering the role of other neurotransmitter systems involved in the normal functioning of the basal ganglia deserves to be taken into account in order to develop effective therapies.

Substantial evidence implicates the nucleus accumbens glutamine-to-glutamate ratio on the prediction of specific components of motivated behavior (34), and glutamine-to-glutamate ratio in the nucleus accumbens predicts effort-based motivated performance in humans (34). These arguments added to preliminary evidence from *post-hoc* and open-label studies showing some improvement in apathy in patients treated with safinamide (27, 28, 35) moved us to formally explore whether a therapeutic strategy using a drug targeting both, dopaminergic and glutamatergic systems, could help to ameliorate apathetic symptoms in PD.

Accordingly, in this study we explored the effects of Safinamide, a multimodal drug with a dual mechanism of action, dopaminergic (reversible mono amine oxidase-B inhibition) and non-dopaminergic [modulation of the abnormal glutamate release(cites)]. It has a predictable beneficial effect on motor fluctuations (35, 36) and was suggested to decrease non-motor symptom burden as well (27, 28). Safinamide has a good safety profile even in special group of PwP with psychiatric complications (37), and was not tested formally in a RCT for apathy in PD.

MATERIALS AND METHODS

Study Design

This was a 24-week, randomized, double-blind, placebo-controlled, add-on, parallel-group study to assess the effect of safinamide on apathy in patients with PD conducted in two centers in Spain. Eligible PwP were randomized (1:1) to 24 weeks of oral treatment with either safinamide 50 mg/day (first 2 weeks) and 100 mg/day (22 weeks) or matching placebo, added to their current, stable PD medications that were to remain unchanged throughout the study.

Sample and Assessments

Inclusion Criteria

Key inclusion criteria were: (1) non-demented PwP with a clinical diagnosis of PD according to the Movement Disorder Society (MDS) PD Criteria (38), aged 45–85 years; (2) Hoehn and Yahr Stage (39) of I to III (mild-to-moderate motor severity) at screening; (3) a total score $\geq$20 on the Montreal Cognitive Assessment scale (MoCA) (40); (3) scoring 1 or more on the Apathy Item of the Neuropsychiatric Inventory (NPI) (41); (4) clinical diagnosis of apathy as defined by Diagnostic Criteria for Apathy in Clinical Practice (42); (5) to be able to speak, read, and understand in the language in which the tests are written; (6) receiving treatment with dopaminergic therapy: levodopa (with or without entacapone) and/or dopamine agonists at a stable dose for at least 4 weeks prior to screening and for the duration of the study; (7) understand and sign the appropriate approved Informed Consent Form of the Study.

Exclusion Criteria

Key exclusion criteria were: (1) diagnosis of moderate-to-severe dementia associated with PD, according to the MDS criteria (43); (2) active psychosis or major hallucinations, severe depression or delirium; history of alcohol or drug abuse for 3

months prior to screening; (3) mental/physical/social condition that could preclude performing efficacy or safety assessments; (4) severe white matter disease, multiple lacunar infarcts, or signs of significant vascular changes on MRI; (5) clinically significant or unstable medical or surgical condition that would, in the opinion of the investigator, preclude participation to the study; (6) currently experiencing significant motor complications (moderate or severe wearing off defined as score >2 on Item 4.4 of MDS-UPDRS Part IV) or disabling dyskinesia (defined as score >2 on Item 4.2 of MDS-UPDRS Part IV) (44); (7) previous treatment with safinamide; (8) treatment with anticholinergic, antidopaminergic medication or acetylcholinesterase inhibitors; and (9) use of MAO-B inhibitors (e.g., selegiline, rasagiline) within 4 weeks prior to screening.

Primary Efficacy Endpoint

The primary efficacy endpoint was the mean change from baseline to week 24 in the 14-item Starkstein Apathy Scale (AS) (45) total score (range 0–42; higher scores indicating more severe apathy).

Secondary Endpoints

Key secondary endpoints were changed from baseline to week 24 in: (1) Parkinson's Disease–Cognitive Rating Scale (PD–CRS) total score; (2) Parkinson's disease–Cognitive Functional Rating Scale (PD–CFRS) total score; (3) NPI; (4) Hamilton Depression Rating Scale (HAM-D); (5) MDS–UPDRS motor subscale (Part III) total score; and (6) Parkinson's Disease Questionnaire (PDQ-39) total score; both (7) Patient's Clinical Global Impression of Change (P–CGI) of Apathy and (8) Clinical Global Impression of Change (CGI) of apathy, were administered at the final visit of the study. Ratings in the P–CGI and CGI were based on a Likert-type scale (0 = not assessed, 1 = very much improved, 2 = much improved, 3 = minimally improved, 4 = no change, 5 = minimally worse, 6 = much worse, 7 = very much worse); maximum score on the scales was 7. Safety and tolerability were assessed through adverse event (AE) reporting and physical examination, body weight and vital signs and electrocardiogram and laboratory test with hematology and biochemistry obtained at baseline, 4, 12, and 24 weeks' visits.

Statistical Analyses

For the planned data analysis, a type 1 error of 5% for the primary hypothesis (alpha 0.05) was assumed. All the efficacy analysis were performed in the modified-intention to treat (ITT) population, therefore, all those subjects randomized and who received at least one evaluation visit were included. We also included all those subjects in the safety analysis who have been randomized and have taken at least one dose of study medication. As a method of imputation of missing values, the Last Observation Carried Forward (LOCF) method was used. The primary efficacy endpoint was the mean change in the AS total score from baseline to week 24. If there were no differences between groups in age, gender, and education, the statistical model to follow was a two-way ANOVA. If there were differences between groups in age, gender, or education, the statistical model to follow was an ANCOVA (if there are differences in a quantitative variable) or three-way ANOVA (if there are differences in a categorical variable). For the analysis of secondary variables, we applied the same model as that for the primary variable. Statistical analysis was performed using the statistical software package SPSS 19.0 for Windows (SPSS Inc., Chicago, IL).

Sample-Size Calculation

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided hypothesis test, we calculated that a sample size of 18 subjects per group ($N = 36$) provided 80% power to detect a difference in mean change of the AS between safinamide and placebo. The SD was assumed in 9, and a dropout rate of 20% was expected among subjects who might discontinue study participation, require safinamide dose suspension or increase dopaminergic dosages.

Ethics

This study (EudraCT 2017-003254-17) was approved by the local Ethics Committee which complies with the regulatory requirements and the Declaration of Helsinki. Written informed consent was obtained before any study procedures from all the patients. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

RESULTS

Early termination of the study due to restrictions caused by the global COVID-19 pandemic precluded the recruitment of the planned sample size ($N = 36$). This was decided in accordance with the Ethics Committee and communicated to the Spanish regulatory authorities. While all the subjects who were active at the beginning of the restrictions were able to complete their pending visits in a timely manner, screening and recruitment of new subjects were stopped because of security reasons. Considerations favoring early termination instead of temporary suspension were: the exploratory nature of the study with the recruitment close to the planned sample size; the uncertainty in the duration of the mobility restrictions and accessing to Hospital facilities; and the relatively close caducity data of the supplied medication and placebo.

Screening, enrollment, and participation information is shown in **Figure 1**. Following screening ($N = 34$), eligible subjects ($N = 30$) were randomized to the safinamide or placebo groups. It supposed six fewer patients than the initially estimated as a total sample. The target dosage (100 mg/day) was achieved on all the participants in the safinamide group except in one subject who discontinued the study at visit 2 for mild dizziness. Other subject in the safinamide group complained of increase in anxiety and left the study at week 10. One subject on the placebo group left the study on week 16 due to hallucinations. According to the estimated dropout rate of 20% of the participants, the resulting sample was considered still valid in terms of sample size. Because

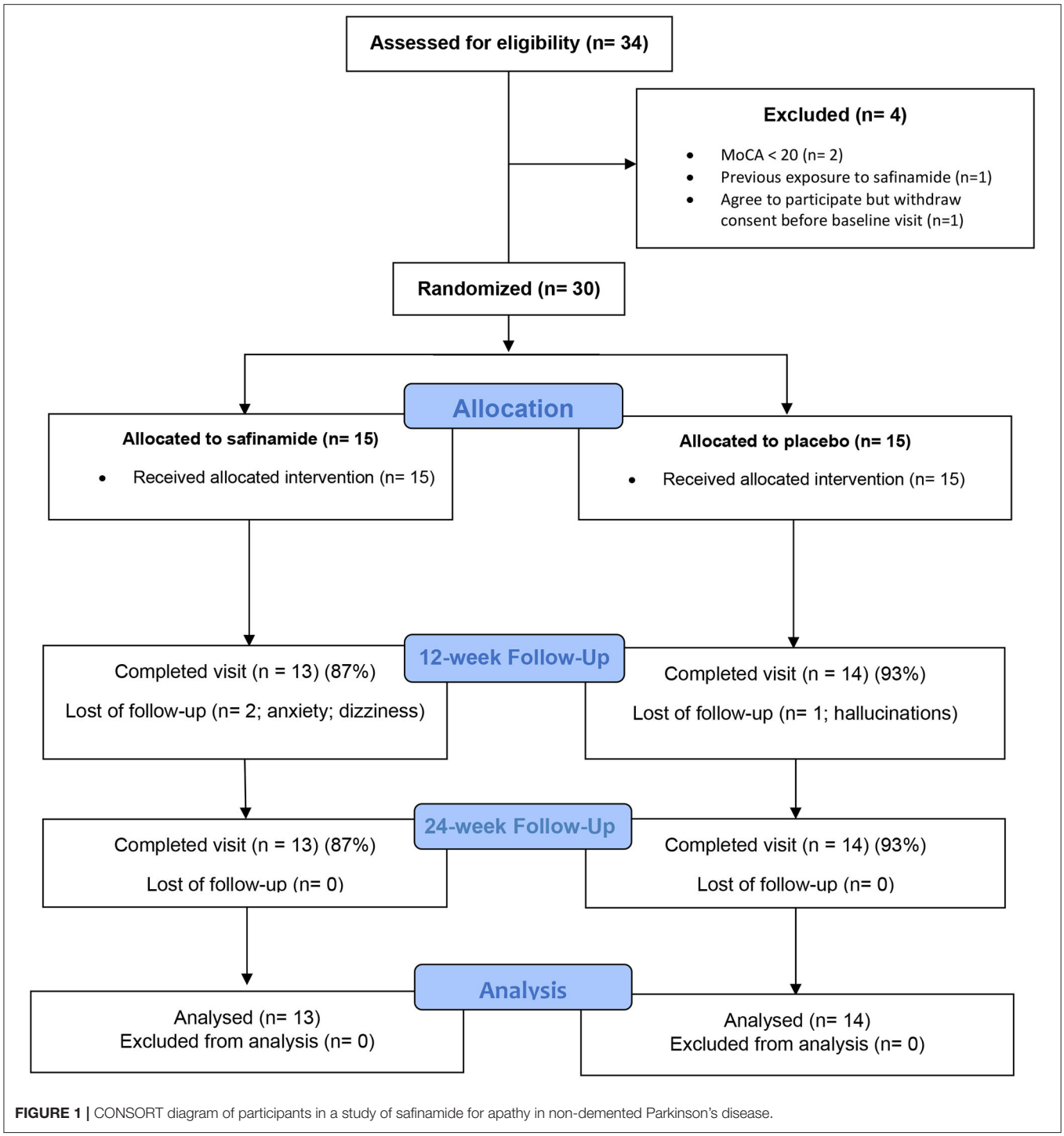

FIGURE 1 | CONSORT diagram of participants in a study of safinamide for apathy in non-demented Parkinson's disease.

of the planned ITT analysis in case of discontinuation, the results from all the 30 subjects participating in the study are reported.

The study sample consisted on 30 patients (mean age = 69.4 ± 9.9 years; mean disease duration = 53 ± 38.6; mean UPDRS-III = 29.9 ± 7.7; mean H&Y = 2 ± 0.4). As per inclusion criteria, all the patients scored ≥1 in the apathy sub-score of the NPI. After randomization, fifteen subjects were allocated to the active treatment (AT) arm and fifteen to the placebo arm. The main clinical and sociodemographic variables at baseline of the whole sample and of the two different treatment groups are described in **Table 1**.

T-tests showed absence of significant between-group differences in the main clinical variables associated with PD. Thus, no differences were found with respect to age, disease

TABLE 1 | Sociodemographic and clinical characteristics of the entire sample and the two treatment groups at baseline.

	Entire sample		Active treatment	Placebo	
	Mean ± SD	Range	Mean ± SD		*p*
Age	69.5 ± 9.8	44–84	66.7 ± 9.2	72.3 ± 10	0.149
Gender (f/m)	9/18	-	5/9	4/9	0.785
Education	12.3 ± 4.06	7–22	14.3 ± 4.6	10.2 ± 2.5	0.009
Disease duration	53 ± 38.6	8–134	57.8 ± 41.1	47.8 ± 36.6	0.512
UPDRS-III	29.9 ± 7.6	11–46	29.9 ± 8.3	29.9 ± 7.5	0.999
H&Y	2.1 ± 0.4	1–3	2.1 ± 0.3	2.1 ± 0.5	0.812
LEDD	609.2 ± 291.6	105–1,400	543 ± 213.8	631 ± 298.7	0.385
MoCA	25 ± 3	20–30	25.8 ± 3	23.9 ± 2.6	0.106
PD-CRS Total	89.1 ± 15.6	59–120	94.5 ± 16.2	81.1 ± 12.1	0.023
PD-CRS frontal-subcortical	59.44 ± 15.5	29–90	66.5 ± 14.6	51.7 ± 12.8	0.010
PD-CRS posterior-cortical	28.67 ± 6.2	22–30	28 ± 2.1	29.3 ± 8.7	0.571
PDQ-39	28.3 ± 17.1	2–59	25.4 ± 15.8	27.5 ± 17.1	0.742
NPI Apathy	4.1 ± 2.5	1–12	4.5 ± 2.9	3.8 ± 2	0.526
AS total score	19.5 ± 7.1	3–34	19.6 ± 7.2	19.3 ± 7.2	0.811
HAM-D	9 ± 5.1	1–23	9.6 ± 5.5	8.4 ± 5.1	0.594
Pharmacological treatment (%)					
Antidepressants	40.7	-	50	30.8	0.310
Anxiolytics	33.3	-	35.7	30.8	0.785
Neuroleptics	0	-	0	0	-
Anticholinergics	3.7	-	0	7.7	0.290
IMAOs	0	-	0	0	-
Amantadine	0	-	0	0	-
Anticholinesterases	7.4	-	7.1	7.7	0.957
Methylphenidate	0	-	0	0	-

duration, H&Y stage, LEDD, pharmacological treatments, and UPDRS-III. Significant differences were found in education level [$t_{(30)} = 2.81$; $p = 0.009$], and in the PD–CRS total [$t_{(30)} = 2.42$; $p = 0.023$] and frontal-subcortical scores [$t_{(30)} = 2.78$; $p = 0.010$] with lower education level and PD–CRS scores in the placebo group. Despite baseline differences in the PD–CRS (used as secondary measure of the study), both the groups were equivalent in terms of global cognitive status measured at baseline with the MoCA. Accordingly, the proportion of patients scoring in the lower range of the PD–CRS was of 6.7% in the AT group and of 7.1% in the placebo group, and the proportion of patients scoring in the medium and higher range was of 93.3% in the AT group and of 92.9% in the placebo group, with no significant differences between the groups.

Both treatment groups showed at baseline an NPI apathy total score (frequency × severity) equal or higher than 1, and a mean AS above the clinical cut-off for apathy (AS ≥ 13), indicating that almost all the patients (75% in the AT groups; 85% in the placebo group; and 80% in the total sample) had clinically significant apathetic symptoms according to the AS, with no differences between groups in the proportion of this prevalence ($\times 2 = 0.361$).

Primary Efficacy Endpoint Analysis

Repeated measures ANOVA applied to explore the primary outcome measure (change in the AS score between 24-week and baseline) showed a trend to a significant group × time interaction [$F_{(1,29)} = 3.06$; $p = 0.059$]. *Post-hoc* analysis showed that this effect was mediated by a more marked, and nearly significant decrease on the AS score in the AT group [$t_{(30)} = -1.95$; $p = 0.062$]. Thus, the mean change from baseline at 24 week was of -7.5 ± 6.9 in the AT group and of -2.8 ± 5.7 in the placebo group. As depicted in **Figure 2**, this effect was observed at 24 week in the AT group, while equivalent scores were obtained in the two groups at baseline and at 12 week.

Paired *t*-test within each group showed that in the AT group, no differences existed between baseline and 12-week AS score [$t_{(13)} = 1.03$; $p = 0.318$], but a significant difference was found between 24 week and 12 week [$t_{(13)} = 4.22$; $p = 0.001$], and between 24 week and baseline [$t_{(13)} = 4.06$; $p = 0.001$]. In the placebo group, no significant differences were found between visits.

When analyzing the change from clinically relevant apathetic symptoms at baseline (AS > 13) to non-apathy (AS < 14) at 24 week, we observed that the significant decrease in the mean

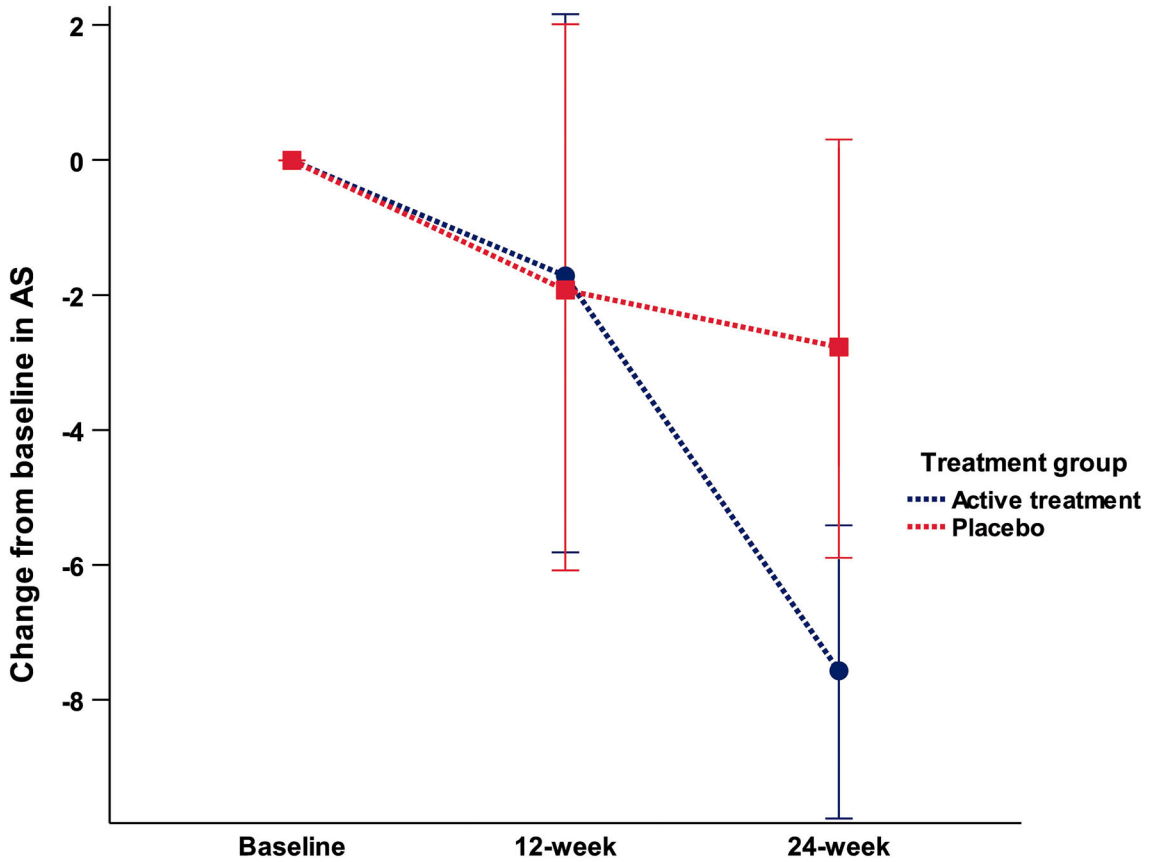

FIGURE 2 | Mean change from baseline in the Apathy Scale in each consecutive visit.

apathy severity score occurred in 46.6% of the subjects in the AT group compared with just 21.4% of those in the placebo group. This difference in the rate of conversion from clinically relevant apathy to non-apathy was significantly different between groups ($x^2 = 0.042$; **Figure 3**).

Secondary Efficacy Endpoints Analysis

Neuropsychiatric Inventory (NPI)

Repeated measures ANOVA showed no significant effects between groups and visits in the NPI total score for apathy (frequency × severity). However, as depicted in **Figure 4A**, *post-hoc* t-test comparison showed a trend to significance [$t_{(30)} = -2.06$; $p = 0.053$] at 24-week mediated by a mean change from baseline of -1.9 ± 2.2 points in the AT group compared with 0 $\pm$ 2.7 in the placebo group. No effects were found with respect to the other neuropsychiatric symptoms covered with the NPI. The statistics for the primary and secondary efficacy endpoints analysis are described in **Table 2**.

UPDRS-III

No significant effects neither trend were found in the repeated measures ANOVA. *Post-hoc* t-test comparisons showed a trend to significance [$t_{(30)} = -1.73$; $p = 0.094$] at 24 week mediated by a mean change from baseline of -3.64 ± 8 in the AT group compared with 2.5 ± 10.5 in the placebo group (**Figure 4B**).

Other Secondary Endpoints

No significant effects or trends were found in the repeated measures ANOVA and *post-hoc* comparisons focusing on cognitive performances (PD–CRS), cognitive-functional status (PD–CFRS), quality of life (PDQ-39), and patient and clinical impression of change (P–CGI, P–CGI–QOL, and CGI).

Safety and Tolerability

Safinamide at the doses of 50 and 100 mg/daily was safe and well-tolerated, and no major or unexpected safety concerns were identified. As reported, early discontinuation occurred in the three patients. One on the safinamide left the study due to mild dizziness at visit 2 without being scaled to receive the 100 mg/day dose, and the other due to increase in anxiety who left the study on week 10 while on 100 mg/day (**Table 3**). The one belonging to the placebo group left the study on week 16 due to hallucinations. No differences were found in vital signs and electrocardiogram, body weight and laboratory test, neither between groups in the baseline visit, nor in the successive follow-ups.

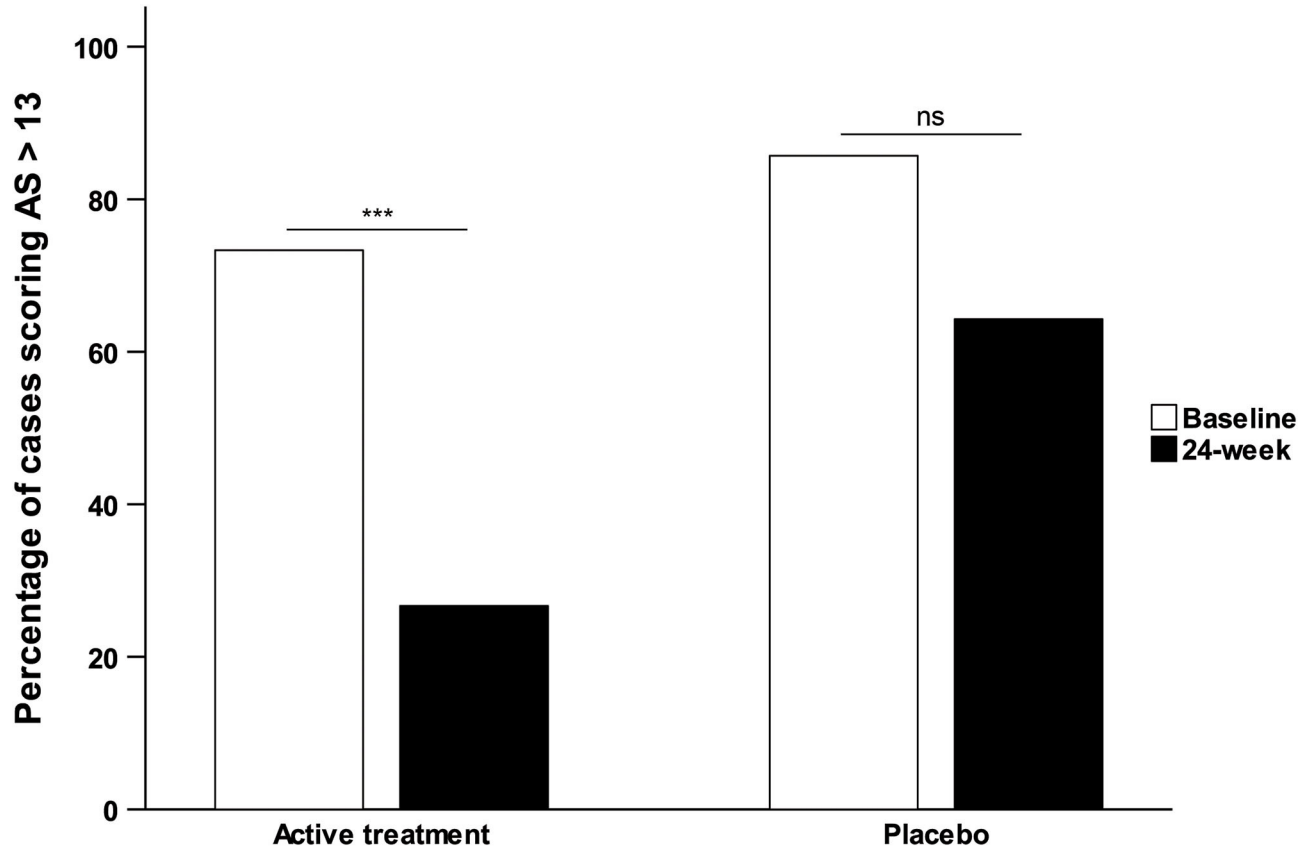

FIGURE 3 | Percentage of participants scoring in the clinical range for apathy based on the Apathy Scale.

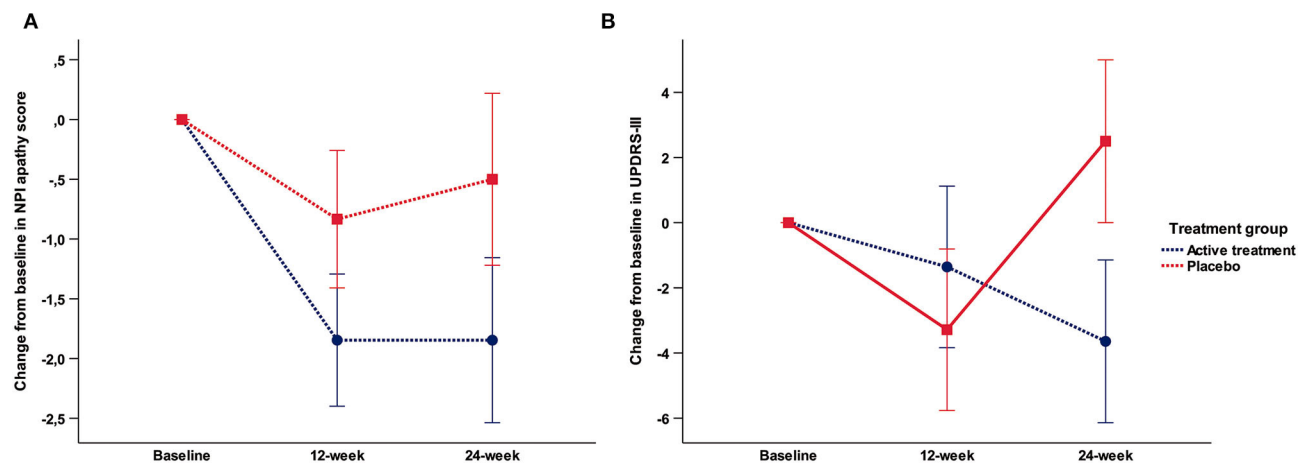

FIGURE 4 | (A) Mean change from baseline in the NPI total apathy score; **(B)** Mean change from baseline in the UPDRS-III.

DISCUSSION

This is the first RCT study exploring the effects of safinamide in non-demented patients with PwP, and one of the few prospective PD studies in which apathy was an inclusion criterion and the primary outcome.

The results of the study are positive in terms of safety, but negative in terms of the effect of Safinamide on apathy. Nevertheless, the results show a tendency toward static significance that we believe deserves consideration. Thus, the addition of safinamide in subjects with PD with significant apathy is well-tolerated and may result in a discrete beneficial

TABLE 2 | Primary and secondary outcome measures analysis.

	Active treatment	Placebo	Difference AT - Placebo	
	Mean ± SD	Mean ± SD	Estimate (95% CI)	*p*
AS total score	−7.5 ± 6.9	−2.8 ± 5.7	−4.71 (−9.68 to 0.25)	0.062
NPI apathy	−1.9 ± 2.2	0 ± 2.7	−1.92 (−3.88 to 0.02)	0.053
PD-CRS total score	1.5 ± 8.9	−4.6 ± 10.6	6.21 (−1.42 to 13.8)	0.306
PD-CFRS	−0.5 ± 2.5	−0.1 ± 2.1	−0.33 (−2.24 to 1.57)	0.722
HAM-D	−1.5 ± 6.6	−0.9 ± 4.1	−0.57 (−4.85 to 3.7)	0.786
UPDRS-III	−3.6 ± 8	2.5 ± 10.5	−6.14 (−13.4 to 1.11)	0.094
PDQ-39	−5.6 ± 19.1	−0.6 ± 12.5	6.95 (−18.6 to 6.1)	0.312
P-CGI	4.08 ± 2.1	4.5 ± 1.9	−0.46 (−2.98 to 1.15)	0.562
P-CGI-QOL	3.08 ± 2.1	2.52 ± 2.1	0.66 (−1.03 to 2.35)	0.428
CGI	4 ± 1.5	3.5 ± 1.5	0.42 (−1.34 to 2.19)	0.607

TABLE 3 | Adverse events.

	Visit (Week)	*N* cases/%	Dosage	Study group
Mild dizziness	4	1/3.33%	50 mg	Active
Anxiety	10	1/3.33%	100 mg	Active
Visual hallucinations	16	1/3.33%	100 mg	Placebo

effect observed in this study in the form of a trend toward significance. Although only reaching a trend to significance in the primary analysis, a beneficial effect of safinamide in comparison to placebo was observed between weeks 12 to 24 in the AT group in the *post-hoc* analysis. This was accompanied by a significant change favoring safinamide in the proportion of subjects moving from clinically significant apathetic symptoms at baseline to not clinically relevant symptoms at the end of the study. No relevant changes were found for any other explored variable, although in consistence with the objective of the study, the only additional statistical trend was a reduction from baseline in the mean NPI apathy score.

In addition to not having observed a statistically significant effect in the primary analysis, a number of lessons supporting further research of safinamide in PD-related apathy can be collected from this exploratory study. The temporal curve showed a trend to significance between weeks 12 and 24 in the safinamide group observed in the exploratory *post-hoc* analysis suggests that the beginning of the eventual positive effect of safinamide can be a delayed one. It is possible that a more consistent effect could have been observed with a longer follow-up and a larger number of patients. Importantly, these positive signals were detected only for the main variable and were not related to motor, mood, or cognitive changes. At last, safinamide was well-tolerated in a cohort of subjects with PD not selected for having levodopa-related fluctuations.

Besides not reaching the planned sample size, other factors related both with the pathogenesis of the apathy syndrome in PD and the characteristics of the tested drug, could have contributed to the modest benefit of associated with safinamide in our study.

Although apathy is highly prevalent in PD from its early stages, the exact pathogenesis of apathy in PD are partially understood at present (46), being likely a combination of progressive alteration of dopaminergic pathways (43, 47), brain atrophy in strategic reward nodes (24) with impaired incentive processing (33), synergistically acting alpha-synuclein and Alzheimer's disease (AD) protein aggregates and increased burden of vascular and inflammatory changes (48) that may limit the response to the pharmacological treatment (1, 49).

While partial correction of an altered neurotransmission may not suffice for apathy to significantly improve in PD, safinamide may have exerted a positive effect on dopamine-dependent apathetic symptoms. Still, considering that its action is not stronger than that of other dopaminergic agents that showed uneven results in improving apathy (49), other factors might concur to explain the partial response of apathy seen in this study.

A glutamate hypothesis for apathy arises from drug trials that suggests a link between the glutamatergic system and apathy symptoms in psychiatric and neurodegenerative diseases other than PD (50–52). While memantine, an agent that blocks the effects of pathologically elevated levels of glutamate, seems not to influence apathetic symptoms in AD, mibampator, a glutamate receptor potentiator, significantly improved apathy in a RCT in AD (50). On this basis, the dual action of a drug that reinforces dopaminergic transmission and blocks the effects of pathologically elevated levels of glutamate, may conceivably improve the synaptic connectivity and trigger the functional recovery of damaged neuronal network, which is typical of apathy.

In this line, blockade of sodium channels and modulation of calcium channels that is the base of the antiglutamatergic activity of safinamide, is not expected to be complete below dosing of 100 mg/day, which were not achieved until the third week of the study. This could explain the significant but delayed reduction in the mean apathy scores compared with the placebo observed

in the *post-hoc* analysis of the second half of our study. Future studies should explore whether higher doses of safinamide and/or more prolonged treatment period have a significant clinical effect.

Consistent with the good safety profile of the drug observed in phase III and large-sample observational studies (37), almost all the apathetic subjects randomized to safinamide treatment completed the study, with a dropout rate of just 13% (two participants). Safinamide was generally well-tolerated over 24 weeks by patients who were receiving polypharmacy without substantial differences in the number or severity of adverse events compared with the placebo. Particularly, adding safinamide in apathetic patients did not worsen motor status, cognition and other important behavioral aspects including mood, hallucinations, or impulse control behavior.

A major strength of our study is that we selected patients accomplishing clinical criteria for apathy and tried to generate high-quality data using a validated instrument as primary outcome to address an important unmet in PD. Consequently, the average apathy rating scale scores obtained at baseline in the AS reflects a PD population with clinically significant apathy. Nevertheless, being apathy scores above the cut-off of apathy (45), they were not in the high range. This may be partially explained by the exclusion of demented patients and the diminished motivation of severely apathetic patients for participating in a research study.

Main limitation of our study was its early termination that precluded the recruitment of the planned sample, and possibly, reaching statistical significance in the primary objective. Nevertheless, our results provide valuable information to inform the design of future trials. A case for a possible favorable response, with a delayed initiation of action and a conceivable more consistent benefit in improving apathy with longer duration of treatment, can be made based on our data.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ERB Hospital de la Santa Creu i Sant Pau. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JK and SM-H: conception and design of the study. JK, SM-H, BP-S, AC, JM-L, HB-k, IA-B, AH-B, and JP: recruitment of participants and assessments. SM-H and AP-D: data analysis. JK, SM-H, and JP: interpretation, draft manuscript, and review. All authors contributed to the article and approved the submitted version.

FUNDING

This work was partially supported by CERCA and CIBERNED funding, and grants from la Marató de TV3 (grants #2014/U/477 and #20142910), Fondo de Investigaciones Sanitarias (FIS) from the Instituto de Salud Carlos III (ISCIII), and Fondo Europeo de Desarrollo Regional (FEDER) grants #PI15/00962 and #PI18/01717. Drug and placebo treatments were facilitated by the Zambon Italy that also provided an unrestricted grant for an independent Investigator-Initiated study. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

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Conflict of Interest: JK has received honoraria for advisory boards or lecturing from: Teva, Zambon, UCB, Bial, General Electric, Sanofi, and Roche. JP has served on advisory or speakers' boards, and received honoraria from: UCB, Zambon, AbbVie, Italfarmaco, Allergan, Ipsen, Bial.

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SYSTEMATIC REVIEW
published: 02 June 2022
doi: 10.3389/fneur.2022.827472

The Application of Deep Brain Stimulation for Progressive Supranuclear Palsy: A Systematic Review

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Progressive supranuclear palsy (PSP) is a rare neurodegenerative disease, and currently no effective symptomatic or neuroprotective treatment is available for PSP. Deep brain stimulation (DBS), as a neurosurgical procedure, plays a role in a range of neurological and psychiatric disorders, and a series of case reports have applied DBS in PSP patients. However, there are no systematic investigations about the application of DBS in PSP patients; we therefore performed a systematic review to evaluate the efficacy of DBS for PSP. PubMed, EMBASE and the Cochrane library were systematically searched from database inception to July 31, 2021. Additionally, the reference lists of included studies were searched manually. Of 155 identified studies, 14 were eligible and were included in our analysis (N = 39 participants). We assessed the data between DBS-OFF and DBS-ON conditions, as measured by the Unified Parkinson's Disease Rating Scale (UPDRS) and other clinical rating scales. A reduction of UPDRS III scores under DBS-ON conditions in most PSP cases was observed, but the differences yielded no statistical significance. There was no sufficient evidence proving DBS was effective for PSP patients, though part of PSP cases could benefit from DBS and our findings could provide up-to-date information about the possible role of DBS in PSP, which would provide design strategies for following clinical trials and might ultimately help to promote the clinical application of DBS in PSP patients.

Keywords: progressive supranuclear palsy, deep brain stimulation, pedunculopontine nucleus, systematic review, Unified Parkinson's Disease Rating Scale (UPDRS)

OPEN ACCESS

Edited by:
Maria Fiorella Contarino, Leiden University Medical Center, Netherlands

Reviewed by:
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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *02 December 2021*
Accepted: *12 April 2022*
Published: *02 June 2022*

Citation:
Wen Y, Jiao B and Zhou Y (2022) The Application of Deep Brain Stimulation for Progressive Supranuclear Palsy: A Systematic Review. Front. Neurol. 13:827472. doi: 10.3389/fneur.2022.827472

INTRODUCTION

Progressive supranuclear palsy is the most common atypical parkinsonian disorder (1) with prominent four-repeat (4R-) tau neuropathology (2), and the classic phenotype termed Richardson's syndrome (PSP-RS, also known as Steele–Richardson–Olszewski syndrome) is characterized by prominent postural instability with repeated unprovoked falls, vertical supranuclear gaze palsy, akinetic-rigid parkinsonism with poor response to dopaminergic agents, and cognitive decline (3, 4). PSP is clinically heterogeneous, and several variant phenotypes have been gradually reported since PSP-RS was introduced in 1964 (3), including PSP-parkinsonism (PSP-P) (5), progressive gait freezing (PSP-PGF, ever referred to pure akinesia with gait freezing, PAGF) (6), and other 7 rare presentations (7, 8). PSP is a uniformly fatal disease

with average disease duration of 8 years (9), and current medicine has limited efficacy in PSP (10). There are still no effective symptomatic or neuroprotective treatment available for PSP despite the transient benefit from levodopa therapy in the early stages of some cases (11).

As a neurosurgical procedure through implanting electrodes into specific targets within the brain and delivering electricity from an implanted battery source (12), deep brain stimulation (DBS) has become an important tool and has been applied to a range of neurological and psychiatric disorders mainly, including Parkinson's disease (PD), essential tremor, dystonia, epilepsy, and major depression (12, 13). PD is a common movement disorder, and muscular rigidity of limbs is an important clinical feature of PD (14), which is distinctive from PSP, the latter predominantly presenting with axial and gait symptoms. The subthalamic nucleus (STN) and globus pallidus interna (GPi) are common stimulating targets for treatments of PD in clinic, especially in cases without response to medication adjustments (15, 16). The pedunculopontine nucleus (PPN) is part of the mesencephalic locomotor region and plays a role in the initiation and maintenance of gait and balance (17). PPN has been proposed as a new target for DBS to treat movement disorders since the first PPN-DBS was carried out in a parkinsonian patient in 2005 (18). Studies have proven that patients with PD treated by PPN-DBS show improvements in gait disorder and falls (15, 19). Moreover, several researches have tried to apply PPN-DBS to treat patients with PSP and proposed PPN as a potential target for PSP (20–22).

The Unified Parkinson's Disease Rating Scale (UPDRS) (23), PSP rating score (PSPRS) (24) and freezing of gait questionnaire (FOG-Q) (25) are widely used clinical rating scales for parkinsonism, among which, UPDRS III and PSPRS are the most common objective assessments applied to reflect the effects of DBS on patients with PSP. Since there is still controversy over surgery benefits between different studies, herein, we carried out a study to evaluate the curative effects and provided a comprehensive summary of DBS for PSP.

METHODS

Information Sources and Search Strategy

This systematic review has been organized according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement guidelines (26) and has been registered at the International Prospective Register of Systematic Reviews (PROSPERO, registration number: CRD42020212628). We performed a comprehensive search of PubMed, EMBASE and the Cochrane library from database inception to July 31, 2021 using the following terms: "progressive supranuclear palsy" or "PSP" in association with "deep brain stimulation" or "DBS." We scanned reference lists of relevant literature for additional potential sources. All publications were restricted to the English language, and all study designs were included.

Study Selection and Data Extraction

Eligible literature had to meet all the inclusion criteria: (1) Subjects: PSP clinical diagnosis [NINDS-SPSP criteria in 1996 (4) and MDS-PSP criteria in 2017 (8) were considered for diagnosis]. (2) Interventions: any types of DBS. (3) Clinical assessments: outcome measures at baseline and follow-up; the UPDRS III is the primary outcome, and other clinical rating scales, including PSPRS, FOG-Q and GF-Q, are secondary outcomes. Reviews, animal research, repeated publications on patients and studies without complete data were excluded. Two independent investigators selected studies through reviewing the titles and abstracts in accordance with the inclusion and exclusion criteria. Disagreements between the two investigators were resolved by a third investigator.

Data were independently extracted by two investigators from each included study on (1) study information (including the first author, year of publication, country of centers); (2) patient characteristics (including age, gender, illness duration, and diagnostic criteria of PSP); (3) intervention (including surgical target for electrode implantation, proper voltage and frequency); (4) assessment of surgery effectiveness [including follow-up time, UPDRS part III scores (UPDRS III), PSPRS and other outcomes]. Additionally, we defined surgery effectiveness as improvement of the clinical rating scales by >30% to better show the surgical efficacy.

Statistical Analysis

We divided the follow-up duration into two parts: short-term (<12 months after DBS) and long-term (≥12 months after DBS). We used the Wilcoxon rank sum test to compare the scores of UPDRS III under different conditions, for example, DBS-OFF vs DBS-ON, before surgery (baseline) vs after surgery (DBS-ON). PSPRS, FOG-Q, GF-Q and other outcomes could not be analyzed due to lack of enough data. Statistical analyses were performed using SPSS 25.0 for Windows, and $p < 0.05$ was statistically significant.

RESULT

Description of Studies

A total of 155 articles of interest were searched and 95 articles identified after duplicates were removed (**Figure 1**). Of these, 62 articles were identified as irrelevant based on their titles and abstracts and were therefore eliminated. Among 33 potentially relevant articles, 10 were excluded because they were the abstracts of poster presentations; patients from five articles (27–31) overlapped with those in other studies (22, 32), and these five articles were excluded; four articles (33–36) had no extracted data and were excluded. A total of 14 articles were finally included in the analysis containing 39 patients with PSP comprising 19 patients with PSP-RS, 7 patients with PSP-P, 5 patients with PSP-PGF, and 8 patients without definite phenotypes. As for surgical targets, 35 patients were treated with PPN-DBS, 1 with STN-DBS, 1 with GPi-DBS, 2 with compound DBS. The basic characteristics of included studies are shown in **Table 1**.

The UPDRS III in PSP Patients

Available data from four studies comprising 10 patients with PSP were included in this analysis comparing UPDRS III in patients with PSP between the DBS-OFF and DBS-ON status

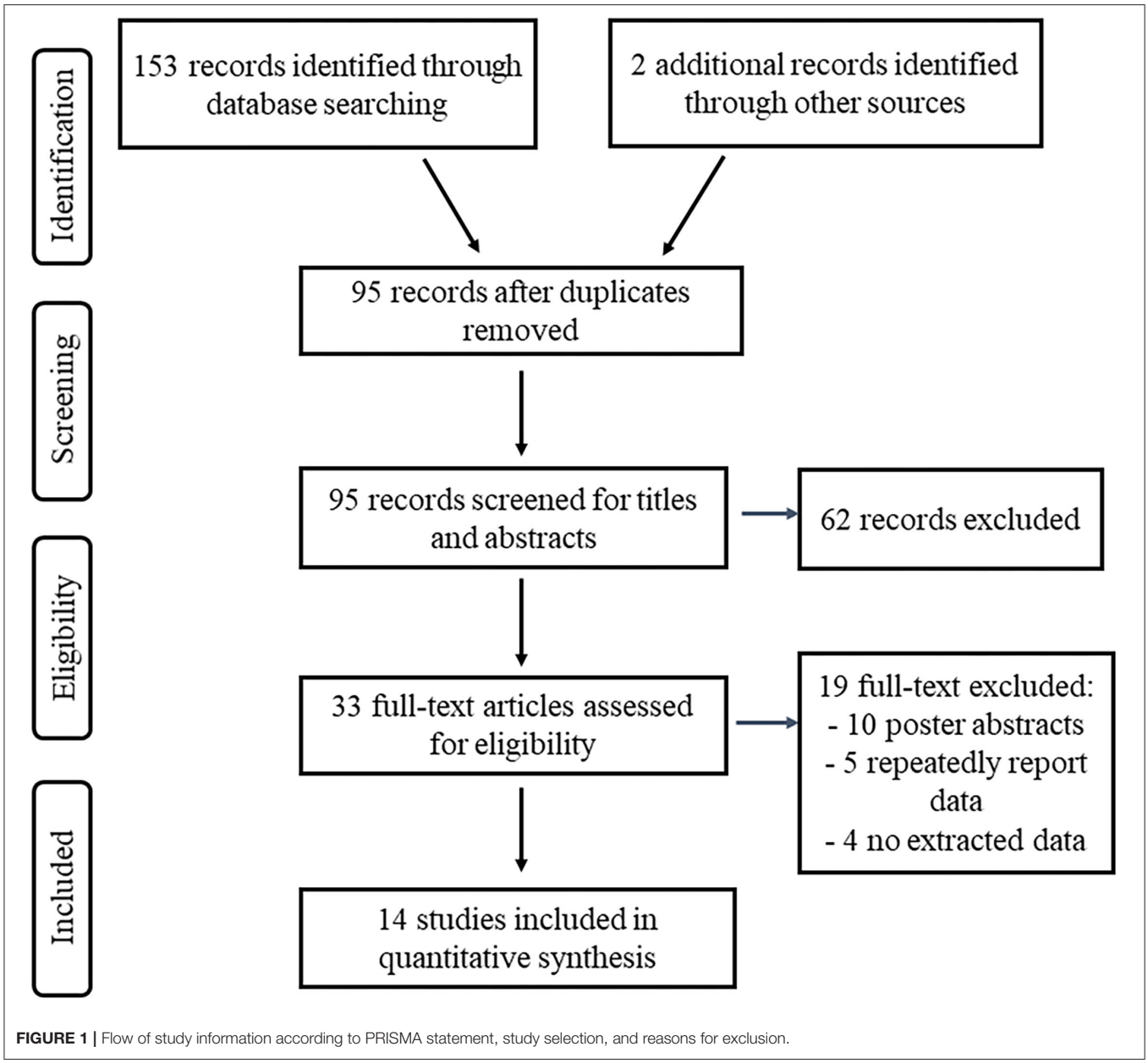

FIGURE 1 | Flow of study information according to PRISMA statement, study selection, and reasons for exclusion.

(**Figure 2**). Mazzone's (22) and Scelzo's studies (32) could not be analyzed since there were no detail scores in each patients with PSP. We divided the follow-up duration into two parts for subgroup analysis: short-term and long-term. In the short-term group, a total of nine PSP cases were analyzed (21, 37, 38, 41), and there was no statistically significant decrease in the UPDRS III scores under DBS-ON status though part of patients showed improvements ($p = 0.051$). Besides, the degree of amelioration was much smaller than those in Mazzone's study where the mean UPDRS III score in four patients with PSP lowered over 40% under DBS-ON conditions (22). In the long-term group, the data from nine patients with PSP were assessed (21, 37, 41) and the differences didn't reach the significance ($p = 0.151$), which was similar to the results of Scelzo's study where a total of eight patients with PSP-RS were treated by unilateral PPN-DBS and no obvious improvements were observed at 6 months or 12 months (32).

On the other hand, we carried out another analysis using data from five articles (17, 20, 21, 38, 41) including 14 patients with PSP, and we compared the UPDRS III scores in these PSP patients pre-operation (at baseline) and post-operation (DBSON) as shown in **Figure 3**. If there were follow-up assessments at different time, we selected the date closest to the operation. No significant differences between these two groups were observed in the Wilcoxon rank sum test where we compared the score after operation to score at baseline ($p = 1.000$).

Considering different PSP clinical phenotypes might have different response to DBS surgery, we performed a simple

TABLE 1 | Baseline characteristics of the included studies.

References	Sample size and gender	Mean age, ys	Duration, ys	Clinical diagnosis	DBS target	DBS parameters			Follow–up	
						Vol. V	Freq. Hz	Pulse width, us	Time, ms	Clinical evaluations
Bergmann et al. (37)	1 F	55	8	PSP–P	bi–STN	L2.5; R3.5	185	60	9/42	UPDRS-III, cognitive tests and levodopa responsiveness
Brusa et al. (38)	1 M	70	3	PSP–P	uni PPN	3.4	25	NA	3/6/9	UPDRS-III, cognitive tests and FOG-Q
Lim et al. (39)	1 F; 1 M	59.5	NA	2 PSP	2 uni PPN	2–2.8	5–30	NA	7/10	Sleep stage distribution
Wilcox et al. (40)	1 M	69	8	PSP–PGF	bi PPN	L2.8–3.3 R3.5–3.8	35	60	2.5/5/7/10/15	FOG-Q and GF-Q
Ostrem et al. (41)	1 M	76	4	PSP–PGF	bi PPN	L4.5–5.1 R4.0–4.4	25	60	3/6/12	UPDRS and FOG-Q
Servello et al. (42)	3 M	68	NA	2 PSP–RS; 1 PSP–P	2 uni PPN; 1 uni PPN + bi GPi	NA	NA	NA	12/14	PSPRS-VI
Doshi et al. (20)	3 F; 1 M	60.8	3	2 PSP–RS; 2 PSP–P	4 bi PPN	0.7–3.5	20–45	60	6/18	PSPRS, UPDRS, PDQ-39 and adverse events
Oliveira Souza et al. (17)	1 F	74	NA	PSP–RS	bi PPN	2–4	20	60	1/3	UPDRS-III
Mazzone et al. (22)	4 NA	NA	NA	4 PSP	4 PPN	4.3–6.9	NA	60	0.5	UPDRS-III, Hoehn and Yahr
Scelzo et al. (32)	8 NA	NA	NA	8 PSP–RS	8 uni PPN	NA	NA	NA	6/12	PSPRS, UPDRS-III and adverse events
Galazky et al. (21)	5 F; 2 M	70	6.2	4 PSP–RS; 2 PSP–PGF; 1 PSP–P	6 bi PPN; 1 PPN + STN	3.5	8–130	60	3/12/24	UPDRS-III, TUG, PSP-QoL, cognitive tests and adverse events
Leimbach et al. (43)	1 F; 1 M	61	5	2 PSP	2 uni PPN	NA	NA	NA	12	Cognitive tests
Orcutt et al. (44)	1 M	75	4	PSP–RS	bi GPi	L 5.3; R 4.7	130	60	12	Improvement of AEO
Dayal et al. (45)	1 F; 2 M	66.7	8.7	1 PSP–RS; 1 PSP–P; 1 PSP–PGF	2 uni PPN; 1 bi–PPN	1.0–9.0	20–30	60	1/6/9/12	PSPRS, FOG-Q, GF-Q and adverse events

DBS, deep brain stimulation; PSP, progressive supranuclear palsy; PSP-P, progressive supranuclear palsy-parkinsonism; PSP-RS, progressive supranuclear palsy-Richardson Syndrome; PPN, pedunculopontine nucleus; STN, subthalamic nucleus; GPi, globus pallidus internus; UPDRS, unified Parkinson's disease rating scale; PSPRS, progressive supranuclear palsy rating scale; FOG-Q, freezing of gait questionnaire; GF-Q, gait and falls questionnaire; PDQ-39, the 39-item Parkinson's disease questionnaire; TUG, timed up and go test; AEO, apraxia of eyelid opening; PSP-QoL, progressive supranuclear palsy quality of life scale.

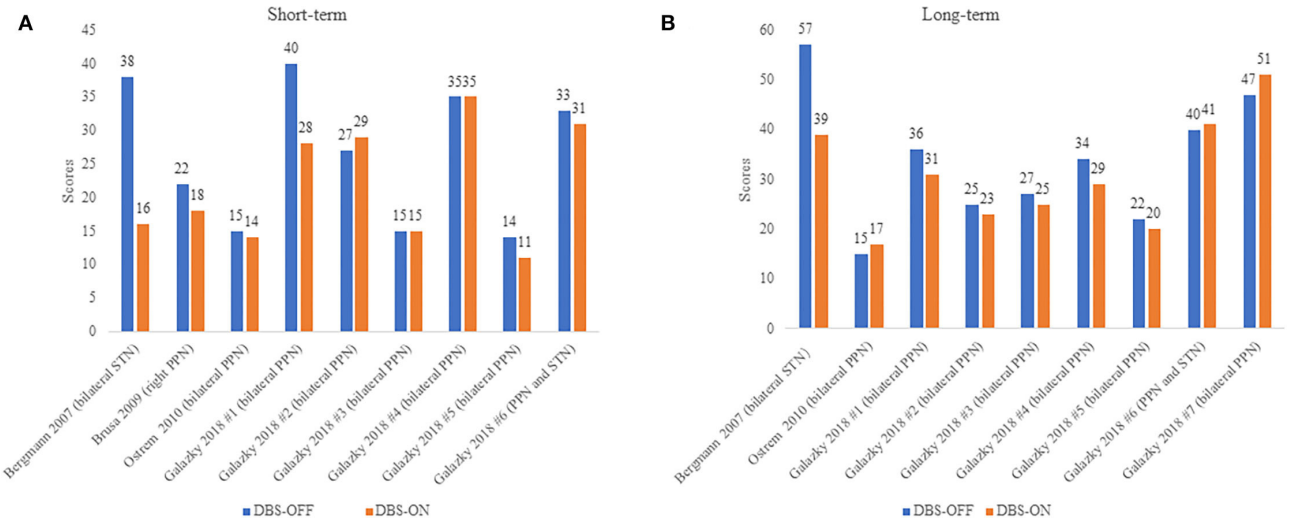

FIGURE 2 | (A,B) UPDRS III scores in PSP patients under DBS-OFF and DBS-ON status. Short-term: <12 months after DBS, long-term: more than or equal to 12 months after DBS. The words in brackets are DBS targets of each patient.

analysis in short-term follow-up of patients presenting as PSP-RS, PSP-P, and PSP-PGF. As **Table 2** shows, complete data were available in nine PSP cases from four studies (21, 37, 38, 41), and the mean improvement in PSP-P was higher than PSP-PS and PSP-PGF. We thus inferred different presentations of PSP might influence the efficacy of DBS, and patients with PSP-P might benefit more from DBS.

Unilateral vs Bilateral PPN-DBS for PSP Patients

A total of 35 PSP cases were treated through stimulating PPN alone; among these, 19 cases were assessed with clinical rating scales at baseline and follow-up and provided detailed information (17, 20, 21, 38, 40–42, 45). We divided these cases into two groups, unilateral PPN-DBS and bilateral PPN-DBS, and compared the improvements of short-term follow-up between these two groups. As **Table 3** showed, the improvement of all five cases in the unilateral PPN group was <30%, while two cases (14.29%) in the bilateral PPN group reached the threshold of effectiveness, which to some degree indicated bilateral PPN stimulations might be more hopeful for PSP patients with mild symptoms than unilateral PPN stimulations. However, the overall surgery effectiveness of PPN-DBS in PSP patients was not very optimistic.

Other Outcomes

Servello et al. followed up three PSP cases that underwent DBS and used PSPRS IV as the main outcome in the long term. They observed a reduction in the number of falls and an amelioration of postural balance in all patients, which was an encouraging result (42). Another three studies also evaluated PSPRS in their cases and reported that there was no obvious improvement (20, 32, 45). In total, four cases from four studies provided available FOG-Q scores: two patients with PSP-P (38, 45) and two PSP-PGF patients (40, 41). The FOG-Q scores among these cases averagely reduced 33.8% at the short-term follow-up visit, with a reduction of more than 50% in a patient with PSP-PGF and a patients with PSP-P. However, the sample size was too small to perform statistical analysis.

One article observed a great improvement of apraxia of eyelid opening (AEO) in a patients with PSP through bilateral GPi stimulations (44). Lim and collaborators proved that PPN-DBS significantly increased nocturnal rapid eye movement sleep in five cases including two patients with PSP (39), which linked PPN with sleep and extended the functions of PPN-DBS. Leimbach et al. focused on the effects of PPN-DBS on cognition through evaluating a comprehensive battery of neuropsychological assessment in five PD cases and two PSP cases. They concluded that PPN-DBS was generally safe from a cognitive perspective though there was no significant change after surgery (43), which was consistent with the results from other studies on cognitive domains (37, 38).

Additionally, four studies mentioned the adverse events related to DBS. Intraoperative bleeding is a major surgical complication worthy strong attention, and it occurred in two patients in PPN-DBS with unknown reasons in Scelzo's cohort where chronic stimulation itself was well tolerated (32), which indicated intracranial hemorrhage during surgery should be better investigated in further studies especially considering the possibility of underreporting due to a negative publication bias. Other surgical adverse events included apathy and a buccofacial apraxia, which were transient and recoverable (21). As for stimulation-related adverse events, paresthesia, oscillopsia, diplopia and dysarthria were observed (20, 21, 45).

DISCUSSION

The aim of this study was to summarize the efficacy of DBS in patients with PSP through analyzing related articles. To our knowledge, this is the first systematic review of DBS for PSP

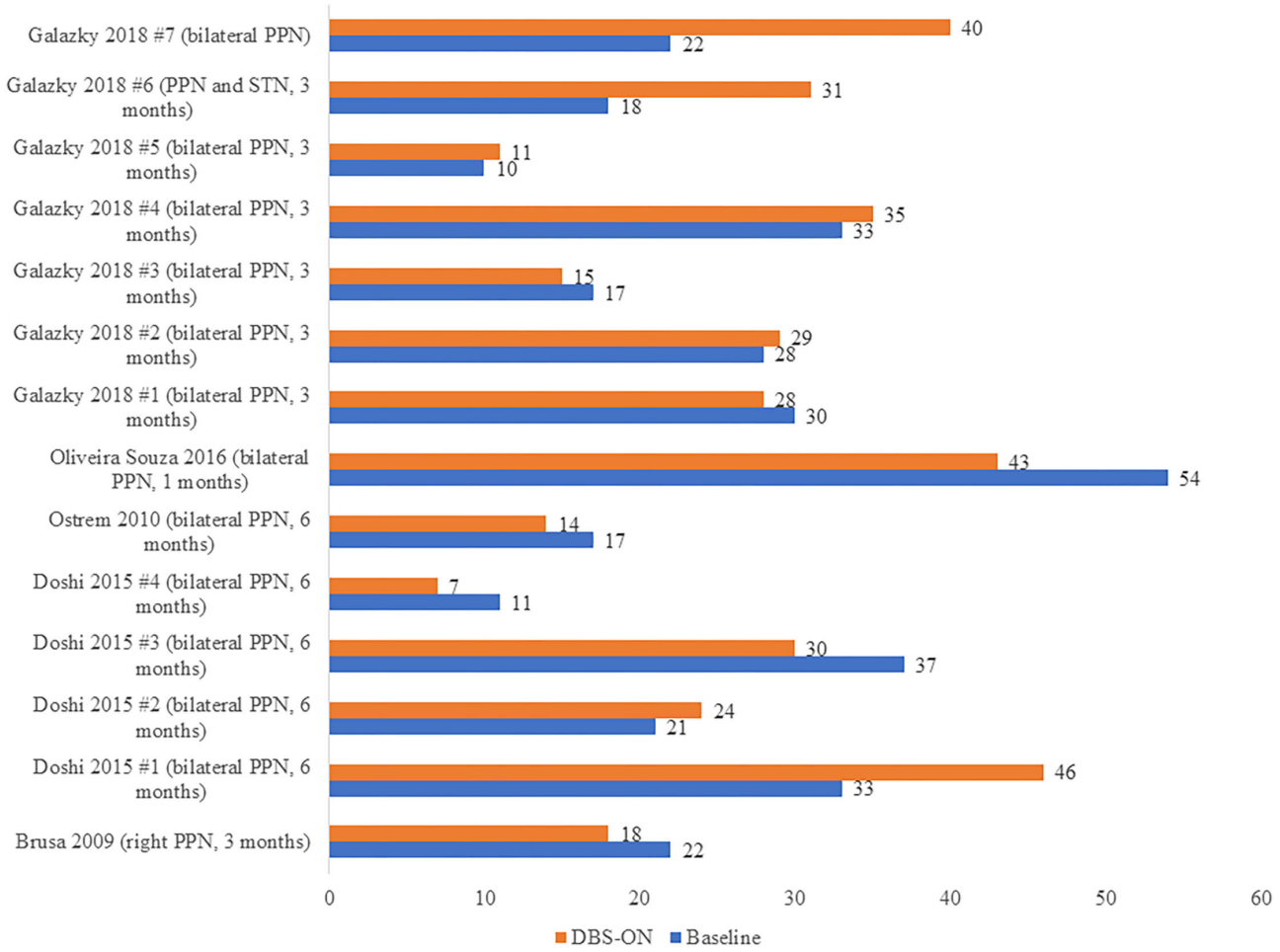

FIGURE 3 | UPDRS III scores in PSP patients before DBS (Baseline) and after DBS (DBS-ON). The words in brackets are DBS targets and postoperative time point at which the "DBS-ON" assessment was performed in each patient.

even though we were only able to combine results from 14 studies. In most cases, the clinical rating scales ameliorated under DBS-ON conditions compared to those under DBS-OFF conditions; however, we found no statistical significances. Additional analyses indicated that the durations of follow-up time, phenotypes of PSP and unilateral or bilateral PPN-DBS might influence the degree of clinical scales improvements. We further found DBS is associated with sleep, AEO and cognitive functions of PSP patients in addition to axial symptoms like falls and gait disorders.

The treatment of PSP is changing since currently, no effective symptomatic or neuroprotective treatment is available for PSP (10), and several clinical trials showed no beneficial effects in PSP patients (46). DBS is a potentially promising tool to provide symptomatic benefit for PSP. Galazky et al. proposed that bilateral PPN-DBS resulted in frequency-dependent effects in PSP patients and they observed low frequency improved cyclic gait parameters while high frequency ameliorated hypokinesia (21), which indicates that choosing proper stimulator parameters for individualized patients is essential. About one PSP case treated by double implanted GPi-PPN gained a better clinical outcome (42). Considering that basal ganglia and brainstem are generally affected in PSP patients (47), there may be an increased synergic effect existing when simultaneously stimulating different nucleus if the patient is tolerant.

PPN is a new target of DBS, and several studies have supported the positive effects of PPN-DBS for PD (15, 19, 48). Garcia-Rill et al. concluded some possible mechanisms of how stimulation in the PPN area could improve gait (49), which mainly results from the complex anatomy and multiple projections of PPN. Pathological study observed that cholinergic and noncholinergic neuronal populations in the PPN were significantly reduced in PSP patients, and this discovery suggested an underlying pathological physiological link could exist between PSP and PPN cell loss (50), which provided evidence for the application of PPN-DBS for PSP patients. Target section within the PPN region could lead to the variability of clinical response (45, 51), which to some extent, can explain why different studies showed variable outcomes. In addition, the variability may be also partly attributable to variations in stimulation parameters,

TABLE 2 | UPDRS III scores of patients with different PSP phenotypes.

Phenotypes	Surgery target	DBS-OFF (scores)	DBS-ON (scores)	Improvement	Mean
PSP-RS	bilateral PPN-DBS (21)	40	28	30.0%	10.00%
	bilateral PPN-DBS (21)	35	35	0.0%	
	bilateral PPN-DBS (21)	15	15	0.0%	
PSP-PGF	bilateral PPN-DBS (21)	27	29	0.0%	9.37%
	bilateral PPN-DBS (21)	14	11	21.43%	
	bilateral PPN-DBS (41)	15	14	6.67%	
PSP-P	PPN- and STN-DBS (21)	33	31	6.06%	27.38%
	right PPN-DBS (38)	22	18	18.18%	
	bilateral STN-DBS (37)	38	16	57.89%	

TABLE 3 | The effectiveness of PPN-DBS for PSP patients.

Stimulation	Clinical rating scales	Baseline (scores)	DBS-ON (scores)	Effectiveness
Unilateral PPN ($N = 5$)	UPDRS III (38)	22	18	No
	PSPRS VI (42)	18	14	No
	PSPRS VI (42)	15	11	No
	PSPRS (45)	50	51	No
	PSPRS (45)	27	31	No
Bilateral PPN ($N = 13$)	FOG-Q (40)	16	7	Yes
	UPDRS III (41)	17	14	No
	PSPRS (45)	39	37	No
	UPDRS III (17)	54	43	No
	UPDRS III (21)	30	28	No
	UPDRS III (21)	28	29	No
	UPDRS III (21)	17	15	No
	UPDRS III (21)	33	35	No
	UPDRS III (21)	10	11	No
	UPDRS III (21)	22	40	No
	UPDRS III (20)	33	46	No
	UPDRS III (20)	21	24	No
	UPDRS III (20)	37	30	No
	UPDRS III (20)	11	7	Yes

Surgery effectiveness was defined as improvement of the clinical rating scales by >30%.

unilateral versus bilateral stimulation, isolated PPN stimulation versus combining the PPN with other targets, duration of follow-up, disease severity and progression, outcome measures used, as well as different PSP phenotypes (45). Therefore, in order to optimize the curative effect of PPN-DBS for PSP, it is important to further understand the anatomy of PPN, improve the localization of the optimal targets and design appropriate parameters.

PSP-P shows a better response to levodopa medications and a more favorable course with longer survival than PSP-RS (52). The present review found PSP-P patients also presented a higher improvement after DBS surgery compared with PSP-RS and PSP-PGF patients, which might result from the various disease severity and different response to levodopa in patients with different phenotypes. On the other hand, we observed that the levodopa equivalent daily dose was largely reduced in a PSP-P

patient in Bergmann's study (37), while part of patients did not reduce levodopa equivalent daily dose after DBS surgery (21), which indicated the effects that the DBS surgery might make on levodopa dose in PSP patients needed further explorations. However, there was not adequate information about levodopa response in included patients, which also restricted the discussion about the interactions between DBS surgery and levodopa response in PSP patients to whether a better response to levodopa leads to better response to DBS and whether DBS surgery changes the response to levodopa in PSP patients.

Cognitive decline is a common clinical symptom in PSP patients, and fronto-executive deficits are the dominated neuropsychological profile of PSP (53). Compared to other parkinsonian syndromes, cognitive progression is more severe and rapid in PSP (53). DBS is generally safe for cognitive function in PD patients (43, 54, 55), and STN-DBS even can improve cognitive function to a certain extent in PD (55). However, there are only a few studies that have investigated the effects of DBS on cognitive condition in PSP patients, and the sample is small and heterogeneous (37, 38, 43). Current evidence indicates PPN-DBS might be safe for PSP patients from a cognitive perspective (38, 43), and more studies are needed to explore the associations between DBS and cognitive function in PSP patients.

This review has several limitations. The major limitation is the relatively small number of included studies as well as the small number of eligible participants. Second, some of included studies are case reports and the data from several studies are incomplete or unavailable, which gains the bias of statistical outcomes and another main limitation for the studies used in this review is possible selection bias: considering PSP could show aggressive progression, relatively benign and early-stage patients might be the candidate for DBS. Moreover, it is an important limitation to analyze the clinical scales, which were performed in different cases where there were no consistent stimulation procedures, DBS parameters, and washout periods. Finally, the outcome of our study is simple: though UPDRS III as the primary outcome was well analyzed, we really desire more motor and non-motor scales to evaluate the DBS for PSP, especially disease-specific outcomes like PSPRS, and the safety of DBS in PSP patients still needs more discussions since only some studies reported adverse events. Thus, more well-designed research with larger cohorts is well needed.

CONCLUSION

This review investigated the application of DBS in PSP patients, however there was not sufficient evidence proving DBS was effective for PSP patients though part of PSP cases could benefit from DBS. Our findings gave up-to-date information about the possible role of DBS in PSP, which would provide design strategies for following clinical trials and ultimately help improve the clinical application of DBS in PSP patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YW searched the articles, selected, and assessed the articles, extracted, analyzed the data, and drafted the manuscript. BJ made contributions to articles selection, data analysis, and manuscript revision. YZ contributed to study design, acquisition of data, assessment of articles, analysis, and interpretation of data, drafting and revising the manuscript, and as well as supporting this study. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported the National Key R&D Program of China (Nos. 2020YFC2008500 and 2018YFC1312003), the National Natural Science Foundation of China (Nos. 81671075, 81971029, 82071216, and 81901171), Hunan Innovative Province Construction Project (No. 2019SK2335), and the Youth Science Foundation of Xiangya Hospital (No. 2018Q020).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 23 June 2022
doi: 10.3389/fneur.2022.895272

Health-Related Quality of Life in Cervical Dystonia Using EQ-5D-5L: A Large Cross-Sectional Study in China

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OPEN ACCESS

Edited by:
Maria Fiorella Contarino, Leiden University Medical Center, Netherlands

Reviewed by:
Cristian Falup-Pecurariu, Transilvania University of Braşov, Romania
Gina Ferrazzano, Sapienza University of Rome, Italy

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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *13 March 2022*
Accepted: *27 May 2022*
Published: *23 June 2022*

Citation:
Liang Y, Lin J, Hou Y, Zhang L, Ou R, Li C, Wei Q, Cao B, Liu K, Jiang Z, Yang T, Yang J, Zhang M, Kang S, Xiao Y, Jiang Q, Yang J, Song W, Chen X, Zhao B, Wu Y and Shang H (2022) Health-Related Quality of Life in Cervical Dystonia Using EQ-5D-5L: A Large Cross-Sectional Study in China. Front. Neurol. 13:895272. doi: 10.3389/fneur.2022.895272

Purpose: The study aimed to evaluate the health-related quality of life (HRQoL) measured by the five-level EuroQol-5 dimensions (EQ-5D-5L) in patients with cervical dystonia, and to explore the determinants of HRQoL in patients with cervical dystonia.

Methods: EQ-5D-5L health state profiles were converted into a single aggregated "health utility" score. A calibrated visual analog scale (EQ VAS) was used for self-rating of current health status. Multiple linear regression analysis was used to explore the factors associated with HRQoL in cervical dystonia.

Results: A total of 333 patients with cervical dystonia were enrolled in the analysis, with an average age of 44.3 years old. The most common impaired dimension of health was anxiety/depression (73.6%), followed by pain/discomfort (68.2%) and usual activities (48%). The median health utility score was 0.80, and the median EQ VAS score was 70.2. Multivariate linear regression analysis indicated that disease duration and the scores of the Hamilton Depression Rating Scale (HDRS), Pittsburgh sleep quality index (PSQI), Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) Part I, and TWSTRS Part III were associated with the health utility scores. After adjusting other parameters, the TWSTRS Part III score and the HDRS score were significantly associated with the EQ VAS scores ($p < 0.05$).

Conclusion: This study evaluated HRQoL in patients with cervical dystonia using the Chinese version of the EQ-5D-5L scale. We found that, besides motor symptoms, non-motor symptoms, including depression, pain, and sleep quality, could be greater determinants of HRQoL in patients with cervical dystonia. Management of non-motor symptoms, therefore, may help improve HRQoL in patients with cervical dystonia.

Keywords: cervical dystonia, HRQoL, EQ-5D-5L, non-motor symptoms, pain, depression

INTRODUCTION

Cervical dystonia (CD) is one of the most common focal dystonias characterized by involuntary contractions of cervical muscles, leading to abnormal movements and posture of head (1). Besides the motor symptoms, non-motor symptoms, such as anxiety, depression, sleep disorders, and pain, are also very common in patients with CD (2). While CD is not a life-threatening disease, it can affect activities of daily living, decrease the quality of life (3), and even cause disability of patients (4).

Most of the previous studies assessed the quality of life in CD using the craniocervical dystonia questionnaire-24 (CDQ 24) or the Short Form-36 Health Survey (SF-36). Additionally, they had usually small sample sizes and yielded inconsistent results. For example, motor severity has been reported to correlate with poor quality of life in CD in some studies (5–7), but not in other studies (8–13).

The five-level EuroQol5-dimensions questionnaire (EQ-5D-5L) is a standardized and more convenient tool to evaluate the health-related quality of life (HRQoL) worldwide (14). It has been extensively used in neurological diseases, such as Parkinson's disease (15), amyotrophic lateral sclerosis (16), and multiple sclerosis (17). The utility values of EQ-5D-5L for Chinese were established in 2017 (18).

Therefore, the aim of this study was to assess HRQoL in patients with CD using the EQ-5D-5L scale in a large Chinese cohort and to explore the determinants of HRQoL in CD.

MATERIALS AND METHODS

Patients Evaluation

We performed a cross-sectional study. All the patients were recruited from the Department of Neurology of West China Hospital of Sichuan University. The patients were diagnosed as CD by neurologists specialized in movement disorders. Only the patients with isolated cervical dystonia were included in the analysis. The patients who had concominant blepharospasm, oromandibular dystonia or dystonia in the limbs or trunk besides CD were excluded in the current study. The study was approved by the Ethics Committee of West China Hospital of Sichuan University (No. 2022-260). All the participants have signed informed consent.

We collected demographic and clinical data of all the participants, including sex, age, age of the onset, and disease duration. All the participants underwent a face-to-face interview by trained movement disorder specialists. Motor and non-motor symptoms were assessed using standard scales. Motor severity was assessed using the Toronto Western Spasmodic Torticollis Rating Scale Part I (TWSTRS-I). Depression was assessed using the Hamilton Depression Rating Scale-24 (HDRS-24) (19). Anxiety was assessed using the Hamilton Anxiety Rating Scale (HARS) (20). Excessive daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS) scale (21). Sleep quality was assessed using the Pittsburgh sleep quality index (PSQI) scale (22). The global cognitive function was assessed using the Montreal Cognitive Assessment (MoCA) scale (23). The Toronto Western Spasmodic Torticollis Rating Scale Part III (TWSTRS-III) was used to assess pain severity. The Toronto Western Spasmodic Torticollis Rating Scale Part II (TWSTRS-II) was used to assess the activities of daily living.

The HRQoL was assessed using the EQ-5D-5L. EQ-5D-5L comprises two parts. The first part of the EQ-5D-5L assesses five dimensions of health, namely, mobility (MO), self-care (SC), usual activities (UA), pain/discomfort (PD), and anxiety/depression (AD). Each dimension has five levels, namely, no problems, slight problems, moderate problems, severe problems, and extreme problems. The scores of these five problems can be converted into a single aggregated "health utility" score according to the Chinese version of the population-based utility values (18). The second part of the EQ-5D-5L is a self-rating calibrated visual analog scale (EQ VAS), with a range of 0 to 100. Score 0 indicates worst possible health state, while score 100 indicates best possible health state.

Statistical Analysis

All continuous variables were presented as the mean and standard deviation (SD), and all categorical variables were presented as numbers and percentages. Spearman's correlation analyses were conducted to explore relationships between EQ-5D-5L values (health utility scores and EQ VAS scores) and clinical variables (sex, age, age of the onset, disease duration, scores of TWSTRS-I, TWSTRS-II, TWSTRS-III, HDRS-24, HARS, ESS, PSQI, and MoCA). The multivariate linear regression model was used to explore the factors correlated with the health utility scores and EQ VAS scores of EQ-5D-5L in CD. The health utility scores and EQ VAS scores of EQ-5D-5L were used as dependent variables.

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22.0, and the R. two-tailed p-values of < 0.05 were considered statistically significant.

RESULTS

A total of 333 patients with CD (118 males) were included in the study. The average age of the patients was 44.3 (SD, 13.3) at the baseline, with a mean disease duration of 3.7 (SD, 5.7) years (**Table 1**).

The median health utility score was 0.80, and the median EQ VAS score was 70.2 for the total patients with CD. Levels 2–5 were considered as impaired for each dimension. The most common impaired dimension of health was anxiety/depression (73.6%), followed by pain/discomfort (68.2%), usual activities (48%), mobility (33.9%), and self-care (20.4%) (**Figure 1**).

Spearman's correlation analyses showed that the health utility scores were significantly associated with disease duration ($r = 0.168$, $p = 0.002$), the TWSTRS-I score ($r = -0.314$, $p < 0.001$), the TWSTRS-II score ($r = -0.625$, $p < 0.001$), the TWSTRS-III score ($r = -0.424$, $p < 0.001$), the HARS score ($r = -0.511$, $p < 0.001$), the HDRS score ($r = -0.590$, $p < 0.001$), and the PSQI score ($r = -0.269$, $p < 0.001$). The EQ VAS scores were significantly associated with disease duration ($r = 0.110$, $p = 0.045$), the TWSTRS-I score ($r = -0.141$, $p = 0.010$), the TWSTRS-II score ($r = -0.353$, $p < 0.001$), the TWSTRS-III score

TABLE 1 | Demographic and clinical features of the recruited patients with cervical dystonia.

	Patients with cervical dystonia
Total number	333
Male sex, No. (%)	118 (35.4%)
Age, years, mean (SD)	44.3 (13.3)
Age of onset, years, mean (SD)	40.6 (13.3)
Disease duration, mean (SD)	3.7 (5.7)
TWSTRS-I score, mean (SD)	13.4 (5.2)
TWSTRS-II score, mean (SD)	8.2 (6.4)
TWSTRS-III score, mean (SD)	3.1 (3.7)
MoCA score, mean (SD)	25.3 (3.5)
HDRS-24 score, mean (SD)	9.7 (8.0)
HARS score, mean (SD)	8.4 (7.1)
ESS score, mean (SD)	4.0 (4.4)
PSQI score, mean (SD)	6.3 (4.1)
EQ-5D-5L health utility score, mean (SD)	0.8 (0.2)
EQ VAS, mean (SD)	70.2 (15.5)

TWSTRS-I, Toronto Western Spasmodic Torticollis Rating Scale Part I;TWSTRS-II, Toronto Western Spasmodic Torticollis Rating Scale Part II; TWSTRS-III, Toronto Western Spasmodic Torticollis Rating Scale Part III; MoCA, Montreal Cognitive Assessment; HDRS-24, Hamilton Depression Scale; HARS, Hamilton Anxiety Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh sleep quality index; EQ-5D-5L, five-level EuroQol 5-dimensions questionnaire; EQ VAS, visual analog scale.

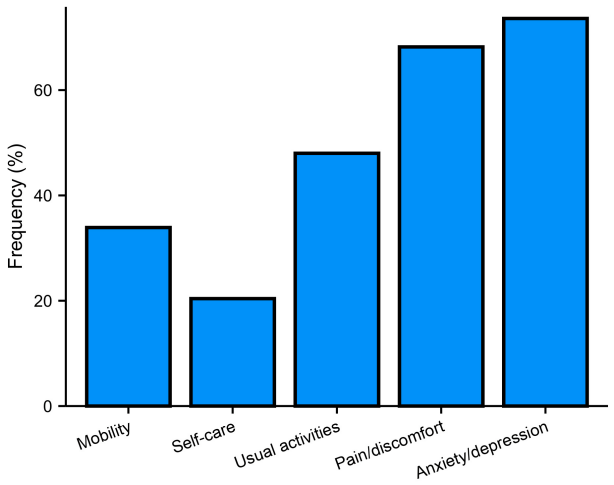

FIGURE 1 | Frequency of reported problems for each of the EQ-5D-5L dimensions.

($r = -0.249$, $p < 0.001$), the HARS score ($r = -0.409$, $p < 0.001$), the HDRS score ($r = -0.484$, $p < 0.001$), and the PSQI score ($r = -0.258$, $p < 0.001$; **Table 2**).

The multivariate linear regression analysis showed that disease duration ($\beta = 0.086$, $p = 0.036$) and the scores of the HDRS ($\beta = -0.458$, $p < 0.001$), the PSQI ($\beta = -0.094$, $p = 0.035$), the TWSTRS Part I ($\beta = -0.216$, $p < 0.001$), and the TWSTRS Part III ($\beta = -0.215$, $p < 0.001$) were associated with the EQ-5D-5L health utility scores. After adjusting other parameters, the TWSTRS Part III score ($\beta = -0.124$, $p < 0.012$) and the HDRS score ($\beta = -0.459$, $p < 0.001$) were significantly associated with the EQ VAS scores ($p < 0.05$; **Table 3**).

TABLE 2 | Spearman's correlation analyses of the EQ-5D-5L healthy utility score and the EQ VAS score in patients with cervical dystonia.

	Healthy utility score	EQ VAS
Sex	0.011	−0.073
Age	0.038	0.029
Age of onset	−0.008	0.025
Disease duration	0.168*	0.110*
TWSTRS-I score	−0.314*	−0.141*
TWSTRS-II score	−0.625*	−0.353*
TWSTRS-III score	−0.424*	−0.249*
MoCA score	0.048	0.042
HDRS-24 score	−0.590*	−0.484*
HARS score	−0.511*	−0.409*
ESS score	−0.007	−0.030
PSQI score	−0.269*	−0.258*

TWSTRS-I, Toronto Western Spasmodic Torticollis Rating Scale Part I; TWSTRS-II, Toronto Western Spasmodic Torticollis Rating Scale Part II; TWSTRS-III, Toronto Western Spasmodic Torticollis Rating Scale Part III; MoCA, Montreal Cognitive Assessment; HDRS-24, Hamilton Depression Scale; HARS, Hamilton Anxiety Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh sleep quality index; EQ-5D-5L, five-level EuroQol 5-dimensions questionnaire; EQ VAS, EuroQol visual analog scale.
**Significant difference.*

TABLE 3 | Stepwise linear regression analysis of the total EQ-5D-5L healthy utility score and the total EQ VAS score in patients with cervical dystonia.

	Variable	Standardized regression coefficient	SE	*P*-value
Healthy utility score	Disease duration	0.086	0.001	0.036*
	HDRS-24 score	−0.458	0.001	<0.001*
	PSQI score	−0.094	0.002	0.035*
	TWSTRS-I score	−0.216	0.001	<0.001*
	TWSTRS-III score	−0.215	0.002	<0.001*
EQ VAS score	HDRS-24 score	−0.459	0.096	<0.001*
	TWSTRS-III score	−0.124	0.204	0.012*

EQ-5D-5L, five-level EuroQol 5-dimensions questionnaire; EQ VAS, EuroQol visual analog scale; HDRS-24, Hamilton Depression Scale; PSQI, Pittsburgh sleep quality index; TWSTRS-I, Toronto Western Spasmodic Torticollis Rating Scale Part I; TWSTRS-III, Toronto Western Spasmodic Torticollis Rating Scale Part III.
**Significant difference.*

DISCUSSION

The current study describes the HRQoL profile in patients with CD in a large Chinese cohort using the EQ-5D-5L scale. The results showed that anxiety/depression (73.6%) and pain/discomfort (68.2%) were the highest reported dimensions impaired in patients with CD. In addition, multivariate linear regression analysis showed that EQ-5D-5L health utility scores were associated with disease duration, motor severity, and non-motor symptoms, including pain, depression, and sleep quality,

while EQ VAS scores were only associated with non-motor symptoms, including pain and depression.

As with our results, non-motor symptoms have been widely reported to play an important role in the decreased quality of life in isolated dystonia, including CD (12, 24, 25). Approximately 55∼90% of the patients with CD have been reported to suffer from pain (1, 26, 27). In the current study, pain/discomfort was reported by 68.2% of the patients with CD. Inconsistent with our results, pain has also been identified to affect the quality of life in patients with CD in several studies (7–9, 11–13). The pain in CD can be relieved by botulinum toxin injection (28). However, the mechanism of pain in patients with CD remains largely unknown. The probable mechanisms include both muscle-based and non-muscle-based mechanisms, such as network changes in the basal ganglia (29).

Depression was another determinant of decreased HRQoL in patients with CD identified in the current study. Mood disorders have been reported to be important determinants of poor quality of life in patients with CD in many previous studies (5, 8–11, 13). Depression is common in patients with CD. A recent meta-analysis has yielded depression prevalence of 31.5% in patients with CD (30). In the current study, anxiety/depression was reported by 68.2% of the patients with CD, indicating that the rate of psychiatric comorbidities in CD might be underestimated. Impairment of the dopaminergic system might be an explanation of the development of depression in patients with CD (31).

In line with our results, sleep disorder has also been found to affect the quality of life in patients with CD by a previous study (10). Sleep disorder is also a very common nonmotor symptom in CD (32). Nearly half of the patients with CD have been found to have poor sleep quality (33, 34), which was in accordance with our results (49.8%). Patients with CD with sleep disorders also had a higher pain burden than those with normal sleep (12).

Several studies reported that motor severity was not associated with quality of life in patients with CD (8–13). However, other studies came to the opposite conclusion (5–7). In addition, a study found that motor symptoms had a small influence only on the physical functioning domain of the HRQoL in CD (24). In the current study, motor severity was associated with EQ-5D-5L health utility scores, but not with EQ VAS scores according to the multivariate linear regression analyses. Therefore, the role of motor severity in the HRQoL of CD needs to be validated by more studies in the future.

The results of the current study offered some indications for the strategies of the decreased quality of life in patients with CD. For example, botulinum toxin injection benefits for both pain and motor severity of CD (28), and it has also been reported to help improve the HRQoL in patients with CD (35). In addition, as non-motor symptoms played an important role in the decreased HRQoL in patients with CD, dealing with these non-motor symptoms might be a good choice for improving the HRQoL in CD.

However, several limitations should be acknowledged in the current study. The first limitation was the lack of the healthy controls. The second limitation was that the treatment choices were not included in the analyses.

In conclusion, our study evaluated the HRQoL in patients with CD using the Chinese version of the EQ-5D-5L scale. The results revealed that, besides motor symptoms, non-motor symptoms, including depression, pain, and sleep quality, could be greater determinants of HRQoL in patients with CD, indicating that management of non-motor symptoms may help improve HRQoL in patients with CD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of West China Hospital of Sichuan University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YL and JL contributed to conception, organization and execution, data collection and statistical analysis, and drafting the manuscript. YH, LZ, CL, QW, BC, KL, ZJ, TY, JY (12th author), MZ, SK, YX, QJ, JY (17th author), WS, XC, BZ, and YW contributed to execution and data collection. RO contributed to conception, organization, execution, and data collection. HS contributed to conception and organization, manuscript review and critique, and was responsible for overall content as the guarantor. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the 1.3.5 project for disciplines of excellence–Clinical Research Incubation Project, West China Hospital, Sichuan University (Grant No. 2019HXFH016).

ACKNOWLEDGMENTS

The authors thank all the participants of this study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 14 July 2022
doi: 10.3389/fneur.2022.939823

Movement Disorders Associated With Cerebral Artery Stenosis: A Nationwide Study

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *09 May 2022*
Accepted: *20 June 2022*
Published: *14 July 2022*

Citation:
Park KW, Choi N, Oh E, Lyoo CH, Baek MS, Kim H-J, Yoo D, Lee J-Y, Choi J-H, Lee JH, Koh S-B, Sung YH, Cho JW, Yang H-J, Park J, Shin H-W, Ahn T-B, Ryu H-S, You S, Choi S-M, Kim BJ, Lee SH and Chung SJ (2022) Movement Disorders Associated With Cerebral Artery Stenosis: A Nationwide Study. Front. Neurol. 13:939823. doi: 10.3389/fneur.2022.939823

Background: Studies of secondary movement disorder (MD) caused by cerebrovascular diseases have primarily focused on post-stroke MD. However, MD can also result from cerebral artery stenosis (CAS) without clinical manifestations of stroke. In this study, we aimed to investigate the clinical characteristics of MD associated with CAS.

Materials and Methods: A nationwide multicenter retrospective analysis was performed based on the data from patients with CAS-associated MDs from 16 MD specialized clinics in South Korea, available between January 1999 and September 2019. CAS was defined as the >50% luminal stenosis of the major cerebral arteries. The association between MD and CAS was determined by MD specialists using pre-defined clinical criteria. The collected clinical information included baseline demographics, features of MD, characteristics of CAS, treatment, and MD outcomes. Statistical analyses were performed to identify factors associated with the MD outcomes.

Results: The data from a total of 81 patients with CAS-associated MD were analyzed. The mean age of MD onset was 60.5 ± 19.7 years. Chorea was the most common MD (57%), followed by tremor/limb-shaking, myoclonus, and dystonia. Atherosclerosis was the most common etiology of CAS (78%), with the remaining cases attributed to moyamoya disease (MMD). Relative to patients with atherosclerosis, those with MMD

developed MD at a younger age ($p < 0.001$) and had a more chronic mode of onset ($p = 0.001$) and less acute ischemic lesion ($p = 0.021$). Eight patients who underwent surgical treatment for CAS showed positive outcomes. Patients with acute MD onset had a better outcome than those with subacute-to-chronic MD onset ($p = 0.008$).

Conclusions: This study highlights the spectrum of CAS-associated with MD across the country. A progressive, age-dependent functional neuronal modulation in the basal ganglia due to CAS may underlie this condition.

Keywords: movement disorders, intracranial artery stenosis, extracranial artery stenosis, moyamoya disease, cerebral artery stenosis

INTRODUCTION

Movement disorder (MD) is considered primary when it occurs as an isolated syndrome and secondary as a symptom of various neurological disorders or systemic diseases (1). The causes of secondary MD include metabolic, infectious, traumatic, toxic, and cerebrovascular diseases. Among them, MD caused by cerebrovascular diseases is one of the most common forms, accounting for up to 22% of all secondary MDs (2).

Previous research on secondary MD caused by cerebrovascular diseases has mainly focused on post-stroke MDs (3). Post-stroke MD refers to movement complications associated with ischemic or hemorrhagic stroke leading to parenchymal destruction (4). Approximately 1–3% of patients with acute stroke develop MDs localizable to the stroke lesion (5, 6). Nevertheless, the clinical profile of post-stroke MD is diverse, both in terms of its forms and prognosis (4).

However, cerebrovascular diseases are not limited to stroke. Cerebral artery stenosis (CAS) and subsequent cerebral hypoperfusion can also cause MD even without overt parenchymal damage due to stroke. MD is also the predominant symptom of moyamoya disease (MMD), a non-atherosclerotic cause of intracranial artery stenosis. However, the clinical profile of MDs associated with arterial stenoses has not been comprehensively characterized due to the heterogeneity of MDs and arterial stenoses, as well as the difficulty in defining the association between the two conditions. These clinical entities are particularly important in the context of East Asia, where the prevalence of intracranial atherosclerosis and MMD is much higher than in Western countries (7, 8).

In this nationwide study, we aimed to characterize the clinical features of MD associated with CAS in Korean patients.

MATERIALS AND METHODS

We performed a nationwide multicenter retrospective analysis on CAS-associated MD cases collected from 16 major tertiary care hospital clinics in South Korea specializing in MDs. The 16 centers were Asan Medical Center, Chungnam National University Hospital, Seoul National University Hospital, Gangnam Severance Hospital, Seoul National University-Seoul Metropolitan Government Boramae Medical Center, Pusan National University Yangsan Hospital, Korea University College of Medicine Guro Hospital, Gachon University Gil Medical Center, Samsung Medical Center, Ulsan University Hospital, Haeundae Paik Hospital, Chung-Ang University College of Medicine, Kyung-Hee University College of Medicine, Kyungpook National University Hospital, Dongsan Medical Center, and Chonnam National University Hospital. The study was approved by the Institutional Review Board of each participating center. Informed consent of patients was waived due to the retrospective nature of this study.

Movement disorder (MD) specialists at each center reviewed the medical records of patients with CAS-associated MDs from their patient registries from January 1999 to September 2019. The inclusion criteria were as follows: (1) age of >18 years; (2) hyperkinetic or hypokinetic MD diagnosis; and (3) CAS diagnosis associated with MD, defined as >50% luminal stenosis of the anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior cerebral artery (PCA), and distal or proximal internal carotid artery (ICA). The association between MD and CAS was determined by specialists according to the following criteria: (1) diagnosis of CAS preceding or coinciding with the onset of MD; (2) absence of other structural or primary neurological disorders better explaining the motor symptoms; (3) with lateralized symptoms, the localization of movement matching with the localization of CAS. The clinical information and the association between MD and CAS were cross-examined by an M.D. specialist from another center. The exclusion criteria were as follows: (1) diagnosis of primary MD during follow-up, including Parkinson's disease; (2) diagnosis of other neurological disorders that may manifest MD as a primary feature, including Wilson's disease, neurodegeneration with iron accumulation in the brain, infectious disease, etc.; (3) history of taking dopamine-blocking agents for more than a month, including neuroleptics and antiemetics.

The clinical information collected included baseline demographics, MD characteristics, CAS characteristics, and the outcome of MD. The baseline demographic data included age, sex, and risk factors for concomitant atherosclerosis, such as hypertension, diabetes mellitus, hyperlipidemia, coronary heart disease, and smoking. MD characteristics included subtype, distribution, and onset. As for the subtype, MDs were classified into one of the following: chorea (with or without ballism), dystonia, parkinsonism myoclonus, tremor or limb-shaking, and a mixed phenotype of the above. The phenotype was determined

by an MD specialist using the generally accepted definitions of each phenotype. With regard to distribution, MDs were classified as focal (affecting one part of the body), unilateral (affecting the ipsilateral arm and leg), or generalized. Based on how MD symptoms developed after the patient first noticed them, the onset of MD was defined as acute (<1 week), subacute (1–4 weeks), or chronic (>4 weeks). According to the outcome of MDs, patients were divided into groups with a good outcome (self-limited, improved with medical treatment, improved with endovascular/surgical intervention) and with a poor outcome (static or progressive). The etiology of CAS was divided into atherosclerosis and MMD.

For statistical analysis, continuous variables were compared with the Student's *t*-test or Kruskal–Wallis test, as appropriate. Categorical variables were compared with the chi-square test or Fisher's exact test, as appropriate. The *p*-value of < 0.05 was considered statistically significant. All statistical analyses were performed using R v.4.1.0.

RESULTS

Baseline Characteristics

A total of 96 cases were initially examined. Fifteen patients were further excluded (age of <18 in 6 patients, insufficient association between MD and CAS in 9 patients), and 81 patients were included in the final analysis.

The mean age of all patients was 60.5 ± 19.7 years, ranging from 18 to 90 years (**Table 1**). Bimodal age distribution was observed in all patients and the subset of patients with chorea (**Figure 1**). Such age distribution pattern was not observed in patients with other MDs, possibly due to a small number of patients with those MDs. The sex ratio was near 1:1, with male patients comprising 57% of the sample. Hypertension was the most common risk factor for atherosclerosis (51%), followed by diabetes (27%) and smoking (22%) (**Table 1**).

Characteristics of MDs

Chorea was the most common MD ($n = 46$, 57%) followed by tremor/limb-shaking ($n = 12$, 15%), myoclonus ($n = 8$, 10%), dystonia ($n = 7$, 9%), and mixed MDs ($n = 5$). About half ($n = 40$, 49%) of the patients had acute onset of the MDs, while a substantial portion of patients ($n = 32$, 40%) had a chronic onset. Most patients had symptoms in one limb ($n = 28$, 35%) or hemibody ($n = 49$, 60%), whereas 4 patients had generalized symptoms (**Table 1**). As shown in **Figure 2A**, there was no predominance of a specific movement phenotype over the stenosis of a particular vessel. The details of each MD phenotype are described below.

In patients with chorea ($n = 46$), symptoms were either in one limb ($n = 12$, 26%) or in the hemibody ($n = 34$, 74%) (**Figure 2**). The onset of symptoms was acute in 25 patients (54%), subacute in 8 patients (17%), and chronic in 13 patients (29%). Atherosclerosis was the etiology of CAS in 39 patients (85%), whereas 7 patients had MMD (15%). Atherosclerosis included intracranial stenosis of the MCA ($n = 17$), PCA ($n = 3$), distal ICA ($n = 3$), proximal ICA ($n = 6$), and multiple arteries ($n = 6$). Eighteen patients had acute infarction lesions in various areas of the basal ganglia and brainstem. These lesions included a single lesion in the putamen ($n = 4$), globus pallidus ($n = 1$), thalamus ($n = 3$), and pons ($n = 2$), or multiple

TABLE 1 | Baseline characteristics of patients ($n = 81$).

Characteristics	
Demographics	
Age, years	60.5 ± 19.7
Sex, male	46 (57)
Risk factors for atherosclerosis	
Hypertension	41 (51)
Diabetes	22 (27)
Hyperlipidemia	13 (16)
Smoking	18 (22)
Coronary heart disease	7 (9)
MD	
MD subtype	
Chorea	46 (57)
Dystonia	7 (9)
Parkinsonism	3 (4)
Myoclonus	8 (10)
Tremor/limb-shaking	12 (15)
Mixed	5 (6)
Distribution of MD	
Focal	28 (35)
Unilateral	49 (60)
Generalized	4 (5)
MD onset	
Acute	40 (49)
Subacute	9 (11)
Chronic	32 (40)
CAS	
Etiology and localization	
Atherosclerosis	63 (78)
MCA	22 (27)
ACA	0 (0)
PCA	6 (7)
Distal ICA	6 (7)
Proximal ICA	9 (11)
Multiple stenoses	20 (25)
Moyamoya disease	18 (22)
Acute stroke lesion	
Present	26 (32)
Absent	55 (68)
Associated symptoms	
Motor weakness	25 (31)
Sensory loss	17 (21)
Dysarthria	19 (24)
Limb ataxia	4 (5)
Others	14 (17)

Data are presented as the mean ± standard deviation or the number of patients (%). ACA, anterior cerebral artery; CAS, cerebral artery stenosis; ICA, internal carotid artery; MCA, middle cerebral artery; MD, movement disorder; PCA, posterior cerebral artery.

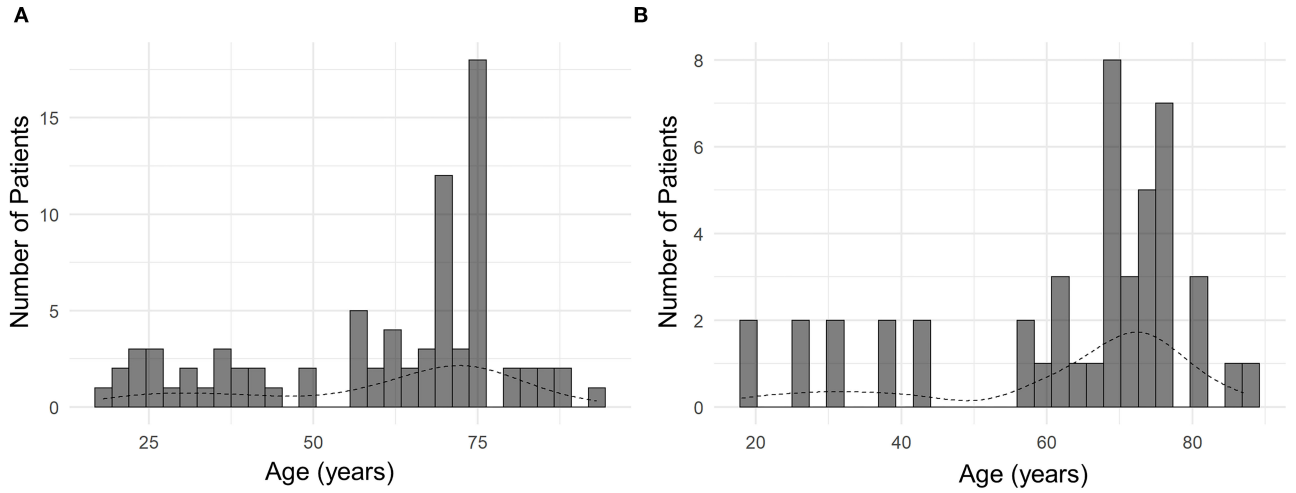

FIGURE 1 | Distribution of patients by age. **(A)** All patients. **(B)** Patients with chorea.

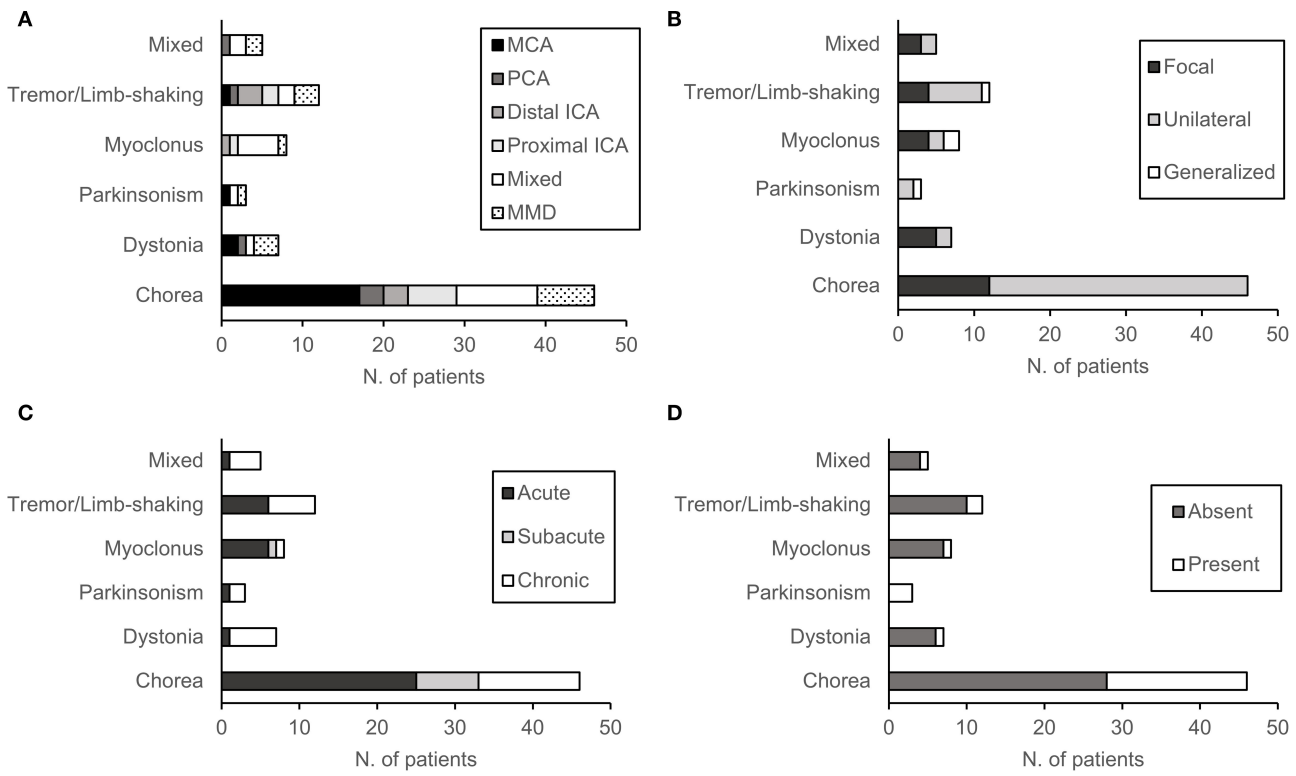

FIGURE 2 | Characteristics of patients according to movement disorder phenotype: **(A)** Location and etiology of cerebral arterial stenosis (CAS), **(B)** mode of onset, **(C)** distribution of symptoms, and **(D)** the presence of acute stroke lesion.

lesions ($n = 9$). Between choreic (n = 46) and non-choreic patients ($n = 35$), there was no statistically significant difference in the baseline demographics and MD or CAS characteristics (**Supplementary Table 1**).

In patients with tremor/limb-shaking ($n = 12$), the second most common form of MD, symptoms were generalized in 1 patient (**Figure 2**). The onset of symptoms was acute in half of the patients ($n = 6$) and chronic in the other half ($n = 6$). Atherosclerosis was the etiology of CAS in 9 tremor/limb-shaking patients, which included stenosis of the MCA ($n = 1$), PCA ($n = 1$), distal ICA ($n = 3$), proximal ICA ($n = 2$), and multiple arteries ($n = 2$). Three patients had MMD. Two patients had

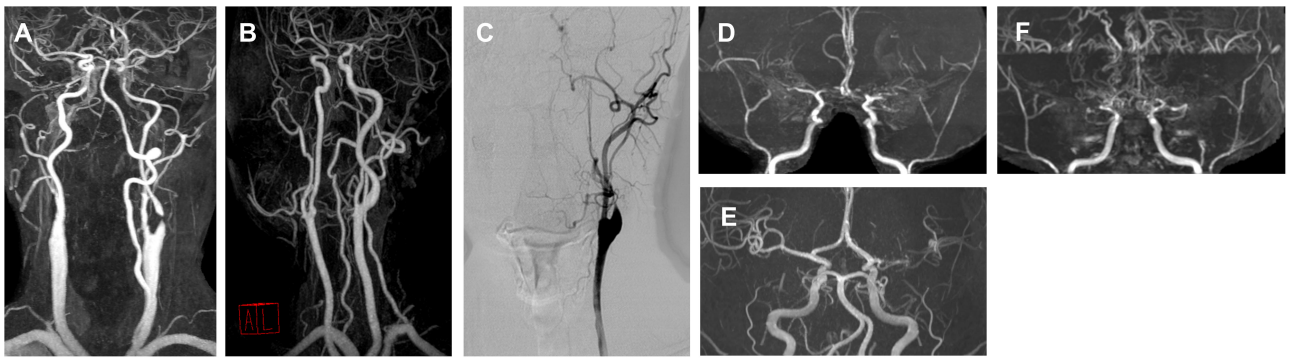

FIGURE 3 | Angiography of representative cases for each movement disorder phenotype. **(A)** A 76-year-old male with right hemichorea and contralateral proximal internal carotid artery (ICA) stenosis. **(B)** A 57-year-old male with limb-shaking of the left arm and contralateral proximal ICA stenosis. **(C)** A 56-year-old male with a myoclonus of the right arm and leg and contralateral proximal ICA stenosis. **(D)** A 24-year-old male with left arm dystonia and moyamoya disease. **(E)** A 46-year-old female with right hemiparkinsonism and contralateral middle cerebral artery (MCA) stenosis. **(F)** A 28-year-old female with a mixed phenotype (left hemichorea and hemidystonia) and moyamoya disease.

acute infarction lesions in the thalamus. Between patients with tremor/limb-shaking ($n = 12$) and the remaining patients with MD ($n = 69$), there was no statistically significant difference in the baseline demographics and MD or CAS characteristics (**Supplementary Table 2**).

In patients with myoclonus (n =8), the third most common form of MD, symptoms were generalized in 25% of patients ($n = 2$). Symptoms were focal in half of the patients (**n** = 4) and unilateral in two patients (**Figure 2**). The onset of symptoms was acute in 6 patients, subacute in one patient, and chronic in 1 patient. In myoclonic patients, the etiology of CAS was atherosclerosis of the distal ICA ($n = 1$), proximal ICA ($n = 1$), multiple arteries (n= 5), as well as MMD ($n = 1$). One patient had acute infarction lesions in the putamen and globus pallidus (**Figure 2D**).

In 7 patients with dystonia, symptoms were either focal (n = 5) or unilateral ($n = 2$) (**Figure 2**). The onset of symptoms was mostly chronic ($n = 6$), except for 1 case with an acute onset. The etiology of CAS was atherosclerosis of the MCA ($n = 2$), PCA ($n = 1$), multiple arteries ($n = 1$), as well as MMD ($n = 3$). One patient had an acute lesion in the putamen.

In three patients with parkinsonism, symptoms were either unilateral ($n = 2$) or generalized ($n = 1$) (**Figure 2**), and 1 patient had developed acute parkinsonism. The etiology of CAS was atherosclerosis of the MCA ($n = 1$), multiple arteries ($n = 1$), as well as MMD ($n = 1$). All three patients had acute ischemic lesions in the putamen.

Finally, there were five patients with mixed MD. Two patients had chorea and dystonia, two had chorea and tremor/limb-shaking, and one had dystonia and myoclonus. These symptoms were either focal (n =3) or unilateral (n = 2) (**Figure 2**). Four patients had a chronic onset of symptoms, and one patient had an acute onset. One patient had stenosis of the PCA, one had multiple intra-/extracranial atherosclerotic lesions, and two patients had MMD. One patient with chorea and dystonia had an acute ischemic lesion in the putamen.

The vascular status of representative cases for each movement phenotype is presented in **Figure 3**, and their clinical characteristics are shown in **Supplementary Table 3**.

Characteristics of CAS

Overall, 78% of patients ($n = 64$) had atherosclerotic disease and the rest had MMD. Among those with atherosclerosis, 34 patients had intracranial atherosclerosis of the MCA (27%), PCA (7%), and distal ICA (7%). Nine patients (11%) had extracranial proximal ICA stenosis, and 21 patients (26%) had multiple atherosclerotic stenoses of various arteries.

Patients with MMD were significantly younger than those with atherosclerosis at symptom onset ($p < 0.001$) (**Table 2**). Most patients with MMD had a subacute or chronic onset of MD symptoms ($n = 15.88\%$), whereas 41% ($n = 26$) of patients with atherosclerosis had an acute onset ($p = 0.001$). Only 1 patient with MMD had an overt stroke lesion at MD onset, while 25 patients with atherosclerosis (40%) also had it ($p = 0.001$). As expected, patients with atherosclerosis had significantly more risk factors for atherosclerosis than MMD patients ($p = 0.013$). However, there was no difference in the ratio of chorea between patients with atherosclerosis and MMD patients.

Acute stroke lesion was present in 26 patients (32%). Motor weakness was the most common neurological symptom associated with MD ($n = 25$, 31%), followed by dysarthria (n = 19, 24%) and sensory loss ($n = 17$, 21%).

Treatment and Outcome

In 20 patients (25%), MD spontaneously improved without medical or surgical intervention. Eight patients (10%) underwent surgical treatment for CAS, and all had full resolution of their MD. Two patients with proximal ICA stenosis underwent carotid endarterectomy, and 6 patients with MMD underwent bypass surgery. The duration of post-intervention follow-up ranged from 4 to 239 months.

Fifty-three (65%) patients received medical treatment, including antichoreic, antidystonic, or antiparkinsonian

TABLE 2 | Comparison between patients with atherosclerosis ($n = 64$) and moyamoya disease ($n = 17$).

	Atherosclerosis ($n = 64$)	Moyamoya disease ($n = 17$)	p-value
Age	66.9 ± 14.6	36.4 ± 17.9	<0.001
Sex			
Male	40 (37)	6 (35)	0.082
Female	24 (63)	11 (65)	
Number of risk factors for atherosclerosis	2 [1-2]	0 [0–0.5]	0.013
MD phenotype			
Chorea	25 (40)	10 (59)	0.235
Non-chorea	39 (61)	7 (41)	
Localization of MD			
Focal/unilateral	60 (94)	17 (100)	0.669
Generalized	4 (6)	0 (0)	
MD onset			
Acute	38 (59)	2 (12)	0.001
Subacute/chronic	26 (41)	15 (88)	
Acute stroke lesion			
Present	25 (39)	16 (94)	0.021
Absent	39 (61)	1 (6)	

Data are presented as the mean ± standard deviation, median [interquartile range], or the number of patients (%). MD, movement disorder.

TABLE 3 | Comparison of demographics, MD characteristics, and CAS characteristics between patients with a good outcome and a poor outcome.

	Good outcome ($n = 64$)	Poor outcome ($n = 17$)	p-value
Age	62.1 ± 18.7	54.6 ± 22.7	0.166
Sex			1.000
Male	36 (56)	10 (59)	
Female	28 (44)	7 (41)	
Number of risk factors for atherosclerosis	2 [1-2]	2 [1-2]	0.830
MD phenotype			1.000
Chorea	36 (56)	10 (59)	
Non-chorea	28 (44)	7 (41)	
Localization of MD			0.669
Focal/unilateral	60 (94)	17 (100)	
Generalized	4 (6)	0 (0)	
MD onset			0.008
Acute	37 (58)	3 (17)	
Subacute/chronic	27 (42)	14 (82)	
Acute stroke lesion			0.542
Present	45 (70)	59 (10)	
Absent	19 (30)	7 (41)	
Etiology			0.964
Atherosclerosis	50 (78)	14 (82)	
MMD	14 (22)	3 (18)	
Localization of CAS			0.114
Intracranial	37 (58)	14 (82)	
Extracranial/mixed	27 (42)	3 (17)	

Data are presented as the mean ± standard deviation, median [interquartile range], or the number of patients (%). CAS, cerebral artery stenosis; MD, movement disorder; MMD, moyamoya disease.

medications along with antithrombotic agents. Among them, 36 patients (44%) showed improvement after pharmacological treatment, while the remaining patients ($n = 17.21\%$) had symptoms that persisted.

In summary, 64 patients (79%) showed a good outcome of MD regardless of whether they received treatment or not, which was characterized by the improvement in movement symptoms. Seventeen patients (21%), however, showed no improvement in MD despite receiving medical treatment. There was no significant association between the outcome and the baseline demographic factors, including age, sex, number of risk factors for atherosclerosis, MD phenotype (chorea *vs.* non-chorea), localization of MD, etiology of CAS, location of CAS, or the presence of acute stroke lesion (**Table 3**). However, patients with a good outcome had a more acute onset of MD ($n = 37$, 58%) than patients with a poor outcome ($n = 3$, 18%) ($p = 0.008$).

DISCUSSION

Given the diverse nature of CAS and hyperkinetic or hypokinetic MDs, few attempts have been made to examine the nature of MD-related CAS in a large series. Our study explored the characteristics of secondary MD associated with CAS across the country. Chorea was the most common type of MD associated with CAS. All movement phenotypes resulted from stenoses of various vascular localization without a specific predominance of the phenotype over a specific area of stenosis. However, we observed that MDs resulting from MMD appear at a younger age, and have a more chronic onset and less acute ischemic lesion compared with MDs caused by atherosclerosis. In our study, patients who received surgical treatment for arterial stenosis showed a good prognosis of MD. In patients with a good outcome of CAS-associated MD, the onset was more acute than in patients with a poor outcome.

In this study, chorea was the most frequent MD associated with CAS, present in 57% of patients. It is also the most frequent movement phenotype among post-stroke MDs, with a prevalence of 36–38% reported in previous studies (5). Traditionally, the subthalamic nucleus was considered to be a typical anatomical correlate for hemichorea and hemiballism (9). However, it has been found that various stroke lesions involved in the striato-pallido-thalamo-cortical feedback loop, including the caudate nucleus, putamen, thalamus, and subcortical white matter, also cause chorea or ballism (10–13). Moreover, there is accumulated evidence of CAS-associated chorea without overt stroke lesions (14–17). These studies uniformly demonstrated striatal hypoperfusion on neuroimaging and chorea reversal after carotid stenting or endarterectomy.

Tremor/limb-shaking was the second most common form of MD in our study. In post-stroke MDs, tremor is usually

associated with lesions of the thalamus or structures of the dentato-rubro-thalamic tract or the cerebello-thalamo-cortical network (4, 18). Limb-shaking, often referred to as "limb-shaking transient ischemic attack (TIA)," was first reported in association with carotid stenosis by Miller-Fisher (19). TIA refers to transient attacks of repetitive brief limb-shaking of the leg or arm associated with carotid stenosis, which lasts from a few seconds to several days (20). Like other vascular paroxysmal dyskinesia, these clinical entities are explained by the hypoperfusion theory. A regional decrease of cerebral blood flow in the dorsofrontal and upper rolandic regions was observed during episodes of limb-shaking (21). Cases show remission of limb-shaking attacks after successful revascularization of carotid stenosis (20–23).

Myoclonus was the third most common phenotype of CAS-associated MD, followed by dystonia. Tremor/limb-shaking and myoclonus are difficult to differentiate, and limb-shaking is sometimes classified as myoclonus (22–24). Theoretically, myoclonus can be the result of any lesion involving the cortical and subcortical white matter (25). Since the basal ganglia are more vulnerable to hypoxia than these areas (26), it seems that myoclonus was not a frequent phenotype.

Dystonia in post-stroke MD is usually reported with stroke lesions in the striato-pallido-thalamo-cortical loop, including the lenticular nucleus, putamen, and thalamus (18). Dystonia is also frequently reported as a movement symptom of MMD without an overt stroke lesion, especially as a form of transient dystonia during hyperventilation (27–29). In our study, MMD was also the cause of dystonia in about half of the cases (3 out of 7).

Taken together, various hyperkinetic MDs including chorea, tremor/limb-shaking, dystonia, and myoclonus, may arise from CAS. Studies have shown hypoperfusion of the basal ganglia and the reversal of movement symptoms with successful revascularization of the stenosed vessel. In all eight patients in our study who underwent surgical treatment to restore blood supply, the remission of MD was observed. The basal ganglia, especially the striatum, are particularly vulnerable to ischemia or hypoxia (26). Hypoperfusion of the basal ganglia by CAS appears to alter the functional balance of motor circuits, leading to various hyperkinetic MDs (30). However, there was no predominance of a specific movement phenotype over a specific localization of stenosis. It can be hypothesized that the motor loop predominantly affected by hypoperfusion (e.g., dentato-rubro-thalamic loop, striato-pallido-cortical loop) determines the dominant motor phenotype.

Then how does hypoperfusion of the basal ganglia alter the motor loop? Experimental studies have shown that energy deprivation causes a surge of dopamine in the striatum (31). In other words, hypoperfusion of the basal ganglia may lead to a relative dominance of the direct pathway over the indirect pathway of the basal ganglia motor circuit. Moreover, glutamatergic activity appears to be altered in animal models of hypoxic-ischemic brain damage (32). In other words, ischemia-trigged glutamatergic excitotoxicity may contribute to the development of CAS-associated MD. In contrast to the usual clinical course of ischemic stroke, 40% of our patients had a gradual onset of symptoms, which further developed within 4 weeks. Overall, these findings suggest a progressive, ongoing functional modulation of neurons in the basal ganglia due to CAS. Such functional modulation may include dopaminergic hypersensitivity, excitotoxicity, and possibly other neurobiological processes such as neuroinflammation. These mechanisms are similar to levodopa-induced dyskinesia in Parkinson's disease (33). The fact that patients with a good outcome have a more acute onset rather than a chronic one may also be in line with the functional modulation hypothesis, suggesting that early initiation of treatment to improve blood supply prevents the perpetuation of MD before ongoing functional neuromodulation prevails. However, the reasons why hypoperfusion of the basal ganglia due to CAS causes MD in only a subgroup of patients, why this phenotype is manifested in patients with various MDs, and why chorea is the most common form of MD should be further clarified.

MD associated with MMD had several notable features. First, MMD was present in more than 20% of patients. The previous case reports on MD associated with MMD mainly present individuals from the East Asian population, which confirms the importance of our study for the region (27, 34–36). Second, the bimodal age distribution was characteristic. MD often occurs as a symptom of MMD and is a poor prognostic factor, especially in children and young adults (34, 37, 38). Moreover, a bimodal age distribution pattern is a distinct epidemiological characteristic of the MMD population (39). According to this pattern, patients with MMD were younger than patients with atherosclerosis and all patients in our study. Third, MD associated with MMD had a more chronic onset and less acute lesion compared to MD associated with atherosclerosis. In other words, synaptic plasticity changes in accordance with the changes in hypoxia that occur with age (40). This finding may support our hypothesis that functional neuronal modulation in the basal ganglia caused by CAS is progressive and age-dependent.

To the best of our knowledge, this is the first nationwide study that systematically examines heterogeneous clinical characteristics of MD associated with CAS. Our study has several limitations. First, the clinical data were collected by an MD specialist who reviewed the patient registry at their respective center. Due to the inherent limitation of a retrospective study, the source of patient registries varied across the centers, including those from MD-oriented registries and those from stroke-oriented registries. Patients who had only minimal MD treated by non-MD specialists could have been overlooked, which might affect epidemiological estimates. Second, the evidence for an association between MD and CAS was based solely on clinical judgment. Since there is no definitive diagnostic work to ascertain the causality of CAS with MD, we have developed clinical criteria ourselves to determine this association. Third, although patients who underwent surgical treatment for CAS showed a good prognosis of MD, this study would not be sufficient to provide recommendations on the treatment guidelines for CAS-associated MD due to its retrospective design. Further prospective studies are warranted.

In conclusion, this study analyzed the nature of CAS associated with MD on a nationwide scale. Progressive, ongoing functional neuronal modulation in the basal ganglia due to CAS may lead to MD. Further studies should focus on the

epidemiology of these clinical entities and the identification of the pathogenic mechanism underlying the heterogeneity of CAS-associated MD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Asan Medical Center, Chungnam National University Hospital, Seoul National University Hospital, Gangnam Severance Hospital, Seoul National University-Seoul Metropolitan Government Boramae Medical Center, Pusan National University Yangsan Hospital, Korea University College of Medicine Guro Hospital, Gachon University Gil Medical Center, Samsung Medical Center, Ulsan University Hospital, Haeundae Paik Hospital, Chung-Ang University College of Medicine, Kyung-Hee University College of Medicine, Kyungpook National University Hospital, Dongsan Medical Center, and Chonnam National University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KP and NC contributed to the conceptualization, data curation, formal analysis, investigation, visualization, and writing of the original draft. MB, DY, J-HC, and SL contributed to the data curation, investigation, and visualization. EO, CL, H-JK, J-YL, JL, S-BK, YS, JC, H-JY, JP, H-WS, T-BA, H-SR, SY, and S-MC contributed to the data curation, methodology, supervision, and review and editing of the original draft. BK contributed to the review and editing of the original draft. SC contributed to the conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, supervision, and review and editing of the original draft. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (HI19C0256).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.939823/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPINION
published: 14 July 2022
doi: 10.3389/fneur.2022.938686

On Disease Modifying and Neuroprotective Treatments for Parkinson's Disease: Physical Exercise

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Keywords: Parkinson's disease, disease modifying agents, neuroprotection, physical activity, preventive treatment

INTRODUCTION

For decades, the concept of "disease-modifying" treatment has been used profusely in several neurodegenerative diseases including Parkinson's disease (PD). This concept has been employed sometimes as an accomplished goal, and many more as a wishful thinking objective. In addition, the related concept of neuroprotective therapy has been widely used and a quick search on Medline shows a steady increase in the number of papers on this issue over time.

As Morant et al. (1) pointed out there is a particular interest in conceptually distinguishing disease-modifying treatments from symptomatic-only treatments.

Perhaps it is time to ponder over these two related concepts: disease modifying and neuroprotective therapies, at least in relation to PD. First, it is important to have (more or less) clear definitions, and then, we can discuss whether we have or we may have (or not) disease modifying and neuroprotective therapy for PD.

To begin with, the definition of "disease-modifying" treatment varies both within and between neurodegenerative disorders, and terminology in current regulatory guidelines also lacks consistency (1). Cummings suggested that disease modification can be defined as treatments or interventions that affect the underlying pathophysiology and have beneficial outcome on the course of the disease (2). Since the pathophysiology of PD is only partially known, we can employ a more clinical approach for disease-modifying measures as "effective treatments that modify the course of PD and maintain or improve patient quality of life" (3).

Here it must be pointed out that, in theory, any disease-modifying treatment may have symptomatic effect as well, possibly masking the modifications produced in the disease. Recently, Vijiaratnam et al. (3) reviewed the crucial issue of why we have failed to demonstrate disease-modifying effect of treatments on PD. Several reason may partly explain this shortcoming, including the complex pathophysiology and heterogeneity of the disease (3), but from a clinical viewpoint, detecting real modification with any given treatment may take an extended period of time.

To date, no disease-modifying drugs has been found, although some promising candidates are still in the pipeline including exenatide and gene therapy (4).

And still, probably, a real disease-modifying treatment for PD already exists and has been used for decades: Physical exercise. As Eric Ahlskog already suggested a decade ago, "often overlooked (…) is the potential benefit of sustained vigorous exercise on PD progression" (5).

OPEN ACCESS

Edited by:
Maria Fiorella Contarino, Leiden University Medical Center, Netherlands

Reviewed by:
Guillaume Lamotte, The University of Utah, United States

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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: *07 May 2022*
Accepted: *07 June 2022*
Published: *14 July 2022*

Citation:
Garcia Ruiz PJ, Luquin Piudo R and Martinez Castrillo JC (2022) On Disease Modifying and Neuroprotective Treatments for Parkinson's Disease: Physical Exercise. Front. Neurol. 13:938686. doi: 10.3389/fneur.2022.938686

In this short review, we collect and summarize, the most relevant data of physical exercise as a symptomatic and preventive measure, and its potential for disease modifying therapy for patients with PD.

PHYSICAL EXERCISE AS SYMPTOMATIC TREATMENT

Although the role of physical exercise as symptomatic treatment for PD was already suggested in classic texts (6), this non-pharmacological approach attracted renew interest in the 90s; Comella et al. (7) carried out a controlled trial testing physical exercise in a group of moderately advanced PD. They authors found statistically significant difference in the experimental group, but also observed that motor improvement was not sustained once normal activity was resumed (7). Many other clinical trials, reviews and meta-analyses have been published on this issue over the last decades (5, 8–14). One particularly noteworthy example is the randomized controlled trial by Corcos et al. (8) which showed that progressive resistance exercise demonstrated a statistically significant reduction in UPDRS-III scores (8). Schenkman et al. (13) in a recent randomized clinical trial, studied the effect of high-intensity endurance exercise on motor symptoms in *de novo* PD patients. They found statistical differences in Unified Parkinson's Disease Rating Scale (UPSRS) motor score in the high-intensity group compared with the usual care group. Another interesting study was carried out by van der Kolk et al. (14). In a double blind randomized controlled trial, the authors studied the effectiveness of home-based supervised aerobic exercise on PD; The off-state MDS-UPDRS motor score revealed a significant difference in favor of aerobic exercise.

In addition, physical exercise showed potential to increase the efficacy of antiparkinsonian medication (9, 11). Recently, in their excellent overview of physical exercise on PD; Mak et al. suggested that exercise training can modify long term motor symptoms in PD (11). Finally, da Silva et al. (10) carried out a systematic review of physical exercise on cognitive function of PD; they suggested that physical exercise promotes significant effects on global cognitive function, processing speed, sustained attention and mental flexibility in PD patients. Even when used only as symptomatic treatment, physical exercise should be widely considered as a fundamental antiparkinsonian measure (5, 8–14).

PHYSICAL EXERCISE AS A DISEASE-MODIFYING TREATMENT

In addition to having confirmed symptomatic antiparkinsonian effect, physical exercise may attenuate and influence the natural history of PD (5, 15–19). An increasing evidence suggests that vigorous exercise may exert a disease-modifying effect on PD (5, 15). A very recent publication from Japan showed that the maintenance of high physical exercise was clearly associated with better clinical course of PD (16). To date, the published evidence is indirect and based mainly on observational cohort studies and/or meta-analyzes (16–18), but it is worth stressing the inverse dose-response association between the amount of physical exercise with evolution and mortality in PD (17, 18).

PHYSICAL EXERCISE AS A PREVENTIVE MEASURE

If any disease-modifying treatment exits, then it would most likely be useful also as a preventive measure for neurodegenerative diseases including PD. Epidemiologic evidence suggests that physical exercise may protect against PD (20–25). Habitual vigorous exercise in midlife reduced the risk of later-developing PD in several cohorts (20–25). Physical exercise also seems to confer protective effect on different neurodegenerative diseases such as PD, Alzheimers's disease, Huntington's disease and degenerative ataxias (26). According to this data, exercise training would be a practical and inexpensive guide to counsel patients at risk of PD, such as LRRK2 carriers and others.

EXERCISE: MECHANISMS OF ACTION

Physical exercise has been recommended since the times of Hippocrates and Galen as a general measure for health and disease prevention (27), but its mechanism has been completely unknown for centuries. At present, some of the potential mechanisms have been studied both in experimental animal models (28–30), and in patients with neurodegenerative diseases as well as controls (31–34).

Potential mechanisms include neuronal survival and plasticity, neurogenesis, epigenetic modifications, angiogenesis, autophagy, and the synthesis and release of neurotrophins (28–35).

Possibly, the most interesting and testable mechanism includes the release of Brain Derived Neurotrophic Factor (BDNF) (28–34), suffice is to recall that BDNF is a crucial neurotrophic factor with multiple roles on regulation of neurophysiological processes (35), including survival of striatal neurons (36). Physical exercise increases plasma BDNF levels in individuals with neurodegenerative disorders (34); and interestingly, BDNF receptor blockade prevents the beneficial effects of exercise in animal models (29).

CONCLUSION

If physical exercise is symptomatically effective, probably prevents neurodegenerative diseases, and has potential neuroprotective mechanisms, why is it not universally used?

This conundrum may be explained by several factors. There are barriers to exercise (37, 38), as Ellis et al. suggested, low outcome expectation from exercise, lack of time, and fear of falling appear to be important perceived barriers to exercise. In addition, optimal benefit requires active and sustained participation of patients and families (37, 38). Finally, as Alberts commented (12), frequently, exercise recommendations lack specificity in terms of frequency, intensity and duration.

In summary, although physical exercise is inexpensive, its use as a treatment requires a complete change of strategy. Exercise training would be considered the first antiparkinsonian measure, even before (or at least at the same time) than drug therapy is added (12, 38, 39). Changes are not easy to implement, although other medical specialties such as endocrinology have well-designed patient education programs for chronic diseases uch as diabetes. Certainly, a long-term prospective studies are needed to confirm the neuroprotective capacity of physical exercise on PD (40). Ongoing Clinical Trials, (Including SPARX3 and CYCLE-II) Have Potential to Further Develop Patient-Specific Exercise Recommendations (12). Confirming this effect of exercise training would revolutionize the way we treat patients with neurodegenerative diseases, and also would open new avenues of basic and clinical research.

Finally, physical exercise would be a practical and inexpensive approach for those patients at risk for PD (such as LRKK2 carriers).

AUTHOR CONTRIBUTIONS

PG conception and design, interpretation of data, drafting the submitted material, and critical review. RL and JM drafting the submitted material and critical review. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We appreciate the editorial assistance of Dr. Oliver Shaw.

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Conflict of Interest: PG received research support from Allergan and UCB, personal compensation as a consultant/scientific advisory board from Italfarmaco, Britannia, Bial, and Zambon and speaking honoraria from Italfarmaco, Zambon, Allergan, and Abbvie. JM has received honoraria as a speaker from AbbVie, Allergan, Bial, Krka, Merz, Ipsen, Italfarmaco, Medtronic, TEVA, and Zambon; travel grants from AbbVie, Allergan, Bial, Italfarmaco, TEVA, Merz, Krka and Zambón; research grants from AbbVie, Allergan, Merz, Italfarmaco, and Zambon; and participated in advisory boards of AbbVie, Allergan, Bial, Merz, Italfarmaco, Lundbeck, Orion, UCB, and Zambon. RL has received honoraria for lecturing and advisory board from AbbVie, Lundbeck, UCB, and Italfarmaco and research grants from the European Commission and Instituto de Salud Carlos III.

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TYPE Brief Research Report
PUBLISHED 05 August 2022
DOI 10.3389/fneur.2022.927573

OPEN ACCESS

EDITED BY
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University of Turin, Italy

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SPECIALTY SECTION
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

RECEIVED 24 April 2022
ACCEPTED 11 July 2022
PUBLISHED 05 August 2022

CITATION
Martinez-Nunez AE, Sidiropoulos C, Wall J, Schwalb J, Air E, LeWitt P, Bulica B, Kaminski P and Patel N (2022) Adjuvant medical therapy in cervical dystonia after deep brain stimulation: A retrospective analysis. *Front. Neurol.* 13:927573. doi: 10.3389/fneur.2022.927573

Adjuvant medical therapy in cervical dystonia after deep brain stimulation: A retrospective analysis

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Background: There is limited information on optimization of symptomatic management of cervical dystonia (CD) after implantation of pallidal deep brain stimulation (DBS).

Objectives: To describe the long-term, "real-world" management of CD patients after DBS implantation and the role of reintroduction of pharmacologic and botulinum toxin (BoNT) therapy.

Methods: A retrospective analysis of patients with focal cervical or segmental craniocervical dystonia implanted with DBS was conducted.

Results: Nine patients were identified with a mean follow-up of 41.7 ± 15.7 months. All patients continued adjuvant oral medication(s) to optimize symptom control post-operatively. Three stopped BoNT and four reduced BoNT dose by an average of 22%. All patients remained on at least one medication used to treat dystonia post-operatively.

Conclusion: Optimal symptom control was achieved with DBS combined with either BoNT and/or medication. We suggest utilization of adjuvant therapies such as BoNT and/or medications if DBS monotherapy does not achieve optimal symptom control.

KEYWORDS

cervical dystonia, deep brain stimulation, medical therapy, botulinum toxin, long-term follow up

Introduction

Dystonia is defined as "a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both" (1). Cervical dystonia (CD) is the most common form of adult focal dystonia characterized by sustained or intermittent muscle contractions of neck muscles that result in involuntary intermittent or sustained posturing of the head.

CD can be associated with significant disability, pain and reduced quality of life. Though botulinum toxin (BoNT) is the standard of care for treatment of CD, up to one-third of CD patients have suboptimal therapeutic response (2). Development of neutralizing antibodies, short duration of benefit between BoNT injections, side effects and delays between injections often contribute to suboptimal treatment response (3). Medications such as anticholinergics, muscle relaxants and benzodiazepines, as well as physical therapy (4), are often used as adjunctive therapies with variable effectiveness (5). Deep brain stimulation (DBS) has become a safe and effective therapy for management of medically refractory CD (6). There are only a few studies describing the long-term effects of DBS on CD beyond 5 years, with reports of an average of at least 25–50% improvement of motor symptoms of CD (7–9). Though this is a clinically meaningful improvement, many patients continue to struggle with pain and spasms in the neck which are not fully controlled with DBS monotherapy. There is limited information related to strategies for optimizing symptomatic benefit in CD beyond DBS monotherapy for those patients experiencing less symptomatic benefit.

We aim to describe the long-term management of CD patients who underwent DBS at the Henry Ford Hospital Movement Disorders Clinic.

Methods

This is a retrospective chart review of medication-refractory CD patients treated with DBS. Patients with isolated CD that were followed up for at least 1 year postoperatively were identified from the Henry Ford Hospital Movement Disorders Clinic database. Patients with hemidystonia or generalized dystonia were excluded from this analysis. All of our patients underwent asleep surgery with intraoperative magnetic resonance imaging (iMRI) following the stereotactic coordinates and techniques previously described by Starr (10). A monopolar review was performed on all of our patients during the first office visit follow-up. This study was reviewed and approved by the Henry Ford Health System Internal Review Board (IRB). This study is conducted according to the declaration of Helsinki.

Demographic data, preoperative Toronto Western Spasmodic Torticollis Scale (TWSTRS) scores (obtained within 1 year prior to DBS implantation), duration of therapy, final programming parameters, pharmacologic and BoNT treatments before and after DBS surgery were captured. Comparison of different BoNT formulations were converted to onabotulinum toxin A equivalents as based upon published guidelines (11). Data was collected retrospectively from the last follow-up visit at Henry Ford Health System.

Descriptive statistics (central tendency measures, proportions) were used to describe demographics, predominant CD phenomenology, motor evaluations, stimulation parameters, and use of adjuvant medication. For comparison of means, we initially ran a normality test (Kolmogorov-Smirnov) to decide whether to use a parametric test (student t-test) or a non-parametric test (Mann-Whitney U test). When comparing two dichotomous variables we calculated an odds ratio (OR) and used the chi-square test to determine independence between categorical polychotomous variables.

TABLE 1 Characteristics of dystonia.

Female	5 of 9 (56%)
Duration of follow up	41.67 ± 15.7 months
Age at onset of disease	46.56 ± 8.2 years
Age at the time of DBS implantation (mean ± standard deviation)	55.8 ± 10.8 years
Duration of CD prior to DBS (mean ± standard deviation)	9.4 ± 8.2 years
Primary direction of dystonic movement	
Laterocollis	5 of 9 (56%)
Torticollis	4 of 9 (44%)
Patients that continue to receive BoNT injections	6 of 9 (67%)

Results

Of the 975 patients with CD in Henry Ford Movement Disorder's clinic database (from January 1, 2014 to April 1, 2020), 11 patients underwent DBS. Two patients were excluded due to their lack of follow-up after the 1st year of surgery. Of the remaining 9 patients, all were implanted with bilateral DBS targeting the globus pallidus internus (GPi). Clinical features of CD are summarized in Table 1. All of our patients were on some form of adjuvant medication and received BoNT injections in cervical muscles pre-operatively, and two of our patients received facial muscles injections for blepharospasm.

All of our patients required continuation of adjuvant therapies in combination with DBS to attain satisfactory control of their dystonia symptoms post-operatively (Table 2). Six patients continued to receive BoNT injections, 4 remained on anticholinergic medications, 3 on muscle relaxants, and 7 on benzodiazepines. Six of the patients were able to reduce their adjuvant therapies post-operatively, and 7 patients were able to reduce BoNT injections (an average dose reduction of 22%, 75.8 ± 14.2 units of onabotulinum toxin A equivalents), of which 3 patients completely stopped their use. The two patients with blepharospasm were able to cease BoNT injections. There was a significant decrease in the mean number of muscles that were injected per BoNT injection session after DBS implantation (8.4 ± 1.5 vs. 6.1 ± 0.7), $p = 0.006$).

A comparative analysis of patients who stopped or decreased BoNT ($n = 7$) vs. patients who kept requiring similar BoNT doses ($n = 2$) was performed. Those patients who were able

TABLE 2 Individual description of each of our patients including disease phenotype, stimulation parameters and adjuvant medication.

Patient	Age at time of surgery (years)	Duration of disease prior to surgery (years)	Follow up after surgery (months)	Dystonia topographic distribution and predominant direction of dystonic pull	Preoperative TWSTRS				Psychiatric comorbidities	DBS settings				Side-effects from stimulation	Adjuvant medication prior to surgery	Adjuvant medication at the last time of follow up
					Total	Motor	Disability	Pain		Lead/ polarity	Amplitude (V)	Pulse width (us)	Frequency (Hz)			
1	59	9	25	Focal / Right laterocollis	-	-	-	-	MDD	Left GPi: C+2–3- Right GPi: C+10–11-	4 4	210 210	130 130	None	Alprazolam 1 mg BID Onabotulinum toxin A (300 U)	Alprazolam 1 mg QID Onabotulinum toxin A (300 U)
2	64	30	56	Focal / Left laterocollis	28	9	16	3	GAD	Left GPi: C+2- Right GPi: C+10-	1.8 2.3	90 90	160 160	Acral dysesthesias	Clonazepam 2 mg TID Onabotulinum toxin A (300 U)	Lorazepam 2 mg QID
3	34	7	51	Segmental (CD and BS) / Right laterocollis	-	-	-	-	ADHD	Left GPi: C+0–1- Right GPi: C+8–9-	4.7 4.6	60 90	130 130	Right arm/ hand cramping and spasms and right foot curling	Trihexyphenidyl 2 mg TID Baclofen 10 mg BID Diazepam 5 mg TID Onabotulinum toxin A (325 U)	Trihexyphenidyl 2 mg TID Baclofen 10 mg qd Onabotulinum toxin A (200 U)
4	59	6	49	Focal / Left laterocollis	39	11	12	16	GAD	Left GPi: C+0–1- Right GPi: C+8–9-	3.6 3.6	180 180	140 140	Blepharospasm	Clonazepam 0.5 mg BID Onabotulinum toxin A (175 U)	Clonazepam 0.5 mg BID Onabotulinum toxin A (255 U)
5	40	11	49	Focal / Left laterocollis and retrocollis	54	24	16	14	None	Left GPi: C+0- Right GPi: C+8-	3 3.3	60 90	130 130	None	Trihexyphenidyl 2 mg TID Rimabotulinum toxin B (17500 U)	Trihexyphenidyl 2 mg TID Baclofen 20 mg TID Diazepam 2 mg TID Onabotulinum toxin A (380 U)
6	64	9	49	Focal / Left torticollis and laterocollis	16	4	4	8	GAD	Left GPi: C+0- Right GPi: C+8-	2.5 2.5	60 60	125 125	None	Trihexyphenidyl 2 MG TID Diazepam 10 mg BID Onabotulinum toxin A (300 U)	Diazepam 10 mg TID

(Continued)

10.3389/fneur.2022.927573

TABLE 2 Continued

Patient	Age at time of surgery (years)	Duration of disease prior to surgery (years)	Follow up after surgery (months)	Dystonia topographic distribution and predominant direction of dystonic pull	Preoperative TWSTRS				Psychiatric comorbidities	DBS settings					Side-effects from stimulation	Adjuvant medication prior to surgery	Adjuvant medication at the last time of follow up
					Total	Motor	Disability	Pain		Lead/ polarity	Amplitude (V)	Pulse width (us)	Frequency (Hz)				
7	53	2	48	Focal / Right torticollis	35	16	11	8	None	Left GPi: C+1- Right GPi: C+9-	3 3	90 90	125 125	None	Trihexyphenidyl 2 mg TID Baclofen 10 mg BID Diazepam 5 mg TID Onabotulinum toxin A (500 U)	Baclofen 10 mg TID Diazepam 5 mg TID Onabotulinum toxin A (500 U)
8	65	3	41	Focal / Right torticollis	31	13	11	7	None	Left GPi: C+1–2- Right GPi: C+9–10-	3 2.6	90 80	130 130	None	Clonazepam 1 mg BID Onabotulinum toxin A (300 U)	Trihexyphenidyl 2 mg TID Onabotulinum toxin A (400 U)
9	65	8	7	Segmental (CD and BS) / Left laterocollis	-	-	-	-	GAD	Left GPi: C+ 3- Right GPi: C+ 2–3-	3.3 3.2	60 60	130 130	None	Trihexyphenidyl 2 mg TID Lorazepam 0.5 mg TID Onabotulinum toxin A (300 U)	Trihexyphenidyl 2 mg TID Lorazepam 0.5 mg TID
Mean	55.89 (±10.83)	9.44 (±8.23)	41.67 (±15.7)	-	33.83 (±16.92)	12.83 (±6.79)	11.67 (±4.41)	9.33 (±4.8)	-	-	3.22 (±0.77)	92.78 (±37.07)	133.33 (±10.57)	-	-	-

TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale; CD, cervical dystonia; BS, blepharospasm; U, units; BID, twice a day; TID, three times a day; QID, four times a day; qd, every day; GPi, globus pallidus pars interna; GAD, generalized anxiety disorder; ADHD, attention-deficit hyperactivity disorder. Standard deviations are in parenthesis.

10.3389/fneur.2022.927573

to decrease or stop BoNT had a longer duration of dystonia symptoms compared to those who remained on similar doses to the pre-operative treatment plan (11.4 ± 8.3 years vs. 2.5 ± 0.7 years, $p = 0.03$).

The average time from implantation to optimization of DBS settings was 11.8 ± 1.7 months. There was no difference in the use of adjuvant medication between patients with or without psychiatric comorbidities (OR = 0.5, CI: 95% −0.3–8.9). Patient 2 and 3 both experienced stimulation induced side effects, localization of the DBS electrodes demonstrated appropriate lead location.

Discussion

Since the first uses of DBS for CD appeared in the medical literature in 2002, the reported individual responses have been varied (9, 12). Though some patients achieve optimal symptomatic control with DBS monotherapy these findings and our clinical experience suggest that many patients do not. Thus warranting the consideration of adjuvant therapies to optimize symptom control. To our knowledge this study is the first to report the "real-world" long-term management of focal and segmental CD patients with bilateral pallidal DBS in patients previously treated with BoTN therapy. Despite achieving clinically meaningful benefit from DBS therapy, each of our patients continued to require at least one adjuvant therapy to optimize symptom control. DBS facilitated the opportunity to reduce the botulinum toxin dose and/or eliminate some of the muscles previously injected while achieving better symptomatic improvement than pharmacological or BoNT therapy. Of note, most of our patients continue to require benzodiazepines for control of their dystonic symptoms, although comorbid generalized anxiety disorder is another factor that could have favored the ongoing use of this medication class.

Similar to our experience, Yamada et al. reported that eight patients in their cohort also continued adjuvant pharmacological therapies post-operatively, apart from one patient who did not receive medications preoperatively (8). However, in this study the authors did not comment on the use of BoNT pre- or post-operatively. In a prospective study of long-term outcomes with pallidal DBS in all types of dystonia, Krause et al. reported that 42% of their patients were able to reduce or stop their medication. Of the 4 patients in this cohort who received BoNT pre-operatively 3 were able to discontinue therapy and 1 was able to reduce the dose BoNT at last follow-up (13). However, the sub-type of dystonia in relation to the use of medication and BoNT was not reported. Similar to our findings, there was a mean delay of 11.8 months for patients to achieve optimal symptomatic benefit with DBS with similar final stimulation parameters (14). In our study long pulse widths were not found to achieve better symptomatic control in our group of CD patients, as reported by others (15).

A surprising result in this study was the correlation between a longer duration of disease pre-operatively and a larger reduction in BoNT dose used post-operatively. This is counterintuitive to reports of longer disease duration impacting the efficacy of DBS in CD (8). Our findings could be attributed to the small sample size and should be interpreted with caution.

There are several limitations to this analysis. This study analyzed an established cohort retrospectively who were managed by five different movement disorder specialists working in a group practice (PL, CS, BB, NP) and two neurosurgeons who implanted DBS (JS, EA). Management of stimulation parameters and adjuvant medications are not standardized between practitioners. Post-operative imaging to confirm lead location was not routinely performed though in the experience of the programming neurologist(s) the effect and side effect profiles suggested appropriate location. Additionally, standardized evaluations of CD were not completed routinely in follow-up which limited our ability to report motor outcomes in our cohort. Given our relatively small sample size, some of the comparisons that were performed were underpowered to demonstrate a difference. Larger prospective studies assessing the role of adjuvant therapies in the care of CD with DBS is recommended.

In this study we report the long-term outcomes of a relatively large cohort of CD patients treated with DBS and the role of adjuvant therapies to optimize symptom control. We recommend considering continuation of adjuvant therapies such as BoNT and medications for those patients whose symptoms are not optimally controlled with DBS monotherapy, especially during the early post-operative period when patient stimulation is not optimized.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Henry Ford Health System Internal Review Board (IRB). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AM-N and NP: conception and design of the study, acquisition of data, analysis, and interpretation of data. AM-N, CS, JW, and NP: drafting the article and revising it critically for

important intellectual content. AM-N, CS, JW, JS, EA, PL, BB, PK, and NP: final approval of the version to be submitted. All authors have approved the final version of this article.

Conflict of interest

Author CS has served on the Scientific Advisory Board of Abbvie, Acorda and Boston Scientific, has served as a consultant for Medtronic and Global Kinetics, and received research grants from Abbvie, Biohaven, and Michigan State University. Author JS receives honoraria from BCBSM for his role as a co-Director of MSSIC, research funding from Medtronic, SetPoint and Neuros, and compensation from NPA for his role on the Steering Committee for RAD-PD. Author EA is a consultant for Stryker, Inc and Functional Neuromodulation, Ltd. Author PL has served as a consultant or investigator in clinical trials sponsored by Acorda Therapeutics, Amneal, Appello, Axovant, Aptynix, Biogen, Biotie, Bukwang Pharmaceuticals, Cavion, Cerevel, Denali Therapeutics, F. Hoffmann LaRoche, Impax Laboratories Inc., Impel Neuropharma, Ipsen, Kyowa Kirin Hakko, Lundbeck A/S, The Michael J. Fox Foundation for Parkinson's Research, Neurocrine Biosciences, Pharma 2B, Mitsubishi Tanabe Neuropharma, Neurocrine Biosciences, NeuroDerm Ltd, Noven, Parkinson Study Group, Prexton Therapeutics, Revance, US WorldMeds, Saccadous, Sun Pharma, and Supernus, Revance Therapeutics, Saccadous, Supernus, and US WorldMeds. He has received speaker honoraria from Acorda Therapeutics, Britannia, the American Academy of Neurology, The International Parkinson's Disease and Movement Disorders Society, Kyowa Kirin Hakko, Neurocrine Biosciences, Pallidan Labs, US WorldMeds, and the World Parkinson Congress. He is compensated for services as editor-in-chief of Clinical Neuropharmacology and serves without compensation on the editorial boards of Journal of Neural Transmission, Translational Neurodegeneration, and Journal of Parkinson's Disease. Author BB has received honoraria as a consultant to Lundbeck and as a speaker for Neurocrine Biosciences. Author NP has received honoraria as a consultant to Acorda Pharmaceuticals, Revance Pharmaceutical and Abbott Laboratories, and as a speaker for Teva Pharmaceuticals.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Neurology

TYPE Review
PUBLISHED 24 August 2022
DOI 10.3389/fneur.2022.912818

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

RECEIVED 04 April 2022
ACCEPTED 26 July 2022
PUBLISHED 24 August 2022

CITATION
Kaji R (2022) Direct cerebello-striatal loop in dystonia as a possible new target for deep brain stimulation: A revised view of subcortical pathways involved. *Front. Neurol.* 13:912818. doi: 10.3389/fneur.2022.912818

Direct cerebello-striatal loop in dystonia as a possible new target for deep brain stimulation: A revised view of subcortical pathways involved

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Dystonia is the second most common movement disorder next to tremor, but its pathophysiology remains unsettled. Its therapeutic measures include anti-cholingerics and other medications, in addition to botulinum neurotoxin injections, and stereotaxic surgery including deep brain stimulation (DBS), but there still remain a number of patients resistant to the therapy. Evidence has been accumulating suggesting that basal ganglia in association with the cerebellum are playing a pivotal role in pathogenesis. Clinical observations such as sensory tricks and the effects of muscle afferent stimulation and blockage suggest the conflict between the cortical voluntary motor plan and the subcortical motor program or *motor subroutine* controlling the intended action semi-automatically. In this review, the current understanding of the possible pathways or loops involved in dystonia is presented, and we review promising new targets for Deep Brain Stimulation (DBS) including the cerebellum.

KEYWORDS

dystonia, tonic vibration reflex, subcortical pathway, deep brain stimulation, cerebellum, target

Introduction

Dystonia is a syndrome in which sustained or repetitive muscle contractions result in twisting and repetitive movements or abnormal fixed postures (1). Usually, those muscle activities are uncontrollable by the subject and classified as an involuntary movement (2). Focal dystonias such as writer's cramp usually affect writing, but not other tasks. Abnormal contractions of muscles start as soon as the subject intend to write, and occur both in agonists (e.g., wrist flexor) and antagonists (e.g., wrist extensor), resulting in freezing of the joint (*co-contraction*), or reciprocally in those muscles (dystonic tremor), in an unintended manner. Distant muscles unnecessary for the task may also be activated (*motor overflow*). There is a discrepancy between the intended motor plan and the resultant movements.

Task specificity and sensory trick

Tasks affected by these focal dystonias are usually performed automatically; writing, playing musical instruments, using a putter in golfing, and so on. The modalities of these tasks are obtained by intensive training with or without psychological stress. Cervical dystonia and blepharospasm are the most prevalent dystonias, and they can be regarded as derangement of head control and blinking, which are acquired after birth (3). These task specificities may be lost as the disease progresses, and the symptoms may spread over other parts of the body. Thus, dystonia is a disorder of motor programs or *subroutines* to perform semi-automatic or fully learned motor acts (3). This of course is entirely different from "supervised" motor learning process of fine control of the limbs, such as pulling a thread through a pin needle hole, which usually requires the subject's highest attention. The latter is presumably controlled by the cortico-cerebellar system (4). The pathway activated in fully learned motor tasks affected in dystonia is classified as "reinforced" learning circuit through the basal ganglia through dopaminergic system (4). As the motor skill and efficiency improve, the shift from supervised to reinforced learning with more involvement of the basal ganglia occurs (5).

Another peculiar characteristic of dystonia is a phenomenon of symptomatic improvements with the aid of sensory input to a particular part of the body when performing the task (*sensory trick*) (6, 7). For instance, touching a part of the face with the subject's hand may straighten the neck in cervical dystonia. This raised a question of stimulating the muscle spindle afferents by a tonic vibration reflex (TVR) maneuver, or blocking them by intramuscular injection of diluted lidocaine, which is known to block gamma-efferents to the spindles selectively in dystonia patients (8). Interestingly the dystonic movements were reproduced by TVR, and abated by muscle afferent block [see videos attached to (8)]. The reflex is spared in extensive cortical lesions in stroke (9). It is therefore concluded that the neural pathways involved in dystonia is mainly subcortical, and there seems to be a conflict between cortical voluntary motor command and the abnormal output from the subcortical structures. Compensatory mechanisms may be possible at the cortical level, as exemplified by the phenomenon of sensory trick, whereby the subject can find a compromise between the two systems.

Based upon the above study of TVR and muscle afferent block, it was proposed that dystonia is a sensory disorder (10). A number of the subsequent studies have explored the abnormalities of the tactile sensory discrimination of the affected or unaffected limbs in dystonia, deciphering the primary somatosensory cortex as the site of primary lesion, but they failed to show conclusive evidence whether the changes are primary or not (11–13). Temporal tactile discrimination, which mirrors the function of somatosensory cortex, was found abnormal even in psychogenic dystonia (14).

Pathophsiology of dystonia

The exact pathology involved in dystonia is still an open question. Traditionally it is regarded as a basal ganglia disorder, since hemi-dystonia is a consequence of contralateral basal ganglia lesions (15), and remarkable histopathological loss of striosome compartment in the striatum is found in dystonic phase of X-linked dystonia-parkinsonism (XDP) (16, 17). Accumulating evidence on the other hand suggests the cerebellum in association with the striatum causing dystonia (18). An autopsy study in a hereditary case of pure dystonia demonstrated exclusive cerebellar atrophy and loss of Purkinje cells in the anterior lobe (19). Dystonia is often a presenting symptom in spinocerebellar atrophies such as SCA6 (20, 21). Of course, secondary involvement of these structures is possible, despite the lack of visibility of the primary lesion. Rare autopsy cases of primary cervical dystonia revealed patchy loss of Purkinje cells, also pointing to the cerebellum as a site of lesion (22), whereas most of the cases of idiopathic dystonia lack such pathology, indicating abnormal synaptic plasticity being the plausible cause.

Classical model of basal ganglia circuit

More than 3 decades ago, Alexander and Crutcher presented a "push-pull" model of basal ganglia circuit which nicely explains hypokinetic and hyperkinetic disorders such as Parkinson's disease and dystonia (23). This model is still useful in understanding dopamine excess causing hyperkinetic states such as dopa-induced dyskinesia and dystonia. There are *direct* or cortico-striato-GPi pathways that exerts excitatory feedback to the cortex (mainly premotor area or PM), and indirect or cortico-striato-GPe(STN)-GPi pathway which feeds back inhibitory background as a surround inhibition (Figure 1A). The net result would be focusing the muscles to be activated for performing tasks. In dopamine deficiency such as in Parkinson's disease, there exists more indirect pathway activity because of the lack of dopamine disinhibits medium spiny neurons (MSNs) in the striatum through D2 receptors. MSNs in the direct pathway on the contrary are inhibited through the D1 receptor. The paucity and slowness of movements (*akinesia* and *bradykinesia*) are the consequences. Dystonia is explained by the excess of dopamine, which favors a direct pathway, which activates muscles unnecessary for performing tasks, as in the case of co-contractions.

Revised model of the basal ganglia

If the dopamine excess is the cause of dystonia, there remain conditions that are unexplained by the classical model; tardive dystonia and dopa-responsive dystonia. As most of the anti-psychotic drugs are termed atypical, causing less tardive syndrome, there still remain a large number of psychiatric patients who suffer from dystonia while using dopaminergic antagonists (24). Of course, many of them start their symptoms after reducing the dose of drugs or even stopping them, which can be explained by the super-sensitivity of the receptors after removing the blockage. Another question is dopa-responsive dystonia, which dramatically benefits from dopa administrations.

The only known pathological finding of dystonia is probably that of X-linked dystonia-parkinsonism (XDP) (16). XDP is a biphasic disease, endemic in the Panai Island of the Philippines, starting with dystonia, which gradually proceeds to Parkinsonism. At the dystonic stage, it typically presents with focal dystonia involving the jaw then generalized to the trunk and the lower limbs. MRI finding of the brain at this stage shows spot-like lesions in the putamen (16, 17, 25). Those afflicted by this condition tend to die by suicide, and autopsy findings of pure dystonia are a reality (16). The striatum consists of two compartments immunohistochemically; *striosome* and *matrix*. The lesions seen on MRI turned out to be exclusively striosome, since all the remaining MSNs are of matrix. Striosome has inputs from the limbic cortex and is related to reward-oriented control of movements (26, 27). There are dopaminergic projections from Substantia Nigra pars compacta (SNc) to the matrix as well as their axonal collaterals to striosomal MSNs with excitatory D1 receptors, which in turn send its GABAergic inhibitory axons back to nigral dopaminergic neurons, thus forming a feed-back control loop of dopamine release to the striatum (Figure 1B) (16, 25, 28). If striosomal MSNs are depleted,

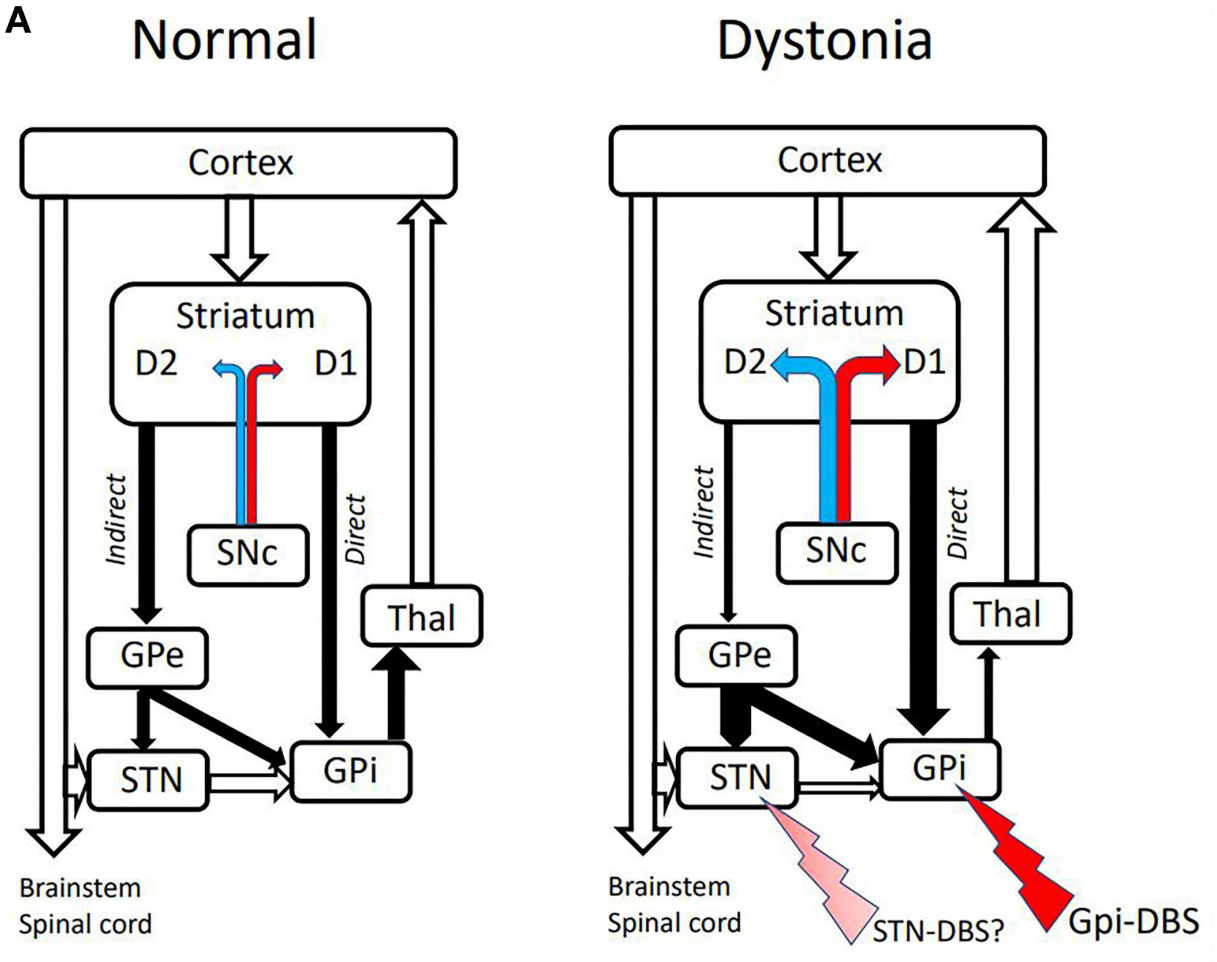

FIGURE 1
Continued

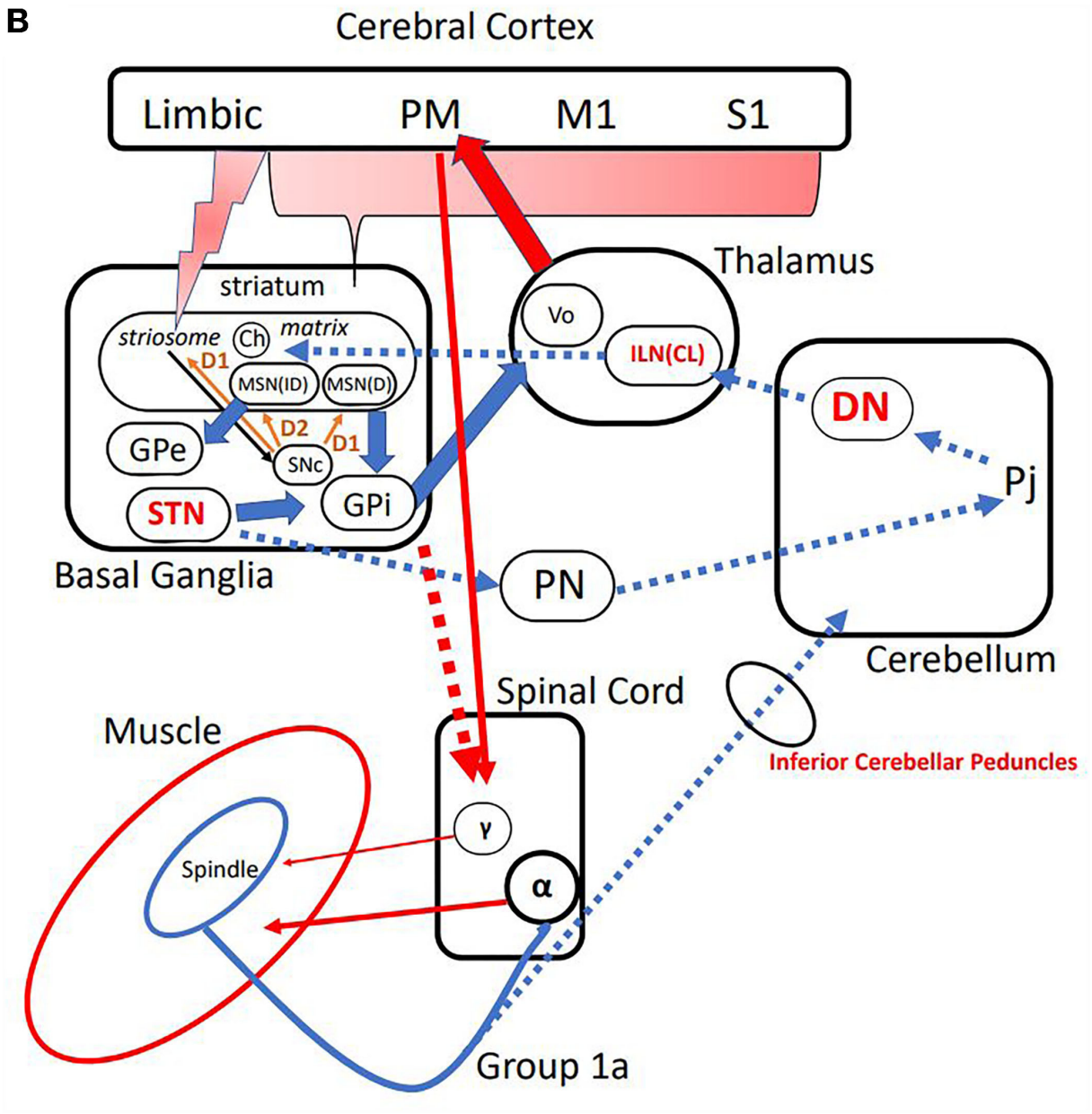

FIGURE 1
(A) Classical model of basal ganglia [Alexander and Crutcher (23)]. Left: Normal condition depicting "excitatory" direct and "inhibitory" indirect pathways. The majority is the inhibitory indirect path, which constitutes "surround inhibition" around the direct path activity of allowing activation of selected muscles. Open arrows are excitatory glutamatergic, and closed arrows are inhibitory GABAergic projections. Dopaminergic projections terminate on medium spiny neurons (MSNs) with excitatory D1 receptor on the direct, and inhibitory D2 receptor on the indirect pathways. Right: Suggested model in dystonia. Relative excess of dopamine from SNc produces direct pathway predominance, and disintegrates surround inhibition. Putative sites of action of DBS are shown with red arrows. SNc, Substantia Nigra pars compacta; GPe, Globus Pallidus externus; STN, Subthalamic Nucleus; GPi, Globus Pallidus internus; Thal, Thalamus. **(B)** Pathways proposed in the pathogenesis of dystonia and TVR-induced dystonic movements. Cerebral Cortex: PM premotor area, M1 primary motor area, S1 primary somatosensory area. Basal Ganglia: Ch cholinergic interneurons, MSN(D) medium spiny neuron in direct pathway, MSN(ID) medium spiny neuron in indirect pathway. Thalamus: Vo thalamic ventral-oralis complex, ILN(CL) intralaminar nuclei in primates or centro-lateral nucleus in rodents. Cerebellum: DN dentate nucleus, Pj Purkinje cells. PN, pontine nuclei. Spinal Cord: α-α motoneuron, γ-γ motoneuron. Broken arrows are putative pathway mediating TVR-induced movements, and possible targets are shown in red.

the control of dopamine content would be deranged, so that relative dopamine excess might result in direct pathway preponderance, causing dystonia. In a model of dopa-responsive dystonia, it was found that tyrosine hydroxylase (TH) content was more depleted in the striosome, accounting for the imbalance between the compartments causing dystonia (29).

TABLE 1 Key references in exploring pathophysiology of dystonia.

Anatomical basis

Marsden CD, Obeso JA, Zarranz JJ, Lang AE. The anatomical basis of symptomatic hemidystonia. *Brain.* (1985) 108(Pt 2):463–83. doi: 10.1093/brain/108.2.463

Model of basal ganglia circuits

Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci.* (1990) 13:266–71. doi: 10.1016/0166-2236(90)90107-L

Sensory aspects in dystonia

Kaji R, Rothwell JC, Katayama M, Ikeda T, Kubori T, Kohara N, et al. Tonic vibration reflex and muscle afferent block in writer's cramp. *Ann Neurol.* (1995) 38:155–62. doi: 10.1002/ana.410380206

Hallett M. Is dystonia a sensory disorder? *Ann Neurol.* (1995) 38:139–40. doi: 10.1002/ana.410380203

Somatosensory cortex

Bara-Jimenez W, Catalan MJ, Hallett M, Gerloff C. Abnormal somatosensory homunculus in dystonia of the hand. *Ann Neurol.* (1998) 44:828–31. doi: 10.1002/ana.410440520

Deep brain stimulation in dystonia

Vercueil L, Pollak P, Fraix V, Caputo E, Moro E, Benazzouz A, et al. Deep brain stimulation in the treatment of severe dystonia. *J Neurol.* (2001) 248:695–700. doi: 10.1007/s004150170116

Dystonia pathology in X-linked dystonia-Parkinsonism

Goto S, Lee LV, Munoz EL, Tooyama I, Tamiya G, Makino S, et al. Functional anatomy of the basal ganglia in X-linked recessive dystonia-Parkinsonism. *Ann Neurol.* (2005) 58:7–17. doi: 10.1002/ana.20513

Cerebellar involvement

Neychev VK, Fan X, Mitev VI, Hess EJ, Jinnah HA. The basal ganglia and cerebellum interact in the expression of dystonic movement. *Brain.* (2008) 131:2499–509. doi: 10.1093/brain/awn168

Direct cerebello-striatal projection in dystonia model

Chen CH, Fremont R, Arteaga-Bracho EE, Khodakhah K. Short latency cerebellar modulation of the basal ganglia. *Nat Neurosci.* (2014) 17:1767–75. doi: 10.1038/nn.3868

Subcortical loop

Kaji R, Bhatia K, Graybiel AM. Pathogenesis of dystonia: is it of cerebellar or basal ganglia origin? *J Neurol Neurosurg Psychiatry.* (2018) 89:488–92. doi: 10.1136/jnnp-2017-316250

Compartmental imbalance has also been implicated in tardive dystonia (24).

Direct connections between the basal ganglia and the cerebellum

Dystonic movements are subconscious since any volitional efforts to correct them are not possible except for the *sensory trick* maneuver. This is in contrast with *tic*, where sensory symptoms to urge movements are usually perceived by the subject, who could volitionally control the movements albeit momentarily. As mentioned above, activation of muscle afferents by high-frequency vibratory stimulation (TVR) could reproduce the dystonic movements, apart from the subject's intention. Patients with pure cerebellar pathology could present with dystonia. It is therefore reasonable to assume a subcortical circuit mediating TVR-induced dystonic movements (9), possibly including the cerebellum, where muscle spindle afferents are utilized as kinesthetic control (30).

Contributions from the basal ganglia and the cerebellum to the genesis of dystonia have been discussed in association with the cerebral cortex as separate loops (Table 1) (17, 18, 31). There has been however ample anatomical evidence showing direct di-synaptic connections between the cerebellum and the striatum or the subthalamic nucleus (32–35). Using the rabies virus as a probe, Hoshi et al. found that the striatum has a di-synaptic input from the dentate through the intralaminar nucleus (CL) of the thalamus (32). Conversely, the subthalamic nucleus (STN) was shown to have di-synaptic output to the cerebellar cortex *via* pontine nuclei (34). Chen et al. confirmed short-latency (∼10 ms) cerebellar modulation of the basal ganglia between the dentate nucleus and the striatum in normal and dystonia model mouse (36). More importantly, they found that high-frequency stimulation of the cortex alone produced long-term depression (LTD), while the concurrent stimulation of the cerebral cortex and the cerebellum produced long-term potentiation (LTP) at the cortico-striatal synapses, providing the direct evidence of cerebellar inputs to the striatum modulating its neuroplasticity. They also explored the pathway in a mouse model of dystonia and found that

the aberrant high-frequency inputs from the cerebellum set the cortico-striatal synapse to favoring abnormal LTP. Severing the link from the cerebellum by silencing the thalamic nuclei abolished the dystonic symptoms. These findings are relevant to the pathogenesis and treatment of dystonia since abnormal LTP or its depotentiation at the cortico-striatal synapses have been shown in a prototypic mouse model of dopa-induced dyskinesia (37) and humans with DYT1 dystonia (38, 39). The termination of the cerebelo-thalamo-striatal projection seems to be cholinergic interneurons, which are located at the border of striosome and matrix compartments, and upregulate dopamine release in the striatum *via* both nicotinic and muscarinic receptors (40, 41). It follows that dystonia can be treated with anti-cholinergics, such as trihexyphenidyl (42), and is aggravated by nicotine (43).

New targets for deep brain stimulation

Targets for deep brain stimulation (DBS) for dystonia have been evolving around internal Globus Pallidum (GPi) and the subthalamic nucleus (STN). The rationale for GPi-DBS is to inhibit the direct pathway through increasing the GABAergic output from GPi to Vo thalamus, which in turn decreases the thalamo-cortical excitatory projections to the premotor area, which is known to be hyper-excitable in dystonia (44). However, STN-DBS in dystonia is less clear. In Parkinson's disease, it is expected to reduce the hyperactive indirect pathway, since STN is located in the indirect pathway, where stimulation is supposed to apply presynaptic inhibition to STN. The stimulation could reduce the ratio of indirect/direct pathways, thus improving the akinesia and bradykinesia on its own and, as a consequence, reduce the doses of anti-Parkinsonian medications. Drug-induced dystonia or dyskinesia can be improved through decreased medication.

It is also known that STN-DBS is equally effective in treating idiopathic and hereditary dystonias compared to GPi-DBS (45–47), although the stimulation parameters could differ from those in Parkinson's disease (48). The rationale for this target is not clearly explained, asin dystonia direct pathway predominance must be met with increasing indirect pathway including STN. It is therefore conceivable that stimulation at STN in dystonia could be excitatory to STN neurons in contrast to the inhibitory nature in Parkinson's. There is also a possibility that STN modulation could affect the direct di-synaptic STN-cerebellar pathway (34).

As discussed, the dentate nucleus or thalamic intralaminar nuclei are promising new targets in the light of their capability of affecting striatal neuroplasticity. In fact, dentate DBS has been reported with preliminary results (49–51). Intralaminar nuclei of the thalamus are also considered as a candidate (52). As in muscle afferent block using diluted lidocaine, the input to the cerebellum through the inferior cerebellar peduncles may be functionally manipulated, although direct evidence is lacking. The stimulation parameters and the precise location in these targets are yet to be determined.

Conclusion

The pathways mediating abnormal motor outputs in dystonia is still undetermined. The classical "push-pull" model of Alexander-Crutcher is still useful, but many revisions must be made considering new clinical and therapeutic features of dystonia or its animal models. More clinical and animal studies searching new and unexplored targets including subcortical cerebello-thalamo-striatal or STN-ponto-cerebellar pathways are needed for better understanding and optimal treatment of dystonia.

Author contributions

RK wrote and reviewed the manuscript.

Funding

This work was funded by an unlimited research grant from Kyowa-Kirin Co. Ltd., Japan (KKCS2020531033) which also covered the publication fee. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Acknowledgments

Prof. Satoshi Goto gave helpful advice.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 26 September 2022
DOI 10.3389/fneur.2022.917187

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

RECEIVED 10 April 2022
ACCEPTED 29 August 2022
PUBLISHED 26 September 2022

CITATION
Kelemen A, Halász L, Muthuraman M, Erőss L, Barsi P, Zádori D, Laczó B, Kis D, Klivényi P, Fekete G, Bognár L, Bereczki D and Tamás G (2022) Clinical parameters predict the effect of bilateral subthalamic stimulation on dynamic balance parameters during gait in Parkinson's disease. *Front. Neurol.* 13:917187. doi: 10.3389/fneur.2022.917187

Clinical parameters predict the effect of bilateral subthalamic stimulation on dynamic balance parameters during gait in Parkinson's disease

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We investigated the effect of deep brain stimulation on dynamic balance during gait in Parkinson's disease with motion sensor measurements and predicted their values from disease-related factors. We recruited twenty patients with Parkinson's disease treated with bilateral subthalamic stimulation for at least 12 months and 24 healthy controls. Six monitors with three-dimensional gyroscopes and accelerometers were placed on the chest, the lumbar region, the two wrists, and the shins. Patients performed the instrumented Timed Up and Go test in stimulation OFF, stimulation ON, and right- and left-sided stimulation ON conditions. Gait parameters and dynamic balance parameters such as double support, peak turn velocity, and the trunk's range of motion and velocity in three dimensions were analyzed. Age, disease duration, the time elapsed after implantation, the Hoehn-Yahr stage before and after the operation, the levodopa, and stimulation responsiveness were reported. We individually calculated the distance values of stimulation locations from the subthalamic motor center in three dimensions. Sway values of static balance were collected. We compared the gait parameters in the OFF and stimulation ON states and controls. With cluster analysis and a machine-learning-based multiple regression method, we explored the predictive clinical factors for each dynamic balance parameter (with age as a confounder). The arm movements improved the most among gait parameters due to stimulation and the horizontal and sagittal trunk movements. Double support did not change after switching on the stimulation on the group level and did not differ from control values. Individual changes in double support and horizontal range of trunk motion due to stimulation could be predicted from the most disease-related factors and the severity of the disease; the latter also from the

stimulation-related changes in the static balance parameters. Physiotherapy should focus on double support and horizontal trunk movements when treating patients with subthalamic deep brain stimulation.

KEYWORDS

Parkinson's disease, dynamic balance, ITUG, subthalamic nucleus, deep brain stimulation, double support, gait, sway

Introduction

The effect of subthalamic deep brain stimulation (STN-DBS) on dynamic balance during gait in Parkinson's disease (PD) has not yet been investigated in detail. Dynamic balance during a movement, e.g., while walking, is one component of the complex balance process, in addition to balance during quiet stance, reactive postural adjustment to external perturbations, and anticipatory postural adjustment in preparation for voluntary movements (1).

The dynamic imbalance in PD derives from several elements. First, it depends on the gait abnormalities characteristic of the disease stage. Reductions in step length and gait speed, reduced swinging of the arms, and increased interlimb asymmetry are frequently reported at the early stage, while turning deficits, gait initiation difficulty, and freezing develop at the mild-to-moderate stage, as well as further gait irregularities due to motor fluctuations and dyskinesias at the advanced stage (2). Second, other disease-related factors were shown to influence dynamic balance during walking, such as even subclinical cognitive impairment, executive dysfunction (3), and fear of falling (1). Although levodopa treatment improves gait speed, facilitates step initiation and anticipatory postural adjustment (4), and reduces gait variability (5), it also raises sway during stance in PD (6). Apparent cholinergic dysfunction was revealed in levodopa-resistant gait abnormalities (7). Third, age is an additive risk factor for poor postural control (8).

The positive effect of STN-DBS on balance tends to taper off after the first nine postoperative months (9). Gait parameters also improve in the first 10 months, especially when STN-DBS is combined with levodopa therapy (10, 11), but deteriorate 3 years after the operation (12, 13).

There is a lack of information about the impact of the STN-DBS on dynamic balance during natural walking. The widely used clinical scales do not assess the different features of gait and balance separately in PD (14) for exploring stimulation-related disturbances (15, 16). Posturography (6, 9, 17) or motion sensor studies (18) on DBS either investigated quiet stances in single (6, 9, 19) or dual-task conditions (6, 17). Gait parameters were analyzed separately with scales (9, 20) or motion sensors (16, 21). The Timed Up and Go test complements the gait analysis by detecting turning and postural transitions, and its total duration time is well correlated to fall risk (22). Its advanced version, the Instrumented Timed Up and Go (ITUG) test, utilizes motion sensors and provides sensitive and reliable gait parameters correlating with the Unified Parkinson's Disease Rating Scale (UPDRS) motor scores (23).

Therefore, we aimed to observe the more than 1-year-long bilateral STN-DBS effect on gait and turning parameters by focusing on the dynamic balance. We hypothesized that gait parameters do not improve with stimulation to the normal control level and that there is a relationship between the stimulation-induced changes in dynamic balance and the disease-related clinical parameters and electrode localization. We also assumed that stimulation-induced changes in dynamic balance during gait are not independent of the postural sway during quiet stance and are measured in sensory conflict situations.

Materials and methods

Participants

We recruited 24 patients with PD treated with bilateral STN-DBS and an age-matched group of 24 healthy controls. The Core Assessment Program for Surgical Interventional Therapies for Parkinson's Disease (24) was followed when indicating the surgery. The inclusion criteria of the patients were as follows: at least 12 months had passed since the operation, stable stimulation parameters, and clinical state for at least 3 months. Exclusion criteria were significant orthopedical/rheumatological disorders or visual disability not correctable with eyeglasses. We excluded four patients because they could not walk in the medication and stimulation OFF state. Finally, 20 patients completed the tasks, and none had levodopa-resistant freezing.

For individual anatomical planning of the surgery, preoperative contrast-enhanced MR (3T Philips Achieva) images and stereotactic contrast-enhanced CT sequences (made on the day of surgery) were merged using the Medtronic

Abbreviations: ITUG, instrumented timed up and go test; ICTSIB, instrumental clinical test of sensory integration and balance; ROM, range of motion; SVR, support vector regression (SVR) analysis.

FrameLink 5 software. Intraoperative electrophysiological mapping was executed with five microelectrodes; macro stimulation controlled clinical symptoms (25).

Ethical approval (reference number: 271/2013) was obtained from the Regional and Institutional Committee of Science and Research Ethics, Semmelweis University, and patients signed informed consent forms.

Measurement protocol

Six wireless Opal monitors (APDM Inc.) (18) consisting of three-dimensional gyroscopes and accelerometers were placed on the chest, the lumbar region, the wrists, and the shins (Figure 1). The sample rate was 128 Hz. The subjects executed the ITUG test with the four major components: sit-to-stand, 7 m long gait, turning, and turn-to-sit tasks (Figure 1). At the beginning of the test, the subject sat on a chair (without an armrest) with their hands placed on their knees. After a sound cue, the patient stood up without using the arms, walked 7 m with a dynamic walking speed until reaching the target line, then turned back and walked back to the chair. Finally, the subject sat back and put their hands on their knees again (23). The average values of three consecutive trials were further analyzed to increase reliability.

We assessed the balance during quiet stance with the Instrumental Clinical Test of Sensory Integration and Balance (ICTSIB) with the following parts: stance on the plain ground with arms folded across the chest with eyes open and closed and stance on foam with arms folded across the chest with eyes open and closed (26).

The patients were on at least a 12-h medication withdrawal before the measurements. They then repeated the ITUG and ICTSIB tasks in four stimulation conditions: bilateral stimulation OFF (OFF), bilateral stimulation ON (StimON), unilateral right-sided (R-StimON), and left-sided (L-StimON) stimulation ON, in counterbalanced order. We stimulated the clinically used contacts during the study, with the stimulation parameters used for therapeutic purposes. A 1-h time interval was maintained as a washout period between testing in two different stimulation conditions. Healthy controls also executed the ITUG and ICTSIB tests three times in one session for averaging. The measurement protocol is presented in Figure 1.

The outcome measures

The ITUG gait parameters were collected and calculated by the Mobility Lab Software (APDM Inc.). We compared the values measured in the four stimulation conditions with the values of the control group, calculated the StimON/OFF improvement, and compared the StimON and OFF parameters in the PD group (Supplementary Table 1). We chose the potential indicator parameters of the dynamic balance as follows: double support (percentage of the gait cycle time that both feet are on the ground, where the gait cycle means the period

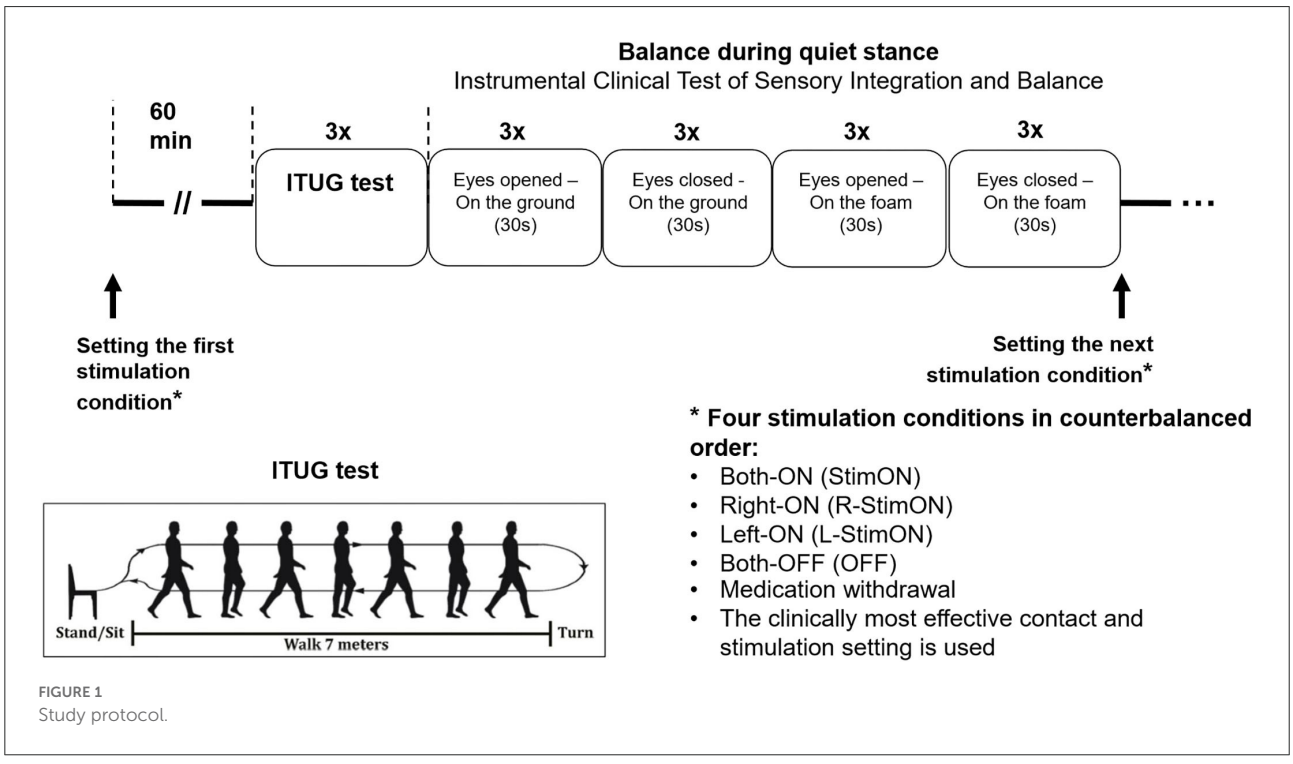

FIGURE 1
Study protocol.

between two consecutive heel-strikes of the right foot); peak turning velocity (peak angular velocity of the trunk during turning), the range of motion (ROM, degree), as well as velocity (degree/s) of the trunk in the horizontal, sagittal, and frontal plane (27).

The potential predictors of clinical factors

We collected the following disease-related parameters: age, disease duration, the pre-and postoperative Hoehn-Yahr stage, the time elapsed since the operation, and the levodopa responsiveness calculated from the rate of UPDRS III scores in preoperative MED ON and OFF states (dopamine agonists were only stopped 1 day before the test because patients did not tolerate the discomfort). In addition, we determined the International Parkinson and Movement Disorders Society MDS-UPDRS III scores in the StimON and OFF stimulation conditions and their ratio to the stimulation responsiveness at the time of measurement.

We collected the sway values (m^2/s^4; the area of the 95% confidence ellipse, an average of the three trials) in the four tasks of the ICTSIB test; their average as combined sway (18, 26) was subsequently used among the potential clinical predictors.

We specified the anatomical location of the active contacts as described in Kelemen et al. (26) in detail. In short, the postoperative CT scans acquired at least 3 months after lead implantation were co-registered with anatomical T1 images. The coordinates of the active contacts were calculated using Euclidean vectorial calculations; the reference point was the mathematical center point of the dorsolateral motor portion of the STN, according to Atlas (28). Distances between the active contacts and the warped motor centers were calculated in each plane and three dimensions in millimeters.

Statistical analyses

The normal distribution of the data was first determined with the Kolmogorov-Smirnov test; according to the results, we used parametric or nonparametric statistical tests. The age of the PD and control group was compared with the Mann–Whitney *U* test. The active contact locations referenced to the center of the dorsolateral STN and the stimulation intensity on the left and right sides were compared with an unpaired Student *t*-test. The parameters of the ITUG test in the different stimulation conditions were compared with control values using the unpaired Student *t*-test; the *p*-value was determined after a Bonferroni correction. Finally, the parameters in the stimulation conditions were compared with ANOVA for repeated measures within the PD group. The determining factor was the STIMULATION CONDITION; we used Tukey's test for multiple comparisons. The level of significance was set at $p < 0.05$.

Support vector regression analyses

We performed a support vector regression (SVR) analysis—representing a machine-learning-based multiple regression method—that could associate the observed and trained values and present the regression coefficient for prediction accuracy (29). This study implemented a data-driven regression model without explicitly stating a functional form, indicating a non-parametric technique.

In short, the algorithm looks for an optimally separating threshold between the two data sets by maximizing the margin between the classes' closest points. The points lying on the boundaries are called support vectors, and the middle of the margin is the optimal separating threshold. Since, in most cases, using a linear separator is not ideal, a projection into a higher-dimensional space was performed, whereby the data points effectively become linearly interrelated. Here, we have used the radial basis function kernel for this projection due to its good performance, as discussed in (30), and used the grid search (min = 1; max = 10) to find the few optimal input parameters, namely, *R* (type of regression algorithm; 1–1,000) and gamma (0.25). A soft-margin classifier of the calculated independent variables was used for every parameter, and a penalty constant *P* weighted spurious correlations. In order to optimize regression accuracy, this was calculated for every regressor. We performed the following steps to demonstrate that no overfitting was attested in our data for the SVR regression algorithm. The results from the SVR are reported here with fivefold cross-validation. Additionally, we used age as a confound in the analyses. We used 70% of the data for training and 30% of the data for testing.

Results

Demographics and clinical parameters

The characteristics of the patient group are summarized in Table 1. The age (median/IQR) of the patients (63/58–68.5 years) and the controls (58/52.3–69 years) did not differ ($p = 0.46$). Five females and 15 males were in the PD group, and 13 females and 11 males were in the control group. The MDS-UPDRS 3.11 scores (freezing of gait) improved in one patient from 3 to 1 while turning the bilateral stimulation on and remained 1 in one patient. No freezing of gait was observed in OFF and StimOn conditions in the rest of the patient group. In the OFF state, four patients had a score of 3, two patients had a score of 2, and four patients had a score of 1 on the MDS-UPDRS 3.12 scale representing postural stability. All other patients had a score of

TABLE 1 Demographics and clinical data of the patients.

Feature		Values; median (IQR)
Disease duration at the time of surgery		11 (9.5–14) years
Time since surgery		19 (13.5–40) months
Levodopa equivalent dose	Preoperative	816 (588–931) mg
	At the study	266 (200–586) mg
Preoperative UPDRS III. score	MED-OFF	29 (23–51) points
	MED-ON	6 (1–11) points
MDS-UPDRS III. score at the study	MED-OFF, BOTH-OFF	37 (22.5–47) points
	MED-OFF, BOTH-ON	15 (7–19) points
Levodopa response	Preoperative	86 (77–100) %
Stimulation response	At the study	65 (50–71) %
Hoehn-Yahr stage	Preoperative	3 (2.5–3)
	1 year after the operation	1 (1–1.5)

0. The latest scores improved in five patients and worsened in four patients after switching on the bilateral stimulation.

The location of the active contacts and parameters of the stimulation

The active contact locations and the stimulation parameters are presented in Table 2. The active contact locations in the three planes, right and left (x: $p = 0.28$; y: $p = 0.8$; z: $p = 0.36$), did not differ. There was no significant difference in the stimulation intensity on the two sides ($p = 0.36$).

Comparison of the ITUG parameters between PD and the control group

The PD group performed worse than controls both in OFF and StimON conditions regarding the following parameters: total duration of the ITUG Test, turn duration, and turn-to-sit duration (Supplementary Table 1).

Effect of bilateral subthalamic stimulation on the parameters of the ITUG test

The majority of the measured parameters were significantly improved by bilateral subthalamic stimulation. The ROM of the left and right arms improved the most in StimON compared to the OFF condition, followed by the arm's velocities and the trunk's ROM and velocity in the horizontal and sagittal planes (Figure 2, Supplementary Table 1). Turning on the stimulation did not affect the double support at the group level, which was not different from the control values.

Individual changes in the parameters of dynamic balance due to stimulation

Double support improved, decreased, or did not worsen in 10 of the 20 patients. In comparison, changes in other parameters were more homogenous (improvement/increase in turn peak velocity: 17/20 patients, trunk ROM horizontal: 17/20 patients, sagittal: 14/20 patients, frontal: 15/20 patients; trunk velocity horizontal: 15/20 patients, sagittal: 19/20 patients, frontal: 16/20 patients).

Prediction analysis of the parameters of dynamic balance

Table 3 presents the accuracy of how the clinical factors predicted the stimulation-induced changes in dynamic balance parameters. The superior-inferior and the anterior-posterior deviations from the motor center of the STN predicted the improvement rate of most parameters. The more posterior and inferior locations in the dorsolateral area improved the dynamic balance. In addition, the changes in the horizontal trunk movements could be predicted from the disease duration, stimulation responsiveness, and the stimulation-induced improvement of the combined postural sway from the ICTSIB test. Stimulation-induced alterations of the double support and the horizontal ROM could be predicted from the largest number of disease-related factors and the active contact location (Table 3). Improvement of double support was associated with the severity of the motor symptoms and the stimulation-induced quiet stance imbalance, in addition to anterior-posterior and superior-inferior contact locations.

TABLE 2 Parameters of the stimulation and distance of the active contact from the motor center of the dorsolateral STN.

Feature		Right	Left
STN stimulation	Amplitude (V; mean ± SD)	2.3 ± 0.8	2.4 ± 2.65
	Frequency (Hz; median and IQR)	130 (130–145)	130 (130–145)
	Impulse width (μs; median and IQR)	60 (60–65)	60 (60–65)
Location distance from center of dorsal STN (mm; mean ± SD)	X	0.46 ± 1.97	0.55 ± 0.34
	Y	−1.44 ± 1.58	−1.24 ± 0.33
	Z	0.48 ± 0.43	0.51 ± 0.53

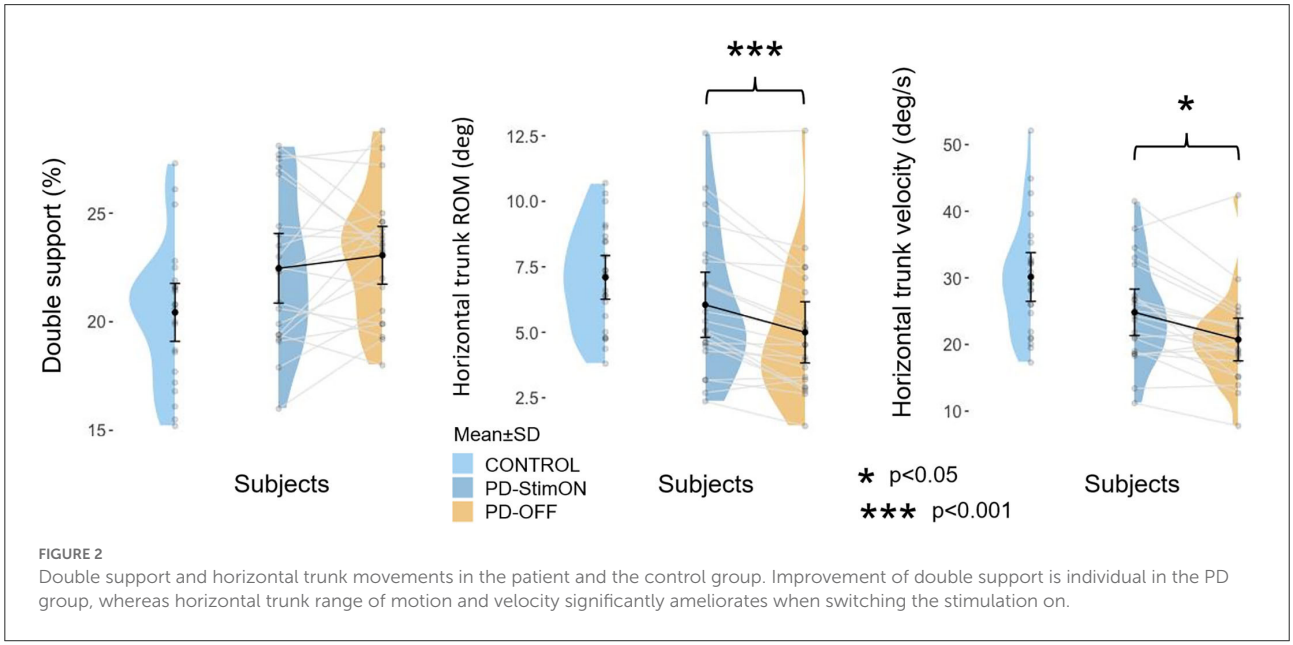

FIGURE 2
Double support and horizontal trunk movements in the patient and the control group. Improvement of double support is individual in the PD group, whereas horizontal trunk range of motion and velocity significantly ameliorates when switching the stimulation on.

TABLE 3 Coefficients of the support vector regression (SVR) analysis.

	StimON/OFF ratio							
	Double support	Turn peak velocity	Trunk ROM			Trunk velocity		
			Horizontal	Sagittal	Frontal	Horizontal	Sagittal	Frontal
Disease duration	0.55	0.52	0.75*	0.58	0.48	0.80*	0.76*	0.68
PreOP levodopa response	0.54	0.52	0.65	0.61	0.64	0.67	0.64	0.62
Stimulation response	0.55	0.58	0.75*	0.57	0.64	0.62	0.62	0.63
MDS-UPDRS III. MED OFF, Stim OFF	0.71*	0.57	0.55	0.57	0.44	0.59	0.47	0.38
StimON/OFF Combined sway	0.78*	0.65	0.42	0.41	0.55	0.71*	0.43	0.47
RX	0.47	0.45	0.42	0.41	0.55	0.46	0.44	0.48
RY	0.75*	0.76*	0.65	0.66	0.55	0.62	0.80*	0.46
RZ	0.72*	0.56	0.73*	0.47	0.72*	0.75*	0.48	0.72*
LX	0.58	0.48	0.47	0.46	0.74*	0.54	0.76*	0.45
LY	0.54	0.70*	0.82*	0.47	0.85*	0.48	0.46	0.82*
LZ	0.66	0.47	0.43	0.42	0.68	0.45	0.42	0.48

Significant predictions are marked with an asterisk.
ROM, range of motion; R, right; L, left; y, anterior (−)-posterior (+), z, superior (+)-inferior (−), x, medial (right + left-)-lateral (right-left+).

Discussion

With our results, we show that highlighted parameters of dynamic balance improve after switching on the STN stimulation, such as trunk range of motion, velocity in the three dimensions, and the turn peak velocity in the ITUG test; however, their values do not achieve the level of the control values. The double support improved the least and did not differ between the OFF and StimON conditions and controls. Its value was individually variable and could be predicted by the absence of medication and off-stimulation motor symptoms. The double support, the horizontal ROM, and the trunk velocity could be predicted by clinical factors that represented the state of the disease, such as the disease duration and postural stance imbalance. We also showed that upper limb movements improved the most with STN-DBS among the ITUG parameters.

Double support

Double support is the percentage of the gait cycle time that both feet are on the ground (27); it is associated with the freezing of gait in PD (7). Levodopa has a positive effect (31, 32) or no effect (33, 34) on double support during both short-term [3–6 months; (31) vs. (34)] and long-term [10–39 months; (32) vs. (33, 35), sequentially] STN-DBS treatment. A combined intervention of medication and stimulation was shown to exert a better effect on the freezing of gait than either treatment alone in a 6–12-months follow-up period (11). Our study has pinpointed that its stimulation-induced change is individually variable and determined by disease-related factors. Results from a large cohort of PD patients (331 patients) support our findings according to which the outcome of STN-DBS on the freezing of gait relates to the severity of the symptoms in the preoperative phase, the severity of the motor fluctuations, the brain atrophy, and the postoperative cognitive performance (36). DBS modulates targeted, selected brain networks, in which dopamine plays a key role (37). In contrast, freezing and falls were associated with cholinergic dysfunction involving the brainstem pedunculopontine nucleus (38), which explains the insufficient effect of STN-DBS treatment on these symptoms. Non-levodopa-responsive axial symptoms appear along with the disease progression (39).

Horizontal trunk movements

This study analyzed trunk movement in three dimensions, showing that the clinical state most influences the horizontal plane's motions. Accordingly, it was reported that the mediolateral sway area during quiet stance is more affected in PD than the anteroposterior, even in the early phase of the disease (40). Levodopa therapy worsens this abnormality, whereas STN-DBS reduces it and stabilizes balance in combination therapy (6, 40). We report for the first time that the stimulation-induced improvement during ITUG can be predicted from the disease duration and the stimulation responsiveness. Besides that, stimulation-induced changes in horizontal trunk velocity could be significantly predicted from the stimulation-induced combined static sway according to their interrelation in the complex balance function (1). Our results confirm that the mediolateral sway is disease-specific (1) and that the disease progression influences the DBS effect on it. It may cause a tendency to fall in the mediolateral direction in PD.

Effect of the active contact location

Dynamic balance could also be predicted from the active contact location in our study. A more superior location on the right side predicted less stimulation-induced improvement of double support and trunk movement range and velocity in the horizontal and frontal plane. The more posterior location was beneficial for most parameters of the dynamic balance. It was earlier demonstrated that high-frequency stimulation of the pedunculopontine nucleus worsens axial symptoms (41). The anatomical arrangement of its associative pathways explains our experiences while stimulating the dorsolateral STN. Ventral STN stimulation impairs gait (42). Stimulation anterodorsal from the STN may reach the Forel's field H2 with the passing pallido-pedunculopontine fibers, resulting in gait disturbances (20, 36).

Effect of STN-DBS on other gait parameters

Stride velocity improved significantly by STN-DBS in agreement with former studies (43), as well as trunk velocity in the three dimensions, the turn peak velocity, the sit-to-stand velocity, and the turn-to-sit velocity. The stride length, the range of motion of the trunk in three dimensions, and the sit-to-stand position transition have also been raised as expected (10). In contrast, the temporal parameters such as cadence and gait cycle time were not influenced by switching the stimulation on, similarly to earlier results (7). Our results confirm that DBS acts more on appendicular than axial movements (42) as the arms' range of motion is most elevated.

Strengths and limitations of the study

The study's strengths include using objective motion analysis to describe gait and dynamic balance. We also measured the anatomical location of the active contacts among the clinical characteristics.

A limitation is the number of recruited patients; it would be beneficial to perform the study in a larger cohort. Furthermore, a more extended washout period between stimulation conditions might be ideal for testing axial symptoms (44). Although, during stimulation OFF or unilateral stimulation, patients feel discomfort and cannot be burdened with this state for hours.

Furthermore, we have used a data-driven machine learning approach in this study, so caution needs to be taken in interpreting the results. The small sample size with the multiple parameter space limits the external validity and needs to be replicated in other centers and larger cohorts of patients.

Conclusion

The improvement of the double support and the horizontal trunk movements by STN-DBS are most affected by disease-related factors. Therefore, these symptoms should be focused on by the physiotherapy of patients with STN-DBS. The detailed kinematic analysis provides new information to plan an appropriate multidisciplinary approach for patient management after DBS implantation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Regional and Institutional Committee of Science and Research Ethics, Semmelweis University (reference number: 271/2013). The patients/participants provided their written informed consent to participate in this study.

Author contributions

AK, LH, MM, and GT: conception and design of the work. AK, LH, LE, PB, DZ, BL, DK, PK, GF, and GT: acquisition. AK, LH, MM, and GT: analysis or interpretation of data for the work. AK, LH, MM, DZ, LB, DB, and GT: drafting the work or revising it critically for important intellectual content. AK, LH, MM, LE, PB, DZ, BL, DK, PK, GF, LB, DB, and GT: provide approval for publication of the content and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank our patients for their committed participation in the study.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.917187/full#supplementary-material

SUPPLEMENTARY TABLE 1
ITUG parameters in the different stimulation conditions in the PD group and controls.

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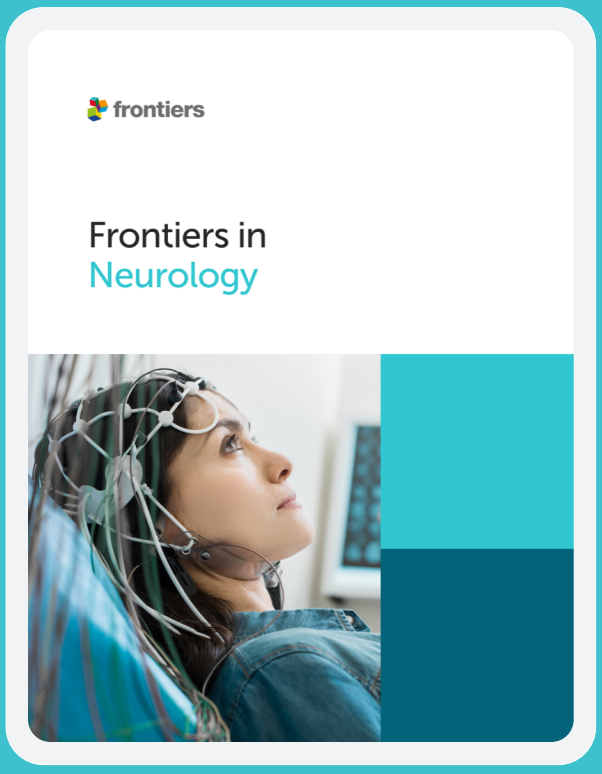

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