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# Insights in parkinson's disease and aging related movement disorders

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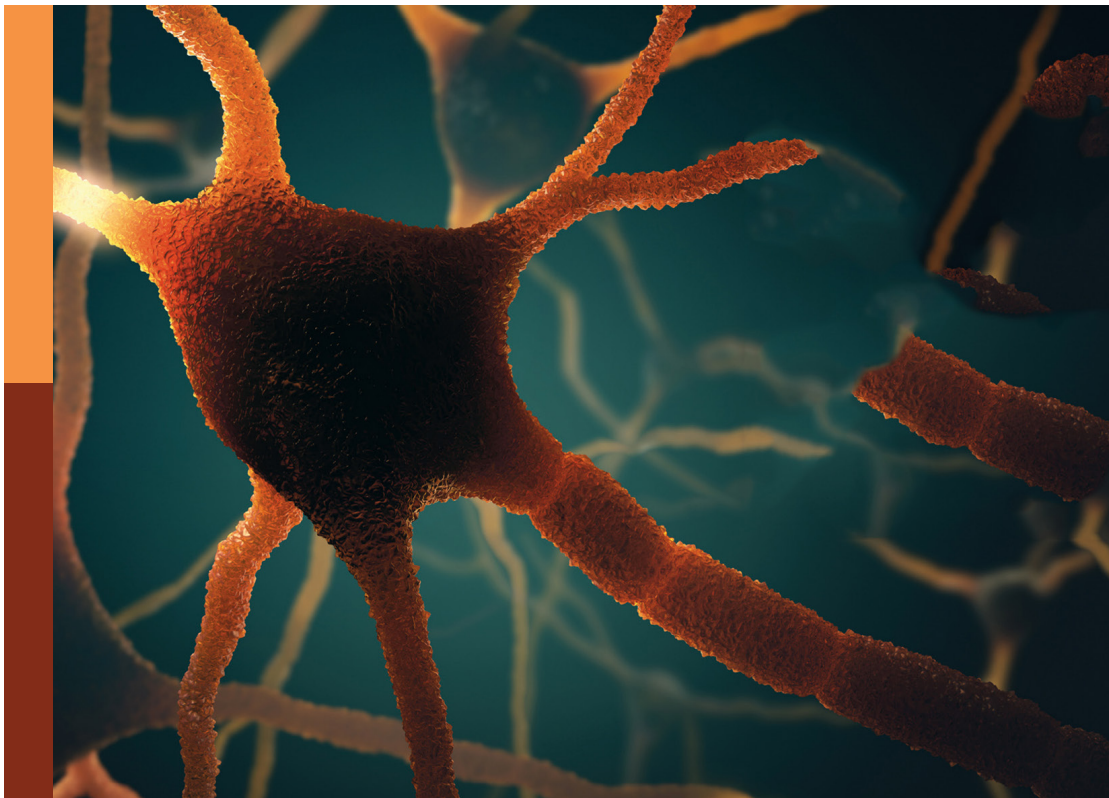

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# Insights in parkinson's disease and aging related movement disorders

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# Editorial: Insights into Parkinson's disease and aging related movement disorders

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KEYWORDS

**Parkinson's disease, ALS, microbiome, mitochondria, sleep**

Editorial on the Research Topic
Insights into Parkinson's disease and aging related movement disorders

As suggested by the title of this Research Topic, this is a broad and diverse group of articles encompassing themes that expand beyond movement disorders into subjects pertinent to neurodegenerative diseases generally. As the second most common neurodegenerative disease, Parkinson's disease is especially important. One of the major challenges in the treatment of neurodegenerative disease is its early diagnosis. There are several challenges surrounding the early diagnosis of Parkinson's disease (Tolosa et al., 2021). An important and underappreciated class of diagnostic criteria is provided by the non-motor symptoms (e.g., constipation, urinary dysfunction, hyposmia) that may be some of the earliest presenting symptoms. The greatest opportunity for successful treatment is during the prodromal stage, underlining the importance of early diagnosis. In addition, there is still a dearth of information about the origins and targets for treating movement disorders. This Research Topic addresses some recent advances including risk factors, novel therapies and targets, assessment of disease progression, and association with other diseases.

As is the case with neurodegenerative disease generally, identifying modifiable risk factors and markers of disease progression is essential. Sleep has emerged as an important factor in neurodegeneration (Musiek and Holtzman, 2016). The original research article by Yi et al. explored the effect of sleep on the progression of Parkinson's disease, addressing both motor and non-motor symptoms. Both categories were found to be negatively impacted by poor sleep hygiene. A separate study by Zhou et al. examined oculomotor function and found that it is an early indicator of Parkinson's disease and can be used to monitor disease progression. An important observation provided by Kang et al. revolves around the association of gait disturbance and cognitive impairment. The results of this study emphasize the importance of evaluating specific aspects of gait disturbance in Parkinson's patients with mild cognitive impairment. Finally, Liu et al. investigated task-generated subjective fatigue in Parkinson's patients associated with regional blood flow changes; fatigue, while one of the most common non-motor symptoms, is underdiagnosed and may in fact be one of the earliest symptoms.

Targeting and treating movement disorders are also crucial efforts. Mitochondria have been implicated in the development of neurodegenerative diseases, and this is true of movement disorders as well. Silvian presented a mini-review on targeting the PINK/Parkin pathway in mitochondrial quality control as a potential treatment for Parkinson's disease. Chen et al. looked specifically at medium spiny neurons and found that a deficiency of Pitx3 resulted in many of the pathological features of Parkinson's disease, suggesting that enhancing Pitx3 may prevent disease and provide a marker for disease diagnosis. Chiu et al. addressed the increased subjective pain sensitivity associated with Parkinson's disease in a rat model. Using fetal tissue allografts, they showed that rats exhibited increased nociceptive thresholds and improved motor function. This study also showed the promise of using fMRI to analyze efficacy. Another major problem associated with movement disorders is fall risk, which can lead to a variety of sequelae including hip fracture. Oddsson et al. demonstrated that a prosthesis provided significant improvement in gait and balance during extended use and suggested that the long-term effect indicates that this is not simply a placebo effect.

While it is well-known that agricultural work is a risk factor for PD, Zhang et al. provided an extensive epidemiological analysis in China that suggests that farmers are at increased risk for ALS. This provides a basis for searching for broader links between pesticide exposure and neurodegenerative conditions beyond PD.

The importance of the gut microbiome to brain health/function is increasingly apparent. In their review, Zeng et al. examined the hypothesis that Parkinson's disease may be derived from the gut, with a focus on whether gastrointestinal diseases are a factor. An interesting observation is that Parkinson's disease and gastrointestinal diseases may have bi-directional correlations, i.e., Parkinson's may predispose to gastrointestinal disease, and gastrointestinal disease may predispose to Parkinson's disease.

It is hoped that this diverse collection of articles will provide focus for future research into Parkinson's disease and age-related movement disorders. Thanks are extended to the authors, editors, and reviewers for helping to assemble and clarify this diverse collection of contributions.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## Conflict of interest

BW was employed by Cleveland Clinic.

The remaining 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



## References

Musiek, E. S., and Holtzman, D. M. (2016). Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science* 25, 1004–1008. doi: 10.1126/science.aah4968

Tolosa, E., Garrido, A., Scholz, S. W., and Poewe, W. (2021). Challenges in the diagnosis of Parkinson's disease. *Lancet Neurol.* 20, 385–397. doi: 10.1016/S1474-4422(21)00030-2

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MINI REVIEW
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# PINK1/Parkin Pathway Activation for Mitochondrial Quality Control – Which Is the Best Molecular Target for Therapy?

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There has been long-term interest in drugging the PINK1-Parkin pathway with therapeutics as a treatment for Parkinson's disease (PD). Despite significant structural data on Parkin as well as the PINK1 kinase and the multiple conformational changes it undergoes, activation of these targets is non-trivial. This review highlights small molecule screening results that suggests that activation of Parkin biochemically does not necessarily translate to activation of Parkin within cells. There are also issues with activation of PINK1 with kinetin analogs, which do not appear to rescue rodent models of PD. The counter-measure of activating the mitophagy pathway with deubiquitinase (DUB) inhibitors such as USP30 inhibitors is progressing in the clinic for kidney disease and the proof of biology for this target will be tested in these trials. An alternative mechanism of activating Parkin in response to oxidative stress via Parkin phosphorylation by the AMPK-ULK1 pathway may be a simpler way to lower the energy barrier Parkin activation.

**Keywords: Parkinson's disease, PINK1-Parkin pathway, molecular mechanisms of activation, AMPK, ULK1 (unc-51 like autophagy activating kinase 1), small molecule, cryoEM (cryo-electron microscopy), crystallography**

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## INTRODUCTION

In response to oxidative stress, the cell uses signaling pathways to remove damaged mitochondria through autophagy (mitophagy) as an important part of quality control; this process can be dysregulated in diseases such as Parkinson's disease (PD). Mitochondria are able to metabolize pyruvate from the glycolytic pathway to generate ATP, which is the energy that powers the brain. Mutations in the PINK1 kinase/PARK6 (Valente et al., 2004) or Parkin E3 ligase/PARK2 (Kitada et al., 1998) gene cause early onset autosomal recessive PD due to loss of dopaminergic neurons of the *substantia nigra pars compacta* (SNc). These genetic mechanisms shed light on the nature of the cellular processes that may go awry in idiopathic PD (Yi et al., 2019).

Overwhelming evidence in mammalian cell culture, Drosophila and other models have linked the PINK1/PARKIN to mitophagy, with PINK1 being the sensor of mitochondrial damage. PINK1 and Parkin work together in a feedforward signaling pathway that mediates mitophagy. Under oxidative stress and other damaging insults, PINK1 is stabilized in the outer mitochondrial membrane where it first auto-activates by phosphorylation (Okatsu et al., 2012; Rasool et al., 2018). It then activates mitophagy by phosphorylation of Ubiquitin (pUb) (Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014) as well as Parkin on its Ubl domain (pParkin) (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012).

Both PINK1 and Parkin targets are examples of a preclinical therapeutic approach to maintain mitochondrial health in PD patients by activating the mitophagy pathway to improve

mitochondrial quality control. An alternative counter-approach toward activating Parkin is to inhibit the mitochondrial deubiquitinase USP30. Knockdown of USP30 rescues the defective mitophagy caused by pathogenic mutations in Parkin (Bingol et al., 2014). Other stress-sensing pathways that involve mitochondrial quality control include the AMPK-ULK1 pathway in which changes to mitochondrial homeostasis can lead to cancer and type 2 diabetes (Cokorinos et al., 2017).

A slate of publications in the last year have increased understanding of the mechanisms of regulation of the PINK1 and Parkin through small molecule therapeutic tools and through crystal and cryoEM structures of PINK1 in different states of activation. Parkin activation has been further elucidated through a high throughput screen to identify small molecule positive allosteric activators that work along with phospho-Ubiquitin to enhance the release of the autoinhibited state (Shlevkov et al., 2022). Furthermore, recent structures of the PINK1 kinase in an activated form from *Tribolium castaneum*, Tc (Rasool et al., 2022) or *Pediculus humanus corporis*, Ph (Gan et al., 2022), have also provided finer details of the step-wise activation mechanism of this kinase.

## PARKIN AS A THERAPEUTIC TARGET

Parkin is a cytosolic Ring-between-Ring E3 ligase that uses a hybrid mechanism of ubiquitin transfer. It does not direct Ubiquitin transfer onto substrate lysines directly, instead the reaction must go through a catalytic cysteine intermediate before transfer to substrate lysines. Parkin's activity is highly regulated–phosphorylation of the Ubiquitin-like (Ubl) domain by PINK1 as well as binding of phospho-Ubiquitin is necessary to fully release its autoinhibited conformation (**Figure 1**). Once activated, the RING2 domain of Parkin containing the catalytic active site cysteine is released and is available for catalytic transfer of Ubiquitin (Tang et al., 2017).

In early studies, researchers seeking small molecules that "activate" Parkin used assays that monitored cellular mitophagy in response to oxidative stress (Garofalo et al., 2017; Johnston and Garofalo, 2017; Springer et al., 2018; Shiba-Fukushima et al., 2020). However, these assays were prone to identifying molecules that actually damage mitochondria, not true Parkin activators, though both types of compounds would have the same readout.

In a recent study, a high throughput *in vitro* biochemical assay was assembled to identify small molecule positive allosteric modulators (PAMs) that worked in conjunction with phospho-Ubiquitin to enhance auto-Ubiquitination of wildtype Parkin (Shlevkov et al., 2022). Several series of compounds were confirmed, and the best compound series (tetrahydropyrazolo pyrizine scaffold) was optimized for potency. The best active compound described (BIO-2007817) achieved the same level of activation as the W403A Parkin positive control, and achieved an $Ec_{50}$ in the ∼100 nM range. It is likely interacting specifically to Parkin as the enantiomer of this compound was inactive in the biochemical assays, suggesting that there is a single binding site. The compounds identified do not accelerate Parkin ubiquitination in the absence of pUb, but do also enhance pParkin autoubiquitination, though to a lesser extent. Using a Parkin-Ub-Vinylsulfone assay to identify the minimal components that the compounds act on, the tools appear to be directly activating Parkin, and not the E1 or E2 present in the original biochemical assay.

However, the compounds did not accelerate mitophagy or accelerate Parkin translocation to mitochondria. Phospho-poly-Ub expression on mitochondrial proteins promotes the translocation of Parkin to mitochondria and serves as an important cellular assay of activation (Shiba-Fukushima et al., 2014). The active compounds did not accelerate Parkin translocation, in contrast to the W403A mutant Parkin, which accelerates Parkin translocation to mitochondria and served as a positive control. Similarly, the compounds failed to enhance mitophagy. The proposed explanation advanced is that these PAMs act on Parkin at a step after the rate-limiting step within the cell, thus their effect appears to be negligible on Parkin translocation or the downstream mitophagy readout.

## PINK1 AS A THERAPEUTIC TARGET

PINK1 (PTEN-induced putative kinase 1) is a highly conserved kinase that is activated by mitochondrial membrane potential depolarization to phosphorylate Ubiquitin and Parkin; its activity on these two substrates triggers the feed-forward loop to mitophagy (Kondapalli et al., 2012). PINK1 has 3 insertions in the N-terminal domain (inserts 1, 2, and 3) that distinguish it from other kinases.

Two recent studies of activated dimeric PINK1 by crystallography (Rasool et al., 2022) and Cryo electron microscopy (Gan et al., 2022) have demonstrated a putative mechanism of kinase activation caused by oxidation and phosphorylation. PINK1 accumulates on the outer mitochondrial membrane in response to mitochondrial depolarization and its interaction with the translocase of the outer membrane (TOM complex) (Tokarew et al., 2021) leads to autophosphorylation of Thr257 as well as Ser228 of the C-loop and Ser402 of the activation loop. The structures demonstrate that dimeric interface of PINK1 requires insert2 and that phosphorylation of the C-loop as well as ordering of insert 3 is necessary for conformational changes in PINK1 required to bind Ubiquitin.

The order of activation is detailed as follows: First, cysteine oxidation in the P-loop of the active site (Cys166 in hPINK1) acts as a redox switch and is responsible for stabilizing the PINK1 dimer. Second, Ser228 within the C-loop of one monomer is phosphorylated which enables a conformational change of the alpha-C helix and structures insert3 to prime it to bind Ubiquitin. Then the second monomer of the dimer is phosphorylated and also primes it to bind Ubiquitin. Third, the PINK1 dimer dissociates into monomers as regulated by reactive oxygen species and Ub can now access and bind to the unique conformation of the autophosphorylated PINK1 N-terminal domain. The Ser65 of the substrate (Ubiquitin or the equivalent residue in the Parkin Ubl) is placed in the active site of the kinase, where it is phosphorylated.

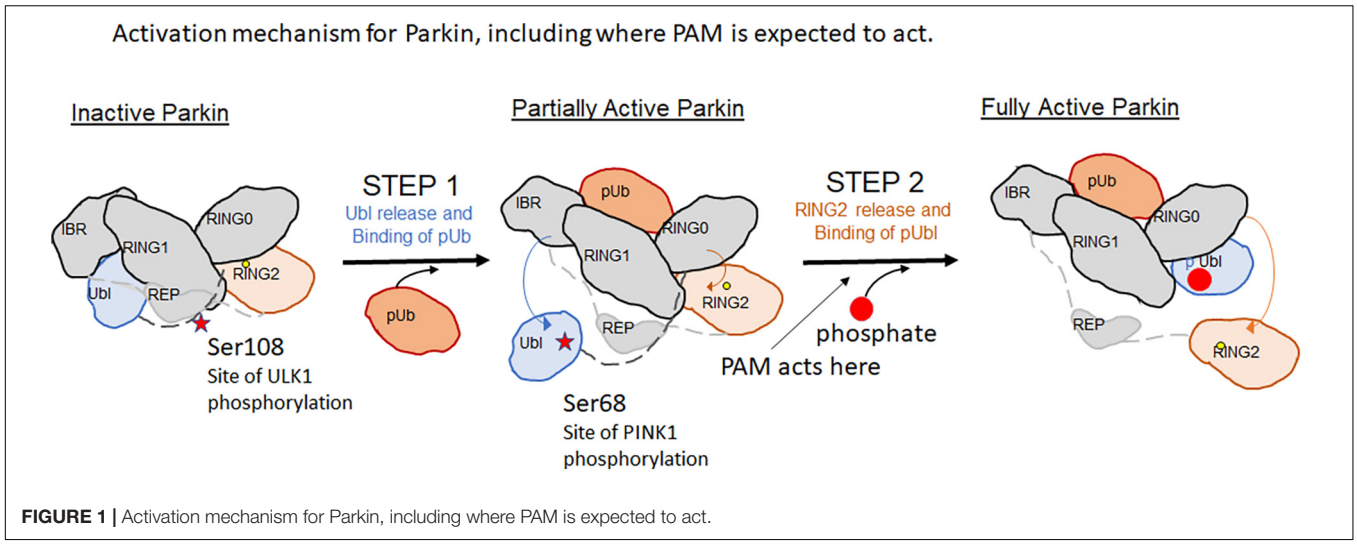


**FIGURE 1 |** Activation mechanism for Parkin, including where PAM is expected to act.

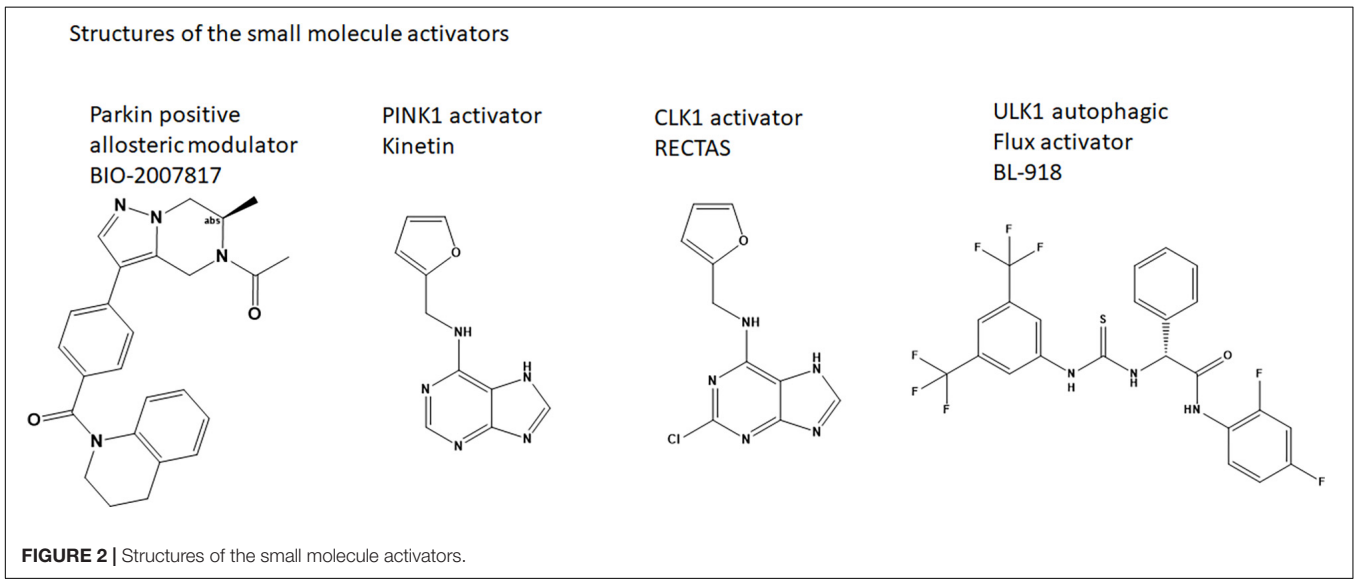


**FIGURE 2 |** Structures of the small molecule activators.

A neosubstrate kinetin (N6-Furfuryladenine; a plant hormone related to Adenosine; **Figure 2**), has been shown to increase the rate of autoactivation of PINK1, increase Parkin's recruitment to depolarized mitochondria, and block mitochondrial motility in axons (Hertz et al., 2013; Osgerby et al., 2017). Kinetin-like analogs have been designed to improve the activation of PINK1—as exemplified in WO2021 1168446 A1. Using the ProTide prodrug technology, these authors showed that a nucleoside monophosphate is the active species for activation (Osgerby et al., 2017). It is tempting to hypothesize that these compounds stabilize the PINK1 dimeric species by binding to its active site and enabling the conformational changes to prime the kinase for Ub binding. A similar compound, RECTAS, a Chloride analog of Kinetin (**Figure 2**), is known as a splice modulator compound that works by activating CLK1, resulting in phosphorylation of the SR (arginine-serine-rich) protein of splicing factors, which leads to splice site inclusion of the IKBKAP-FD exon 20 (Ajiro et al., 2021). While it is not clear exactly how RECTAS activates CLK1, CLK1 also has a unique, flexible N-terminal domain that enables oligomerization and specific binding to RS-domain containing substrates.

Despite its promise as a small molecule activator of PINK1, long-term studies of oral kinetin has been shown to not protect against synuclein-induced neurodegeneration in rodent models of PD (Orr et al., 2017).

## USP30 as a Therapeutic Target

The counter-strategy to activating Parkin and enhancing the mitophagy pathway is to inhibit enzymes that de-ubiquitinate mitochondrial protein. Deubiquitinating enzymes (DUBs) such as USP30 (Bingol et al., 2014), USP8 (Mizuno et al., 2005 #143; Durcan et al., 2014; #141) USP15 (Cornelissen et al., 2014, 2018), and Ataxin-3 (Durcan and Fon, 2011; Durcan et al., 2012) have been identified as ones that deubiquitinate mitochondrial proteins as summarized recently (Chakraborty and Ziviani, 2020). One

of the challenges in this as a therapeutic approach is the difficulty of blocking many distinct DUBs. The pleotropic nature of this deubiquitination phenomenon means that it may not be possible to target one DUB without others substituting in its place.

USP30 and Parkin have common targets such as TOM20 and MIRO1 and USP30 has a negative feedback effect on Parkin. Several UPS30 inhibitors have been advanced and are recently reviewed (Wang et al., 2022). The most advanced compounds are substituted cyanopyrrolidines which has been reported as entering clinical studies by Mission Therapeutics (US20180086708A1). Their first DUB program (MTX652) is being tested in kidney disease starting in April 2022. If successful in proof of biology, a brain-penetrant DUB may be required for testing in Parkinson's disease. FORMA therapeutics similarly has generated patents for cyanopyrrolidine inhibitors of USP30 (US20200317658). Genentech also has described Usp30 inhibitors (US2014041111A1). Mitobridge, acquired by Astellas, has also generated USP30 inhibitors such as M-094, a benzenesulonamide which appears to be very specific for USP30 (Kluge et al., 2018). The efficacy and safety of DUB inhibitors to act as proof-of-concept therapy to improve mitochondrial health remains to be tested in the clinic.

## AMPK-ULK1 AS A THERAPEUTIC TARGET

Interestingly, Parkin also serves to connect downstream mitophagy to the AMPK-ULK1 pathway, which senses cellular stress. AMPK activates ULK1 through physical interaction and phosphorylation of multiple sites, which results in translocation of ULK1 to the mitochondria (Laker et al., 2017). Recent studies demonstrate that ULK1 (UNC-51-like kinase 1) phosphorylates the ACT domain within Parkin, resulting in enhanced mitophagy (Hung et al., 2021).

In fact, mutation of a 3 residue cluster Ser108/Ser109/Ser110 within the Parkin ACT domain to Alanine or Threonine leads to delays in the rate of mitophagy. In particular, Ser 108 is the substrate for phosphorylation by ULK1. The ACT domain was previously shown to be disordered in the autoinhibited Parkin structure; but in the active pParkin-pUb structure, this region is thought to enable Ring2 release by mimicking interactions of Ring2 on the UPD and shielding the UPD once the Ring2 domain is released. Looking at greater detail at the phospho-Parkin/pUb complex structure (Gladkova et al., 2018)(PDBID:6GLC), while the Ser108 residue was poorly ordered, it docks near some highly basic residues like R256, W183. If phosphorylation of Ser108 activates Parkin, it is possible that a unique pocket for the phosphorylated Ser108 could help fully stabilize the Ring2-released, activated state of Parkin. The Hung et al. (2021) study suggests that even small amounts of mitochondrial stress can cause quick phosphorylation of this site within 5 min (Hung et al., 2021) unlike activation of PINK1 which occurs 30–60 min after mitochondrial damage. Thus the phosphorylation of the ACT domain by ULK1 is an early "alert signal" of mitochondrial damage.

This connection suggests that activation of AMPK, which senses cellular stress, could also be a potential therapeutic approach for neurological disease such as PD. An oral AMPK activator, Metformin, is approved for type 2 diabetes and reduces the risk of cancer. Studies have shown that metformin can also penetrate the blood-brain barrier and protect neurons. Repurposing of anti-diabetic agents for the treatment of cognitive impairment and mood disorders has been proposed (Cha et al., 2016).

Potentially even more specifically as a Parkinson's disease therapy, activation of ULK1 by a small molecule may sensitize Parkin for activation and enable mitophagy. A small molecule (33i, BL-918) (**Figure 2**) has been identified. The compound enabled autophagic flux in SH-SY5Y cells and reversed MPP + induced cell death (Ouyang et al., 2018). It is possible that this compound is also indirectly activating Parkin.

## DISCUSSION

In summary, the cell uses multiple regulation mechanisms to ensure that damaged mitochondria are cleared. By generating tool compounds and testing phosphorylation sites in PINK1 and Parkin, the field is getting closer to a molecular understanding of how dysregulation of this pathway could lead to dopaminergic neuron loss in PD or poor mitochondrial clearance in diabetes. But therapeutic agents that can reverse the damage by activating the pathway are in short supply. There are disconnects between *in vitro* biochemical and cell-based biochemical outcomes for Parkin activators, and disconnects between cell-based and mouse model outcomes for PINK1 activators. While it is generally accepted that activation of a target downstream in the cascade provides the better specificity than activating targets upstream, possibly the best therapeutic approach requires a pivot from activating Parkin to activating ULK1 or AMPK upstream.

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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## REFERENCES


Ajiro, M., Awaya, T., Kim, Y. J., Iida, K., Denawa, M., Tanaka, N., et al. (2021). Therapeutic manipulation of IKBKAP mis-splicing with a small molecule to cure familial dysautonomia. *Nat. Commun.* 12:4507. doi: 10.1038/s41467-021-24705-5.

Bingol, B., Tea, J. S., Phu, L., Reichelt, M., Bakalarski, C. E., Song, Q., et al. (2014). The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* 510, 370–375. doi: 10.1038/nature13418

Cha, D. S., Vahtra, M., Ahmed, J., Kudlow, P. A., Mansur, R. B., Carvalho, A. F., et al. (2016). Repurposing of anti-diabetic agents for the treatment of cognitive impairment and mood disorders. *Curr. Mol. Med.* 16, 465–473. doi: 10.2174/1566524016666160429121737

Chakraborty, J., and Ziviani, E. (2020). Deubiquitinating enzymes in Parkinson's disease. *Front. Physiol.* 11:535. doi: 10.3389/fphys.2020.00535

Cokorinos, E. C., Delmore, J., Reyes, A. R., Albuquerque, B., Kjobsted, R., Jorgensen, N. O., et al. (2017). Activation of skeletal muscle AMPK promotes glucose disposal and glucose lowering in non-human primates and mice. *Cell Metab.* 25, 1147–1159.e10. doi: 10.1016/j.cmet.2017.04.010

Cornelissen, T., Haddad, D., Wauters, F., Van Humbeeck, C., Mandemakers, W., Koentjoro, B., et al. (2014). The deubiquitinase USP15 antagonizes Parkin-mediated mitochondrial ubiquitination and mitophagy. *Hum. Mol. Genet.* 23, 5227–5242. doi: 10.1093/hmg/ddu244

Cornelissen, T., Vilain, S., Vints, K., Gounko, N., Verstreken, P., and Vandenberghe, W. (2018). Deficiency of parkin and PINK1 impairs age-dependent mitophagy in Drosophila. *Elife* 7:e35878. doi: 10.7554/eLife.35878

Durcan, T. M., and Fon, E. A. (2011). Mutant ataxin-3 promotes the autophagic degradation of parkin. *Autophagy* 7, 233–234. doi: 10.4161/auto.7.2.14224

Durcan, T. M., Kontogiannea, M., Bedard, N., Wing, S. S., and Fon, E. A. (2012). Ataxin-3 deubiquitination is coupled to Parkin ubiquitination via E2 ubiquitin-conjugating enzyme. *J. Biol. Chem.* 287, 531–541. doi: 10.1074/jbc.M111.288449

Durcan, T. M., Tang, M. Y., Perusse, J. R., Dashti, E. A., Aguileta, M. A., McLelland, G. L., et al. (2014). USP8 regulates mitophagy by removing K6-linked ubiquitin conjugates from parkin. *EMBO J.* 33, 2473–2491. doi: 10.15252/embj.201489729

Gan, Z. Y., Callegari, S., Cobbold, S. A., Cotton, T. R., Mlodzianoski, M. J., Schubert, A. F., et al. (2022). Activation mechanism of PINK1. *Nature* 602, 328–335. doi: 10.1038/s41586-021-04340-2

Garofalo, A. W., Johnston, J., and Fatheree, P. R. (2017). *Triazole Benzamide Derivatives and the Compositions and Methods of Treatment Regarding the Same.* Patent WO/2017/210694. Washington, DC: U.S. Patent and Trademark Office.

Gladkova, C., Maslen, S. L., Skehel, J. M., and Komander, D. (2018). Mechanism of parkin activation by PINK1. *Nature* 559, 410–414. doi: 10.1038/s41586-018-0224-x

Hertz, N. T., Berthet, A., Sos, M. L., Thorn, K. S., Burlingame, A. L., Nakamura, K., et al. (2013). A neo-substrate that amplifies catalytic activity of parkinson's-disease-related kinase PINK1. *Cell* 154, 737–747. doi: 10.1016/j.cell.2013.07.030

Hung, C. M., Lombardo, P. S., Malik, N., Brun, S. N., Hellberg, K., Van Nostrand, J. L., et al. (2021). AMPK/ULK1-mediated phosphorylation of Parkin ACT domain mediates an early step in mitophagy. *Sci. Adv.* 7:eabg4544. doi: 10.1126/sciadv.abg4544

Johnston, J., and Garofalo, A. W. (2017). *Pyradazinone Derivatives and the Compositions and Methods of Treatment Regarding the Same.* Patent WO/2017/21068. Washington, DC: U.S. Patent and Trademark Office.

Kane, L. A., Lazarou, M., Fogel, A. I., Li, Y., Yamano, K., Sarraf, S. A., et al. (2014). PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J. Cell Biol.* 205, 143–153. doi: 10.1083/jcb.201402104

Kazlauskaite, A., Kondapalli, C., Gourlay, R., Campbell, D. G., Ritorto, M. S., Hofmann, K., et al. (2014). Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem. J.* 460, 127–139. doi: 10.1042/BJ20140334

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., et al. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392, 605–608.

Kluge, A. F., Lagu, B. R., Maiti, P., Jaleel, M., Webb, M., Malhotra, J., et al. (2018). Novel highly selective inhibitors of ubiquitin specific protease 30 (USP30) accelerate mitophagy. *Bioorg. Med. Chem. Lett.* 28, 2655–2659. doi: 10.1016/j.bmcl.2018.05.013

Kondapalli, C., Kazlauskaite, A., Zhang, N., Woodroof, H. I., Campbell, D. G., Gourlay, R., et al. (2012). PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol.* 2:120080. doi: 10.1098/rsob.120080

Koyano, F., Okatsu, K., Kosako, H., Tamura, Y., Go, E., Kimura, M., et al. (2014). Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 510, 162–166. doi: 10.1038/nature13392

Laker, R. C., Drake, J. C., Wilson, R. J., Lira, V. A., Lewellen, B. M., Ryall, K. A., et al. (2017). Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nat. Commun.* 8:548. doi: 10.1038/s41467-017-00520-9

Mizuno, E., Iura, T., Mukai, A., Yoshimori, T., Kitamura, N., and Komada, M. (2005). Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. *Mol. Biol. Cell* 16, 5162–5174. doi: 10.1091/mbc.e05-06-0560

Okatsu, K., Oka, T., Iguchi, M., Imamura, K., Kosako, H., Tani, N., et al. (2012). PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nat. Commun.* 3:1016. doi: 10.1038/ncomms2016

Orr, A. L., Rutaganira, F. U., de Roulet, D., Huang, E. J., Hertz, N. T., Shokat, K. M., et al. (2017). Long-term oral kinetin does not protect against alpha-synuclein-induced neurodegeneration in rodent models of Parkinson's disease. *Neurochem. Int.* 109, 106–116. doi: 10.1016/j.neuint.2017.04.006

Osgerby, L., Lai, Y. C., Thornton, P. J., Amalfitano, J., Le Duff, C. S., Jabeen, I., et al. (2017). Kinetin riboside and Its ProTides activate the Parkinson's disease associated PTEN-induced putative kinase 1 (PINK1) independent of mitochondrial depolarization. *J. Med. Chem.* 60, 3518–3524. doi: 10.1021/acs.jmedchem.6b01897

Ouyang, L., Zhang, L., Zhang, S., Yao, D., Zhao, Y., Wang, G., et al. (2018). Small-molecule activator of UNC-51-like kinase 1 (ULK1) that induces cytoprotective autophagy for Parkinson's disease treatment. *J. Med. Chem.* 61, 2776–2792. doi: 10.1021/acs.jmedchem.7b01575

Rasool, S., Soya, N., Truong, L., Croteau, N., Lukacs, G. L., and Trempe, J. F. (2018). PINK1 autophosphorylation is required for ubiquitin recognition. *EMBO Rep.* 19:e44981. doi: 10.15252/embr.201744981

Rasool, S., Veyron, S., Soya, N., Eldeeb, M. A., Lukacs, G. L., Fon, E. A., et al. (2022). Mechanism of PINK1 activation by autophosphorylation and insights into assembly on the TOM complex. *Mol. Cell* 82, 44–59.e6. doi: 10.1016/j.molcel.2021.11.012

Shiba-Fukushima, K., Imai, Y., Yoshida, S., Ishihama, Y., Kanao, T., Sato, S., et al. (2012). PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. *Sci. Rep.* 2:1002. doi: 10.1038/srep01002

Shiba-Fukushima, K., Inoshita, T., Hattori, N., and Imai, Y. (2014). PINK1-mediated phosphorylation of Parkin boosts Parkin activity in Drosophila. *PLoS Genet.* 10:e1004391. doi: 10.1371/journal.pgen.1004391

Shiba-Fukushima, K., Inoshita, T., Sano, O., Iwata, H., Ishikawa, K. I., Okano, H., et al. (2020). A cell-based high-throughput screening identified two compounds that enhance PINK1-Parkin signaling. *iScience* 23:101048. doi: 10.1016/j.isci.2020.101048

Shlevkov, E., Murugan, P., Montagna, D., Stefan, E., Hadzipasic, A., Harvey, J. S., et al. (2022). Discovery of small-molecule positive allosteric modulators of Parkin E3 ligase. *iScience* 25:103650. doi: 10.1016/j.isci.2021.103650

Springer, W., Fiesel, F. C., and Caulfield, T. R. (2018). *Small Molecule Activators of Parkin Enzyme Function.* Patent WO 2018/023029. Washington, DC: U.S. Patent and Trademark Office.

Tang, M. Y., Vranas, M., Krahn, A. I., Pundlik, S., Trempe, J. F., and Fon, E. A. (2017). Structure-guided mutagenesis reveals a hierarchical mechanism of Parkin activation. *Nat. Commun.* 8:14697. doi: 10.1038/ncomms14697

Tokarew, J. M., El-Kodsi, D. N., Lengacher, N. A., Fehr, T. K., Nguyen, A. P., Shutinoski, B., et al. (2021). Age-associated insolubility of parkin in human

midbrain is linked to redox balance and sequestration of reactive dopamine metabolites. *Acta Neuropathol.* 141, 725–754. doi: 10.1007/s00401-021-02285-4

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., et al. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304, 1158–1160. doi: 10.1126/science.1096284

Wang, F., Gao, Y., Zhou, L., Chen, J., Xie, Z., Ye, Z., et al. (2022). USP30: structure, emerging physiological role, and target inhibition. *Front. Pharmacol.* 13:851654. doi: 10.3389/fphar.2022.851654

Yi, W., MacDougall, E. J., Tang, M. Y., Krahn, A. I., Gan-Or, Z., Trempe, J. F., et al. (2019). The landscape of Parkin variants reveals pathogenic mechanisms and therapeutic targets in Parkinson's disease. *Hum. Mol. Genet.* 28, 2811–2825. doi: 10.1093/hmg/ddz080

**Conflict of Interest:** LS is employed by company Biogen.

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# Perfusion Imaging of Fatigue and Time-on-Task Effects in Patients With Parkinson's Disease

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Fatigue is a highly prevalent and debilitating non-motor symptom in Parkinson's disease (PD), yet its' neural mechanisms remain poorly understood. Here we combined arterial spin labeling (ASL) perfusion functional magnetic resonance imaging (fMRI) with a sustained mental workload paradigm to examine the neural correlates of fatigue and time-on-task effects in PD patients. Twenty-one PD patients were scanned at rest and during continuous performance of a 20-min psychomotor vigilance test (PVT). Time-on-task effects were measured by the reaction time changes during the PVT and by self-reported fatigue ratings before and after the PVT. PD subjects demonstrated significant time-on-task effects, including progressively slower reaction time on the PVT and increased post-PVT fatigue ratings compared to pre-PVT. Higher levels of general fatigue were associated with larger increases in mental fatigue ratings after the PVT. ASL imaging data showed increased CBF in the right middle frontal gyrus (MFG), bilateral occipital cortex, and right cerebellum during the PVT compared to rest, and decreased CBF in the right MFG at post-task rest compared to pre-task rest. The magnitude of regional CBF changes in the right MFG and right inferior parietal lobe correlated with subjective fatigue rating increases after the PVT task. These results demonstrate the utility of continuous PVT paradigm for future studies of fatigue and cognitive fatigability in patients, and support the key role of the fronto-parietal attention network in mediating fatigue in PD.

**Keywords: psychomotor vigilance test, Parkinson's disease, fatigue, time-on-task effect, ASL perfusion fMRI, frontoparietal network**

## INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting millions of people worldwide. PD patients typically show a resting tremor, rigidity, bradykinesia, and postural instability (Hoehn and Yahr, 1967; Tandberg et al., 1995). While these motor signs are considered cardinal features of PD, there is mounting evidence that PD is a systemic disease that

also includes non-motor signs and symptoms (Hoehn and Yahr, 1967; Friedman and Friedman, 1993; Pfeiffe, 2016). Of these non-motor symptoms, fatigue, both mental and physical, is one of the most common and disabling (Pfeiffe, 2016). Among PD patients, the prevalence of fatigue is 33–70% (Friedman and Friedman, 1993; van Hilten et al., 1993; Friedman et al., 2011; Herlofson et al., 2012), and it may even precede the onset of motor symptoms (Hagell and Brundin, 2009). About one-third of PD patients report that fatigue is the most disabling symptom and significantly impairs their daily activities and reduces their overall quality of life (Herlofson and Larsen, 2003; Friedman et al., 2007; Kluger et al., 2014). However, fatigue tends to be under-diagnosed, and there are no effective treatments. Currently, in clinical settings, fatigue assessments rely largely on subjective patient self-reports, but there is a growing interest in objective assessments of fatigue in PD for earlier identification of physical indicators of fatigue. Furthermore, understanding of the neural mechanisms underlying fatigue in PD could potentially provide significant insights for developing prevention and management strategies.

Fatigue includes both physical and mental/cognitive manifestations. However, progress in PD fatigue research is partly hinded by issues of definition and measurement of fatigue, particularly its cognitive aspects. Kluger et al. suggested distinguishing subjective sensations of exhaustion (i.e., perceived fatigue) from objective decrement in cognitive performance or physiological capacity induced by continued activity (i.e., fatigability) (Kluger et al., 2013). Cognitive fatigability is often measured as declines in either reaction time (RT) or accuracy over time on continuous cognitive tests. Perceived fatigue experience and fatigability may be dissociable. For example, although PD patients demonstrate heightened fatigability on both cognitive and motor tasks, such fatigability does not correlate with subjective fatigue complaints (Lou, 2009). This highlights the importance of considering both objective and subjective measurements of fatigue in PD patients. More studies are needed to establish the relationship between these two components. It is possible that the appropriate choice of performance metric may improve our ability to examine the associations between self-reported fatigue and objectively measured cognitive fatigability (Wang et al., 2014).

Several studies have examined the neural bases of subjective fatigue complaints in PD. For example, reduced perfusion in the prefrontal cortex and decreased serotonergic activity in the basal ganglia and limbic regions has been reported in fatigued PD patients (Abe et al., 2000; Pavese et al., 2010). PD patients with fatigue also showed abnormal metabolic changes in the in the salience network and default mode network (DMN) regions (Cho et al., 2017). Using blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI), Tessitore et al. (2016) found that primary PD-related fatigue is associated with increased connectivity within the prefrontal and posterior cingulate hubs of the DMN in drug-naive patients (Tessitore et al., 2016). These findings are consistent with the model of central/cognitive fatigue proposed by Chaudhuri and Behan (2000), which hypothesizes that central fatigue is associated with impairment of basal ganglia non-motor functions and negatively impacts the striatal-thalamic-frontal cortical system.

In contrast to subjective patient fatigue studies, few if any studies examined the neural correlates of objective cognitive fatigability in PD patients. To the best of our knowledge, there are no reported brain imaging studies that have probed the neural underpinnings of cognitive fatigability in PD from mentally demanding tasks. Early imaging studies on fatigue typically employed positron emission tomography (PET) or BOLD fMRI (Tessitore et al., 2016; Cho et al., 2017). However, PET has poor temporal and spatial resolution in localizing regional brain activity while BOLD fMRI has limited utility in tracking slow neural activity changes observed in fatigability studies (Aguirre et al., 1997, 2002).

Using magnetically labeled water in arterial blood as the diffusible tracer to provide quantification of regional cerebral blood flow (CBF), arterial spin labeling (ASL) perfusion fMRI is capable of non-invasively evaluating slow variations in neural activation patterns with high spatial and temporal resolution over long periods of time (Detre and Wang, 2002). ASL fMRI shows high stability over hours and days making it well suited to image fatigue and cognitive fatigability in PD, and it has been successfully used to assess brain function during both sustained cognitive tasks and task-free resting baselines (Wang et al., 2005; Kim et al., 2006; Olson et al., 2006; Rao et al., 2007a,b,c).

We have used ASL perfusion imaging and measured brain fatigue and time-on-task effects from sustained mental workload from continuous performance of the psychomotor vigilance test (PVT) in healthy individuals (Lim et al., 2010). The PVT is a simple reaction time task which provides a simple, reliable, and highly sensitive paradigm for capturing deficits in sustained attention and performance (Dorrian et al., 2005; Lim and Dinges, 2008). The attentional requirements in the PVT are free of aptitude or learning effects and are undiluted by elements of selectivity such as spatial orientation or executive decision-making, allowing it to be applied to all individuals and without adjustments for experience. These task features allowed us to isolate the effect of fatigability without having to account for potential confounding effects of differing visual stimuli, aptitude, strategy shifts or learning. Using the PVT in healthy subjects, we observed progressively slowing of reaction time (i.e., time-on-task effects) during task performance and increased mental fatigue ratings after the task (Lim et al., 2010). Moreover, regional CBF was significantly reduced in right fronto-parietal regions during post-task rest compared to pre-task rest, and the degree of regional CBF decrease correlated with the degree of performance decline, suggesting the key role of the fronto-parietal attention network in mediating brain fatigue from sustained mental workload.

In the present study, we employed the continuous 20-min PVT paradigm with ASL perfusion imaging to assess fatigue, cognitive fatigability, and brain activity changes in a cohort of PD patients. We aimed to examine whether PD patients with fatigue would (1) have difficulty completing the PVT due to motor deficits (van Rooden et al., 2009); (2) demonstrate cognitive fatigability and time-on-task effects in response to a sustained

PVT task, and (3) show analogous neural changes as had been obtained in healthy adults (Lim et al., 2010).

# MATERIALS AND METHODS

## Participants

A total of 21 PD patients (11 male, age range 52 to 80 years, mean age = 68 years) participated in this study. All participants were recruited from a large cohort of PD patients enrolled in the Udall Center at the University of Pennsylvania. PD participants had Montreal Cognitive Assessment (MoCA) score greater than 22, and reported normal sleep pattern (6–9 h range), as confirmed by the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). Patients were excluded if they had claustrophobia, depression, epilepsy, or other chronic debilitating medical conditions. Before the fMRI study, participants were required to have 7–8 h of sleep each night to minimize the potential effects of sleep debt on behavior and brain function. The Fatigue Severity Scale (FSS) was used to measure perceived general fatigue over recent period of time, day-to-day life. All subjects were compensated for participation in the study. Written consent was obtained according to the University of Pennsylvania Institutional Review Board.

## The PVT Task

The PVT (Dinges et al., 1997; Durmer and Dinges, 2005; Lim et al., 2010) was used as the sustained attention task in this study. This 20-min test is a simple reaction time test with varying and random inter-stimulus intervals (ISI) which range from 2 to 10 s (mean ISI = 6 s, including a 1s delay after each button press for subjects to read their reaction time). Participants were instructed to focus their attention on a red, rectangular box subtending 2 × 1.3 degrees of visual angle in the middle of a black screen. They were instructed to quickly stop the counter with a button press as soon as they saw a number displayed, but avoiding false starts. Immediately before and after administration of the PVT, patients rated their subjective mental fatigue score on the 9-point Visual Analog Scale (VAS). The following indexes were extracted as the measures of PVT performance, including mean reaction time (RT), median RT, standard deviation of RT, and number of lapses (defined as RT exceeding 500 ms). To assess time-on-task effects and performance fatigability, we divided the 20-min PVT into five 4-min quintiles and compared the median RT in the first quintile to that of the last quintile for each subject.

## Imaging Data Acquisition and Analyses

Functional Imaging data were acquired on a Siemens Magnetom 3T Prisma whole body scanner (Siemens AG, Erlangen, Germany), using a 64-channel head coil. All fMRI scans were conducted at the Hospital of the University of Pennsylvania. A spiral 3D pseudo-continuous arterial spin labeling (ASL) sequence was used for the perfusion scan with the following parameters: TR = 4 s, TE = 10 ms, flip angle = 90°, image matrix = 64 × 64, FOV = 240 mm, labeling time = 1.8 s, post labeling delay = 1.7 s. A total of 34 slices with 3.75 mm slice thickness were acquired in interleaved manner from anterior to posterior. High resolution T1-weighted structural images were acquired using a 3D MPRAGE sequence with the following parameters: TR = 2,400 ms, TE = 2.22 ms, flip angle = 8°, 208 slices with slice thickness of 0.80 mm, image matrix = 300 × 320, FOV = 256 mm. The baseline rest ASL protocol lasted for 6 min before and after PVT task and consisted of 36 acquisitions each. The PVT lasted for 20 min with 150 acquisitions.

Image data analysis was performed using Statistical Parametric Mapping (SPM 12) software (Wellcome Department of Cognitive Neurology, London), based in Matlab R2012b (Mathworks Inc., Natick, MA, United States). The ASL data processing was performed using fMRI Grocer toolbox[1] and in house scripts (Dolui et al., 2019). Five subjects were excluded for imaging data analyses. Specifically, one subject was excluded due to missing VAS fatigue data. Two subjects were excluded due to large head movements greater than the size of a voxel (3.75 mm × 3.75 mm × 3.75 mm) and two other subjects were excluded due to coverage problems and CBF signal losing from ASL scans.

Pre-processing steps included motion correction, coregistration, normalization and smoothing. Motion correction was done by aligning all the functional images to the mean image of the time series for each run to correct for the effects of head movements. Next, all the realigned images were coregistered to individual subject's own T1 structural image. Then, perfusion weighted image series were generated by pair-wise subtraction of the label and control images (Wang et al., 2005). The resulting CBF-weighted images were averaged to obtain a mean CBF image for each condition. The mean CBF image was smoothed using a three-dimensional, 8 mm full width at half maximum Gaussian kernel and normalized to a 2 mm × 2 mm × 2 mm Montreal Neurological Institute (MNI) template. Similar to our previous study (Lim et al., 2010), the 20-min PVT data were divided into five 4-min quintiles, as follows: PVTq1 (the first quintile), PVTq2 (the second), PVTq3 (the third), PVTq4 (the fourth), and PVTq5 (the fifth). Each quintile resulted in its own smooth and warped mean CBF-weighted image. All the warped mean CBF maps were visually checked for optimal sensitivity, good contrast and intensity (see **Figure 1** for an example of CBF maps). These mean CBF images were then entered into the whole brain voxel-wise general linear model (GLM) analysis. Contrasts were generated to compare the PVT with rest baselines (PVT vs Rest), the post-task resting baseline with the pre-task resting baseline (Rest2 vs Rest1), and the last quintile with the first quintile of PVT (PVTq5 vs PVTq1). Activation clusters were identified at a significance level of whole brain FWE corrected $p < 0.05$ with cluster size larger than 30 voxels.

In order to examine whether PD patients show analogous neural changes as had been obtained in healthy adults, region of Interest (ROI) analysis was also performed using *a priori* ROIs were defined from our previous study of fatigue and time-on-task effects in healthy subjects (Lim et al., 2010). These *a priori* ROIs included bilateral middle frontal gyrus (MFG), anterior cingulate cortex (ACC), superior temporal cortex (STC) and the right inferior parietal lobe (IPL). Regional

[1]https://www.nitrc.org/projects/fmri_grocer/

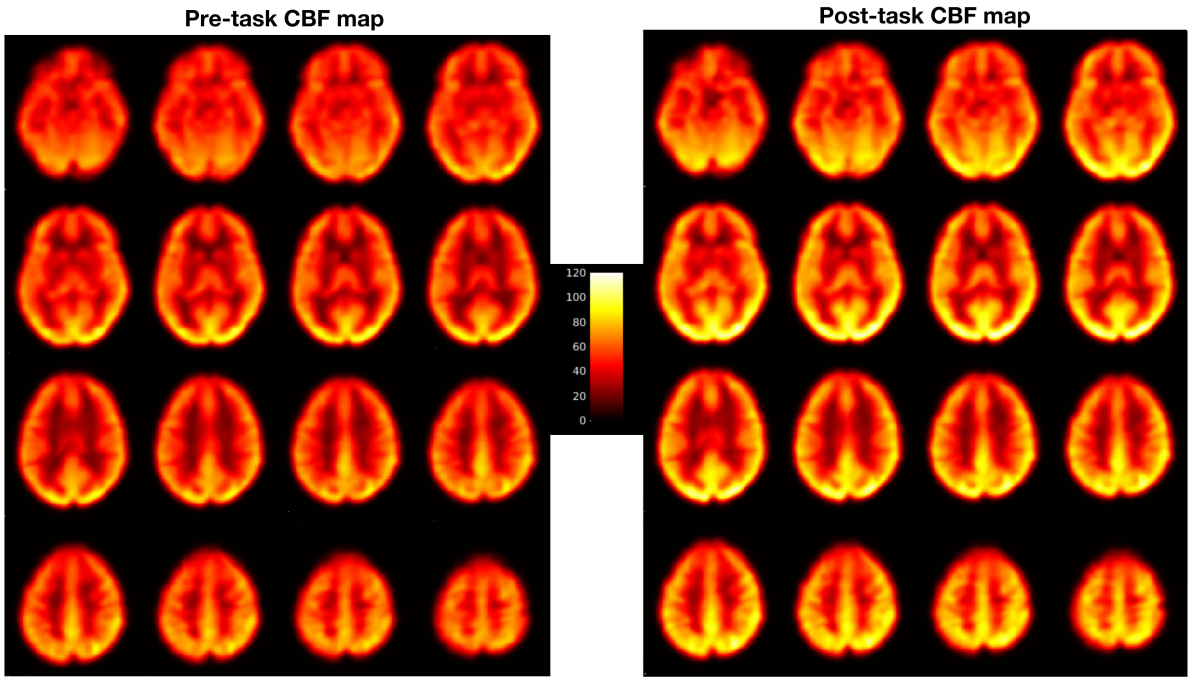


**FIGURE 1 |** Pre- and post-task CBF maps of one representative PD patient.

CBF values were extracted for each ROI for both pre and post-task baselines. CBF changes (adjusting for global CBF difference) were calculated and correlated with performance changes and subjective fatigue measurements.

# RESULTS

## Behavioral Data

The median and mean RT for the PVT across all participants and all trials were 360.22 and 428.69 ms, respectively. On average subjects showed a steady increase in RT over 20 min of the PVT with median RT increasing from 331 to 389 ms and mean RT increasing from 352 to 569 ms (**Figure 2A**). Because there was less variance with median RT compared to mean, median RT was used to conduct subsequent analyses. One-way analysis of variance (repeated measures) of median RT over the five 4-min quintiles showed a significant effect of time ($F_{4,105} = 2.67$, $p < 0.05$). Similar to previous studies, we also observed large inter-individual differences. For instance, as shown in **Figure 2B**, subjects 1 and 17 showed a monotonic increase in RT, subjects 2, 4 and 5 appeared to reach plateau at 10 min but then continued to increase RT over the final 2 quintiles, and subjects 3 and 13 showed very little change in RT from the 1st quintile to the 5th quintile.

Patients also reported significantly increased mental fatigue rating on the after the 20-min PVT than before the PVT (**Figures 2C**, $p < 0.05$) and self-reported fatigue change significantly correlated with the FSS score (**Figures 2E**, $r = 0.690$, $p = 0.001$). These findings suggest that subjects with higher levels of perceived general fatigue have greater worsening of their fatigue while performing a sustained vigilance task. However, there was no correlation between RT change and self-reported mental fatigue change (**Figure 2D**, $r = -0.101$, $p = 0.66$), suggesting a dissociation between subjective fatigue and cognitive fatigability. In addition, there was a non-significant trend of correlation between RT change to the FSS score (**Figure 2F**, $r = 0.393$, $p = 0.078$).

## Imaging Results

**Figure 3** displays the results from whole brain analysis. When comparing the PVT to resting baselines, increased CBF was found in the right middle frontal gyrus (MFG), bilateral occipital cortex, and right cerebellum (see **Figure 3A** and **Table 1A**). When comparing post-task resting baseline to pre-task resting baseline, decreased CBF was found in the right MFG (BA 9, 10) while no increased CBF were observed (see **Figure 3B** and **Table 1B**). When comparing the first quintile of PVT to the last quintile of PVT, increased CBF was found in the right superior temporal cortex (STC; **Figure 3C** and **Table 2**).

**Figure 4** displays the results from ROI analyses. Among the seven ROIs showing significantly reduced CBF in healthy adults (Lim et al., 2010), only the right STC (**Figure 4**, $p = 0.030$) showed a significant reduction in CBF between the pre-task and post-task baseline scans in PD patients. Regional CBF changes in the right IPL and the right MFG showed significant positive correlations with subjective fatigue (VAS) changes (IPL: $p = 0.025$, **Figure 5A**; MFG: $p = 0.046$, **Figure 5B**). There was also a trend of positive correlation between CBF changes in the right IPL and the FSS scores ($p = 0.054$, **Figure 5C**). There were no significant

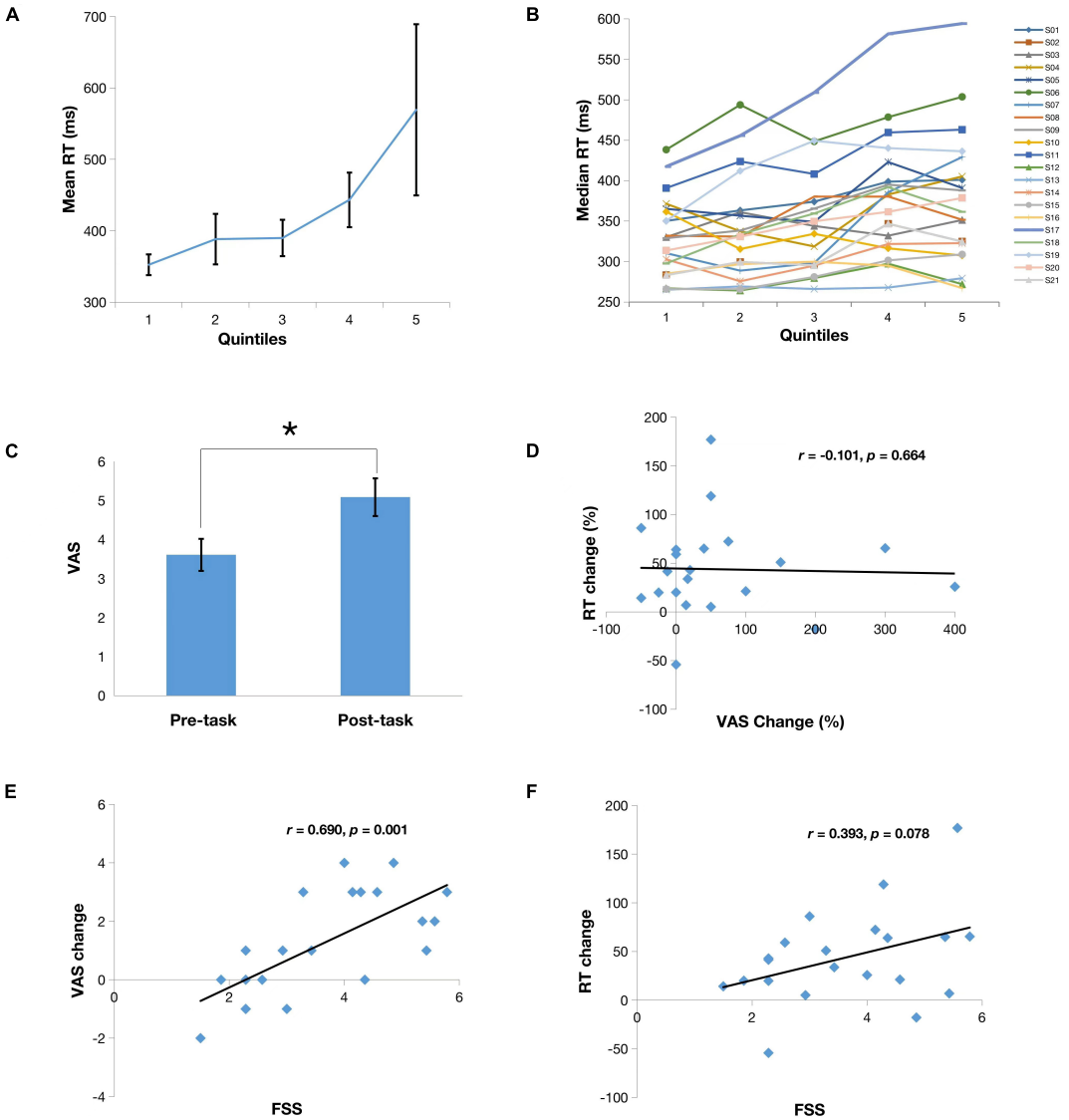


**FIGURE 2 | (A)** Means of reaction time (RT) from the first to the last quintile. Note there is significant differences between mean RT in the last quintile and the first three quintiles ($p < 0.05$); **(B)** Median RT series of all subjects from the first to the last quintile; **(C)** Mean scores on a 9-point Visual Analog Scale (VAS) reported by subjects before and after the PVT. Note there is a significant mental fatigue increase after PVT compared to prior PVT (*$p < 0.05$); **(D)** Correlation of VAS change (%) to RT change (%); **(E)** Correlation of VAS change to Fatigue Severity Scale (FSS); **(F)** Correlation of RT change to FSS.

correlations between CBF changes in the right MFG and the FSS scores ($p = 0.139$, **Figure 5D**), and RT changes and regional CBF changes from pre-task to post-task baselines in any of the other ROIs (all $p > 0.1$).

## DISCUSSION

This study evaluated fatigue and cognitive fatigability induced by performing a mentally challenging sustained attention task in a clinical sample of PD patients. As expected, PD patients in this study reported greater fatigue ratings after the task and displayed progressively increasing RT over the course of the PVT. These findings demonstrated that performance of a sustained attention task is associated with decrements in task performance and an increase in subjective fatigue in PD patients, suggesting that the research paradigms employed in this study can be used successfully to study induced fatigue in this population.

Previous research has suggested that PD patients might have difficulty completing the PVT due to motor deficits caused by the disease (van Rooden et al., 2009). However, our study demonstrated that, even though some PD patients had significant number of lapses, nearly all patients were able to complete the 20-min continuous PVT. Berard et al. (2020) also observed more lapses during the 20-min PVT in multiple sclerosis (MS),

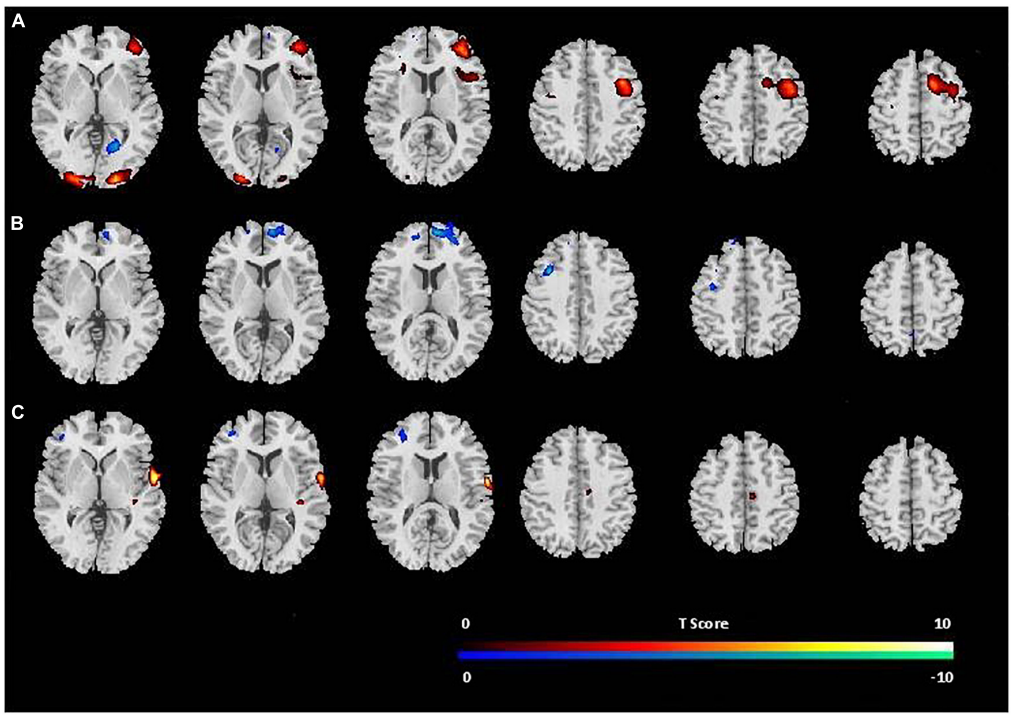


**FIGURE 3 |** Brain areas with increase (red) or decrease (blue) in CBF **(A)** during PVT performance in comparison to the resting baselines; **(B)** post-task resting baseline in comparison to pre-task resting baseline; **(C)** during fifth quintile PVT scores in comparison to first quintile PVT scores. The threshold of display was set as uncorrected $p < 0.001$.

**TABLE 1 |** Brain areas showing **(A)** significant activation (CBF increases) and deactivation (CBF decreases) to the PVT compared to resting baselines (PVT vs. Rest) and **(B)** CBF decreases for post-task resting baseline compared to pre-task resting baseline (Rest 2 vs. Rest 1).

| Region | Cluster size | MNI coordinates | | | Peak Z | Peak *p* (uncorrected) |
|---|---|---|---|---|---|---|
| | | x | y | z | | |
| **(A) CBF increases: PVT vs. Rest** | | | | | | |
| R. Middle frontal gyrus | 976 | 32 | 40 | 20 | 5.55 | < 0.001*** |
| R. Occipital | 386 | 20 | −94 | 0 | 5.03 | < 0.001* |
| R. Middle frontal gyrus | 1218 | 36 | 8 | 42 | 4.73 | < 0.001*** |
| L. Occipital | 384 | −26 | −94 | 4 | 4.65 | < 0.001* |
| R. Cerebellum | 310 | 40 | −56 | −22 | 3.74 | < 0.001* |
| R. Inferior parietal gyrus | 71 | 52 | −40 | 34 | 3.88 | < 0.001 |
| R. Insula | 126 | 48 | 16 | 14 | 3.64 | < 0.001 |
| **CBF decreases: PVT vs. Rest** | | | | | | |
| R. Occipital | 213 | 22 | −60 | −6 | 3.95 | < 0.001 |
| **(B) CBF decreases: Rest 2 vs. Rest 1** | | | | | | |
| R. Middle frontal gyrus | 317 | 10 | 58 | 18 | 4.03 | < 0.001* |
| L. Middle frontal gyrus | 59 | −34 | 12 | 44 | 3.87 | < 0.001 |

*Thresholds were set as uncorrected $p < 0.001$. ***The clusters meet FWE corrected $p < 0.001$, *The clusters meet FWE corrected $p < 0.05$.L, left, R, right.*

compared to healthy controls. It is possible that the 500 ms window, which was the lapse threshold defined by studying healthy volunteers, may need to be adjusted when studying clinical populations, who may have limiting motoric or sensory deficits. Notwithstanding this concern, our study suggests that the 20-min PVT is sufficient to invoke statistically significant differences in perceptions of fatigue and performance fatigability and supports the utility of continuous PVT as an appropriate paradigm to induce and examine fatigue in PD.

There were significant positive correlations of patients' experience of fatigue in the week preceding the study (i.e., *via* the FSS) with their self-reported mental fatigue changes assessed immediately before and after the task (i.e., *via* a VAS), as well as a trend of correlation with performance declines

**TABLE 2 |** Brain areas showing CBF changes for the last quintile of the PVT comparing to the first quintile of the PVT (PVTq5 vs. PVTq1).

| Region | Cluster size | MNI coordinates | | | Peak Z | Peak *p* |
|---|---|---|---|---|---|---|
| | | x | y | z | | (uncorrected) |
| **CBF increases: PVTq5-PVTq1** | | | | | | |
| R. Superior Temporal Gyrus | 374 | 58 | −2 | 0 | 3.89 | < 0.001* |
| R. Hippocampus | 57 | 34 | −24 | −6 | 3.43 | < 0.001 |
| **CBF decreases: PVTq1-PVTq5** | | | | | | |
| L. Cerebellum | 169 | −2 | −64 | −22 | 3.80 | < 0.001 |
| L. Occipital Cortex | 113 | −50 | −58 | −6 | 3.54 | < 0.001 |
| L. Middle Frontal Gyrus | 64 | −30 | 26 | 30 | 3.53 | < 0.001 |
| L. Middle Frontal Gyrus | 80 | −30 | 40 | 16 | 3.42 | < 0.001 |

*Thresholds were set as uncorrected p < 0.001. *The clusters meet FWE corrected p < 0.05.L, left, R, right.*

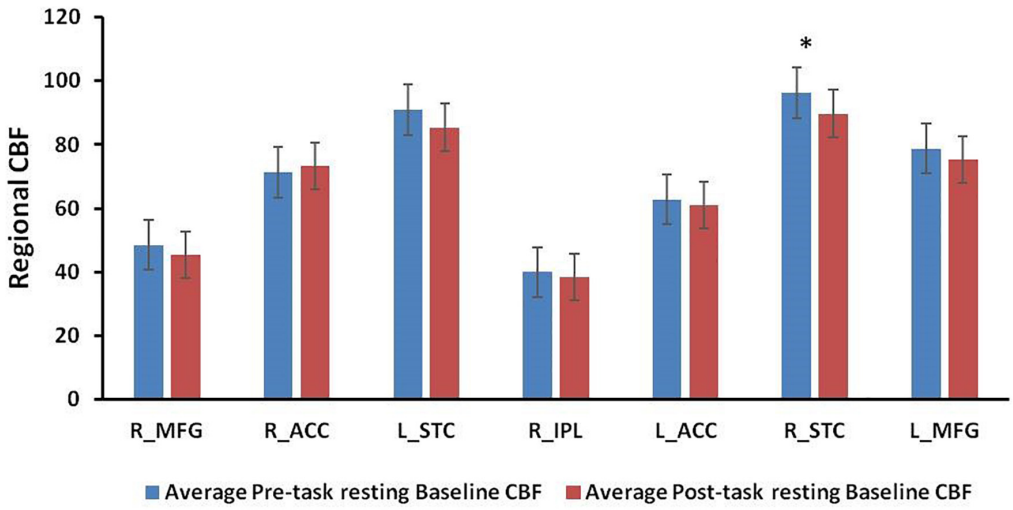


**FIGURE 4 |** Regional CBF values at pre-task and post-task resting baselines in the region-of-interests (ROIs) from previous healthy control study. *$p < 0.05$.

during the PVT. However, mental fatigue changes did not correlate with task performance declines. These findings are consistent with a previous study reporting that PD-related fatigue is not associated with performance in a motor task without cue (Martino et al., 2016). However, they reported a link between PD-related fatigue and performance in an externally cued, attention-demanding version of the same task. These results are consistent with the notion that task-induced subjective fatigue and objective fatigability are two independent processes, though both appear to be influenced by perceived general fatigue before the task. That is, individuals with higher levels of general fatigue are more susceptible to the detrimental effects of sustained mental demands.

Significant task-related CBF changes were observed in the right MFG, bilateral occipital cortex, and the right cerebellum using ASL fMRI. The MFG activation in PD subjects is similar to the activation we found in healthy volunteers (Lim et al., 2010), indicating the critical role of the prefrontal cortex in tasks requiring vigilance and continuous performance in both the healthy and clinical populations. Similar activation of this area has also been reported in a variety BOLD fMRI studies employing comparable attention tasks (Fan et al., 2005; Ogg et al., 2008). The current study also found activation in cerebellum, which was not reported in our previous study. While primarily associated with motor control, the cerebellum has also been implicated in range of cognitive functions including attention (Brittain and Brown, 2013; Buckner, 2013). Thus, it is not surprising for the involvement of the cerebellum when performing the PVT in PD.

Compared to the pre-task resting baseline, post-task resting baseline scans showed significant deactivation in the right MFG, an area that overlapped with above PVT activated frontal region, indicating a persistent effect of brain fatigue in this area. Increased right MFG activation during the PVT is consistent with a previous study reporting compensatory hyper-activation in the fronto-parietal regions in PD patients to maintain attention performance and executive functions (Gerrits et al., 2015). The right MFG deactivation may reflect some residual cerebral effort in PD patients after a prolonged mental demanding task, which needs to be examined in future studies.

We also examined ROIs from our previous study where CBF decreases were observed from pre-task to post-task baselines in healthy subjects (Lim et al., 2010). In the current

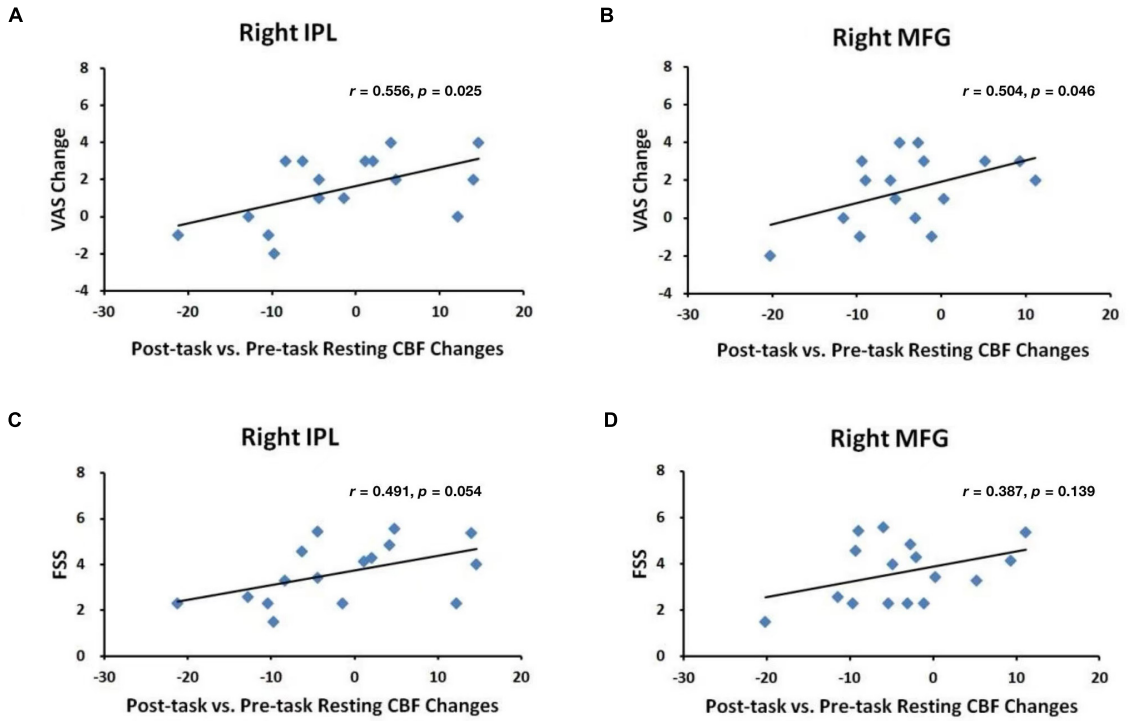


**FIGURE 5 |** Correlations between VAS changes and post-task regional CBF changes in the **(A)** right IPL and **(B)** right MFG and between FSS scores and post-task regional CBF changes in the **(C)** right IPL and **(D)** right MFG.

study, unlike healthy subjects, PD patients did not show significant CBF reduction from pre-task baseline to post-task baseline in most of these ROIs. Moreover, PD patients and healthy subjects showed differential correlations between changes in regional CBF, fatigue ratings, and PVT performance. In PD patients, regional CBF changes in the right MFG and the right IPL were significantly correlated with self-reported mental fatigue increases, but not RT increases. In contrast, regional CBF changes in these fronto-parietal regions were significantly correlated with RT increases, but not self-reported mental fatigue increases in healthy subjects (Lim et al., 2010). A potential explanation is that healthy subjects have the ability to consciously allocate their available cognitive and brain resources to preserve better performance over time, but such ability in PD patients may be impaired due to overload in their sustained attentional capacity during continuous time-on-task effects. As our findings demonstrated, the mean RT of the first quintile for PD patients is close to that of the last quintile in healthy subjects. The overloaded cognitive and brain activities may inevitably cause patients to experience fatigue. Taken together, these findings suggest that fronto-parietal regions may play different roles in mediating self-reported fatigue experience and cognitive fatigability in PD patients and healthy subjects, respectively.

Our study highlights the role of fronto-parietal attention network in mediating cognitive fatigue in PD, which are consistent with the fatigue literature. For example, Sepulcre et al. (2009) suggested an association between the symptom of fatigue and a disruption of brain networks involved in cognitive/attentional processes in MS. Our findings also suggest that using cerebral activity as a gauge for fatigability may result in improved correlations with subjective fatigue changes in persons with PD than performance declines.

The current study has several limitations. First, given the small sample size, our results may not be generalized to more heterogeneous samples. It is also unclear whether these findings are specific to PD patients. For example, fatigue in PD could be influenced by depressive symptoms (Katsarou et al., 2007). Even though those patients with major depressive disorder have been excluded form the present study, we cannot fully rule out the effect of depressive symptoms on the results. Thus future studies are needed to replicate these findings with a much larger sample size and better controlling for different confounding factors. Second, because no control group was included in this study, we were unable to compare the differential findings between PD patients and our previous study of healthy subjects (Lim et al., 2010). For example, CBF decrease from pre-task to post-task baseline scans was correlated with healthy individual's ability to better maintain performance (Lim et al., 2010), while in the current study, we did not find such correlation in PD patients. Nevertheless, we speculate that this difference may be due to the minimal level of perceived general fatigue in healthy populations before the study. On the other hand, ages of PD patients in this study are much older than ages of healthy controls in our

previous study (Lim et al., 2010), therefore we cannot be certain whether fatigue levels, demographic variables such as age, or their interaction ultimately explains the discrepancy. Future studies including both PD patients and age-matched healthy controls are necessary to understand how fatigue specifically manifests in the PD-affected brain under the same conditions as the healthy brain.

In summary, the current study utilized behavioral, psychological, and brain imaging measurements and demonstrated that the continuous PVT paradigm could successfully induce subjective fatigue and performance fatigability. We found that CBF changes in the right fronto-parietal network between the resting periods before and after the PVT were correlated with subjective fatigue changes, which suggest the critical role of this network in mediating fatigue in PD. These findings support the utility of continuous PVT paradigm to induce cognitive fatigue in PD and ASL perfusion imaging as a reproducible method for quantification of fatigue-related brain activity changes from sustained mental workload. Given that PD is a major global health concern affecting over 9 million people worldwide (Maserejian et al., 2020) and fatigue is one of the major non-motor symptoms of PD that impairs daily activity for PD patients, our study sheds light on the importance of understanding the brain mechanisms underlying fatigue to potentially help develop more effective treatment and prevention strategies.

## 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 the University of Pennsylvania Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

HR conceived the overall project and edited the manuscript. WL, RB, and EM conducted the study and analyzed the data. WL, JL, and RB drafted the original manuscript. All other authors reviewed and edited the manuscript for important scientific content and final approval.

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## REFERENCES

Abe, K., Takanashi, M., and Yanagihara, Y. (2000). Fatigue in patients with Parkinson's disease. *Behav. Neurol.* 12, 103–106. doi: 10.1155/2000/580683

Aguirre, G. K., Detre, J. A., Zarahn, E., and Alsop, D. C. (2002). Experimental design and the relative sensitivity of BOLD and perfusion fMRI. *Neuroimage* 15, 488–500. doi: 10.1006/nimg.2001.0990

Aguirre, G. K., Zarahn, E., and D'Esposito, M. (1997). Empirical analyses of BOLD fMRI statistics. II. Spatially smoothed data collected under null-hypothesis and experimental conditions. *Neuroimage.* 5, 199–212. doi: 10.1006/nimg.1997.0264

Berard, J. A., Fang, Z., Walker, L. A. S., Lindsay-Brown, A., Osman, L., Cameron, I., et al. (2020). Imaging cognitive fatigability in multiple sclerosis: objective quantification of cerebral blood flow during a task of sustained attention using ASL perfusion fMRI. *Brain Imaging Behav.* 14, 2417–2428. doi: 10.1007/s11682-019-00192-7

Brittain, J. S., and Brown, P. (2013). Oscillations and the basal ganglia: motor control and beyond. *NeuroImage.* 85, 637–647. doi: 10.1016/j.neuroimage.2013.05.084

Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron.* 80, 807–815. doi: 10.1016/j.neuron.2013.10.044

Buysse, D. J., Reynolds, C. F. III, Monk, T. H., Berman, S. R., and Kupfer, D. J. (1989). The pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213. doi: 10.1016/0165-1781(89)90047-4

Chaudhuri, A., and Behan, P. O. (2000). Fatigue and basal ganglia. *J. Neurol. Sci.* 179, 34–42. doi: 10.1016/s0022-510x(00)00411-1

Cho, S. S., Aminian, K., Li, C., Lang, A. E., Houle, S., and Strafella, A. P. (2017). Fatigue in parkinson's disease: the contribution of cerebral metabolic changes. *Hum. Brain Mapp.* 38, 283–292. doi: 10.1002/hbm.23360

Detre, J. A., and Wang, J. (2002). Technical aspects and utility of fMRI using BOLD and ASL. *Clin. Neurophysiol.* 113, 621–634. doi: 10.1016/s1388-2457(02)00038-x

Dinges, D. F., Pack, F., Williams, K., Gillen, K. A., Powell, J. W., Ott, G. E., et al. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep* 20, 267–277.

Dolui, S., Tisdall, D., Vidorreta, M., Jacobs, D. R. Jr., Nasrallah, I. M., Bryan, R. N., et al. (2019). Characterizing a perfusion-based periventricular small vessel region of interest. *Neuroimage Clin.* 23:101897. doi: 10.1016/j.nicl.2019.101897

Dorrian, J., Rogers, N. L., and Dinges, D. F. (2005). "Psychomotor vigilance performance: a neurocognitive assay sensitive to sleep loss," in *Sleep Deprivation: Clinical Issues, Pharmacology and Sleep Loss Effects*, ed. C. Kushida (New York, NY: Marcel Dekker, Inc), 39–70.

Durmer, J. S., and Dinges, D. F. (2005). Neurocognitive consequences of sleep deprivation. *Semin Neurol.* 25, 117–129. doi: 10.1055/s-2005-867080

Fan, J., McCandliss, B. D., Fossella, J., Flombaum, J. I., and Posner, M. I. (2005). The activation of attentional networks. *NeuroImage.* 26, 471–479. doi: 10.1016/j.neuroimage.2005.02.004

Friedman, J., and Friedman, H. (1993). Fatigue in Parkinson's disease. *Neurology.* 43, 2016–2018. doi: 10.1212/WNL.43.10.2016

Friedman, J. H., Abrantes, A., and Sweet, L. H. (2011). Fatigue in parkinson's disease. *Exp. Opin. Pharmacother.* 12, 1999–2007. doi: 10.1517/14656566.2011.587120

Friedman, J. H., Brown, R. G., Comella, C., Garber, C. E., Krupp, L. B., Lou, J. S., et al. (2007). Fatigue in Parkinson's disease: a review. *Mov. Disord.* 22, 297–308. doi: 10.1002/mds.21240

Gerrits, N. J., van der Werf, Y. D., Verhoef, K. M., Veltman, D. J., Groenewegen, H. J., Berendse, H. W., et al. (2015). Compensatory fronto-parietal hyperactivation during set-shifting in unmedicated patients with Parkinson's disease. *Neuropsychologia.* 68, 107–116. doi: 10.1016/j.neuropsychologia.2014.12.022

Hagell, P., and Brundin, L. (2009). Towards an understanding of fatigue in Parkinson disease. *J. Neurol. Neurosurg. Psychiatry.* 80, 489–492. doi: 10.1136/jnnp.2008.159772

Herlofson, K., and Larsen, J. P. (2003). The influence of fatigue on health-related quality of life in patients with Parkinson's disease. *Acta. Neurol. Scand.* 107, 1–6. doi: 10.1034/j.1600-0404.2003.02033.x

Herlofson, K., Ongre, S. O., Enger, L. K., Tysnes, O. B., and Larsen, J. P. (2012). Fatigue in early Parkinson's disease. Minor inconvenience or major distress? *Eur. J. Neurol.* 19, 963–968. doi: 10.1111/j.1468-1331.2012.03663.x

Hoehn, M. M., and Yahr, M. D. (1967). Parkinsonism: onset, progression, and mortality. *Neurology.* 17, 427–442. doi: 10.1212/wnl.17.5.427

Katsarou, Z., Bostantjopoulou, S., Hatzizisi, O., Giza, E., and Solercardona, A. (2007). Immune factors or depression? fatigue correlates in parkinson's disease. *Rev. Neurol.* 45, 725–728.

Kim, J., Whyte, J., Wang, J., Rao, H., Tang, K. Z., and Detre, J. A. (2006). Continuous ASL perfusion fMRI investigation of higher cognition: quantification of tonic CBF changes during sustained attention and working memory tasks. *NeuroImage.* 31, 376–385. doi: 10.1016/j.neuroimage.2005.11.035

Kluger, B. M., Brown, R. P., Aerts, S., and Schenkman, M. (2014). Determinants of objectively measured physical functional performance in early to mid-stage Parkinson disease. *PMR.* 6, 992–998. doi: 10.1016/j.pmrj.2014.05.013

Kluger, B. M., Krupp, L. B., and Enoka, R. M. (2013). Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. *Neurology.* 80, 409–416. doi: 10.1212/WNL.0b013e31827f07be

Lim, J., and Dinges, D. F. (2008). Sleep deprivation and vigilant attention. *Ann. N Y Acad Sci.* 1129, 305–322. doi: 10.1196/annals.1417.002

Lim, J., Wu, W. C., Wang, J., Detre, J. A., Dinges, D. F., and Rao, H. Y. (2010). Imaging brain fatigue from sustained mental workload: an ASL perfusion study of the time-on-task effect. *Neuroimage.* 49, 3426–3435. doi: 10.1016/j.neuroimage.2009.11.020

Lou, J. S. (2009). Physical and mental fatigue in Parkinson's disease: epidemiology, pathophysiology and treatment. *Drugs Aging.* 26, 195–208. doi: 10.2165/00002512-200926030-00002

Martino, D., Tamburini, T., Zis, P., Rosoklija, G., Abbruzzese, G., Ray-Chaudhuri, K., et al. (2016). An objective measure combining physical and cognitive fatigability: correlation with subjective fatigue in Parkinson's disease. *Park. Relat. Disord.* 32, 80–86. doi: 10.1016/j.parkreldis.2016.08.021

Maserejian,, N., Vinikoor-Imler, L., and Dilley, A. (2020). Estimation of the 2020 Global Population of Parkinson's Disease (PD) [abstract]. *Mov. Disord.* 35(Suppl. 1), S79–S80.

Ogg, R. J., Zou, P., Allen, D. N., Hutchins, S. B., Dutkiewicz, R. M., and Mulhern, R. K. (2008). Neural correlates of a clinical continuous performance test. *Magn. Reson Imaging.* 26, 504–512. doi: 10.1016/j.mri.2007.09.004

Olson, I. R., Rao, H., Moore, K. S., Wang, J., Detre, J. A., and Aguirre, G. K. (2006). Using perfusion fMRI to measure continuous changes in neural activity with learning. *Brain Cogn.* 60, 262–271. doi: 10.1016/j.bandc.2005.11.010

Pavese, N., Metta, V., Bose, S. K., Chaudhuri, K. R., and Brooks, D. J. (2010). Fatigue in Parkinson's disease is linked to striatal and limbic serotonergic dysfunction. *Brain.* 133, 3434–3443. doi: 10.1093/brain/awq268

Pfeiffe, R. F. (2016). Non-motor symptoms in Parkinson's disease. *Park. Relat Disord.* 22(Suppl. 1), s119–s122.

Rao, H., Gillihan, S. J., Wang, J. J., Korczykowski, M., Sankoorikal, G. M. V., Kaercher, K. A., et al. (2007b). Genetic variation in serotonin transporter alters resting brain function in healthy individuals. *Biol. Psychiatry.* 62, 600–606. doi: 10.1016/j.biopsych.2006.11.028

Rao, H., Wang, J., Tang, K., Pan, W., and Detre, J. A. (2007a). Imaging brain activity during natural vision using CASL perfusion fMRI. *Hum. Brain Mapp.* 28, 593–601. doi: 10.1002/hbm.20288

Rao, H., Wang, J. J., Giannetta, J., Korczykowski, M., Shera, D., Avants, B. B., et al. (2007c). Altered resting cerebral blood flow in adolescents with in utero cocaine exposure revealed by perfusion functional MRI. *Pediatrics.* 120, e1245–e1254. doi: 10.1542/peds.2006-2596

Sepulcre, J., Masdeu, J. C., Goni, J., Arrondo, G., de Mendizabal, N. V., Bejarano, B., et al. (2009). Fatigue in multiple sclerosis is associated with the disruption of frontal and parietal pathways. *Mult. Scler.* 15, 337–344. doi: 10.1177/1352458508098373

Tandberg, E., Larsen, J. P., Nessler, E. G., Riise, T., and Aarli, J. A. (1995). The epidemiology of Parkinson's disease in the county of Rogaland, Norway. *Mov. Disord.* 10, 541–549. doi: 10.1002/mds.870100503

Tessitore, A., Giordano, A., Micco, R. D., Caiazzo, G., Russo, A., Cirillo, M., et al. (2016). Functional connectivity underpinnings of fatigue in "Drug-Naïve" patients with Parkinson's disease. *Mov. Disord.* 31, 1497–1505. doi: 10.1002/mds.26650

van Hilten, J. J., Weggeman, M., van der Velde, E. A., Kerkhof, G. A., van Dijk, J. G., and Roos, R. A. C. (1993). Sleep, excessive daytime sleepiness and fatigue in parkinson's disease. *J. Neural Transm. Park. Dis. Dement.* 5, 233–244. doi: 10.1007/BF02257678

van Rooden, S. M., Visser, M., Verbaan, D., Marinus, J., and van Hilten, J. J. (2009). Motor patterns in Parkinson's disease: a data-driven approach. *Mov. Disord.* 24, 1042–1047. doi: 10.1002/mds.22512

Wang, C., Ding, M., and Kluger, B. M. (2014). Change in intraindividual variability over time as a key metric for defining performance-based cognitive fatigability. *Brain Cogn.* 85, 251–258. doi: 10.1016/j.bandc.2014.01.004

Wang, J., Rao, H., Wetmore, G. S., Furlan, P. M., Korczykowski, M., Dinges, D. F., et al. (2005). Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. *Proc. Natl. Acad. Sci. U.S.A.* 102, 17804–17809. doi: 10.1073/pnas.0503082102

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# Worse Sleep Quality Aggravates the Motor and Non-Motor Symptoms in Parkinson's Disease

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**Backgrounds:** Sleep disorders are the most common and disabling symptoms in patients with Parkinson's disease (PD). Understanding the associations between sleep characteristics and motor and non-motor symptoms (NMSs) in PD can provide evidence to guide therapeutic interventions and nursing strategies. We aimed to investigate the association between sleep characteristics and motor function and NMSs in PD using multiple approaches.

**Methods:** A total of 328 participants were included, and all participants underwent Pittsburgh Sleep Quality Index (PSQI) evaluation and clinical assessments of PD symptoms. We conducted Spearman's correlation to evaluate the associations between sleep and PD symptoms, nonlinear regression to assess the relationships between sleep habits and PD, and mediated analyses to test the effects of NMSs on global PSQI and PD severity, quality of life, and motor symptoms.

**Results:** Poor sleep was associated with more severe PD symptoms. In addition, the reflection point for bedtime was around 21:52, associated with motor symptoms, and insufficient and excessive total time spent in bed and nocturnal sleep duration were correlated with higher NMS burdens. The optimal points were 8–9.2 and 6.2–6.9 h, respectively. It was also discovered that NMSs played the mediating roles in global sleep with the quality of life, PD stages, and motor symptoms to a varying range of 6.8–95.4%.

**Conclusions:** Sleep disorders have a significant effect on the burden of PD symptoms. The current findings provide new insights into the monitoring and management of sleep and PD and need to be further explored in the future studies.

**Keywords: Parkinson's disease, sleep characteristics, motor symptoms, non-motor symptoms, Pittsburgh Sleep Quality Index**

## INTRODUCTION

Parkinson's disease (PD), the second most common neurodegenerative disease in the elderly, is brought into focus since it cannot be cured; therefore, an increasing number of studies pay close attention to early diagnosis of PD (Stefani and Högl, 2020). The main motor manifestations of PD are resting tremor, bradykinesia, muscle rigidity, and walking and gesture abnormalities. The appearance of non-motor symptoms (NMSs) has been proven earlier than that of motor

symptoms. NMSs are the most disabling symptoms of PD and are often neglected. Therefore, it is of great value to explore the clinical characteristics of NMSs for early diagnosis and intervention (Sveinbjornsdottir, 2016). Sleep disorders are among the most frequent NMSs, with a prevalence of 20–90% (Cai et al., 2019), and are also regarded as life-changing symptoms due to their significant impact on the quality of life (Jasti et al., 2018). It needs to be identified and managed early to improve motor symptoms.

Sleep–wake disturbances have been reported to precede the diagnosis of Parkinsonism in prodromal PD (Al-Qassabi et al., 2017). The pathophysiology is unknown and may be caused by multiple factors, including the effects of motor function and NMSs on sleep. PD-related disturbed sleep is largely attributed to central sleep regulation center degeneration in the thalamus and brainstem, including difficulty falling asleep, decreasing total sleep duration and efficacy, frequent awakenings, and increasing daytime sleepiness (Zhu et al., 2016). Furthermore, dopaminergic therapies can precipitate sleep disturbances (Breen et al., 2014), and changes in non-dopaminergic neurotransmitter levels due to PD progression affect sleep (Diederich et al., 2005). According to the previous studies, sleep maintenance insomnia due to disrupted sleep occurs most commonly, followed by early morning awakening (Ylikoski et al., 2015). Studies using polysomnography have also revealed that patients with PD have shorter total sleep time and lower sleep efficiency using polysomnography (Yong et al., 2011; Selvaraj and Keshavamurthy, 2016). Moreover, worsening of sleep disturbances and other neuropsychiatric complaints may contribute to the progression of NMSs, and a broad range of sleep disorders are seen, with potential interactions existing in various disorders (Bargiotas et al., 2016). For instance, sleep disorders are related to emotional and cognitive impairment, as well as decreased quality of life, which augments the morbidity and mortality in the PD population (Pandey et al., 2016; Bohnen and Hu, 2019). Sleep quality is defined in terms of subjective complaints, and self-reported sleep measures are quick, brief, and cost-effective ways to measure sleep disturbances (Zea-Sevilla and Martínez-Martín, 2014). A host of scales has been applied for the evaluation of sleep disorders; however, only a few scales can be used to assess sleep quality in patients with PD (Högl et al., 2010). The Pittsburgh Sleep Quality Index (PSQI) is the most widely used scale in clinical practice and allows the rate of sleep quality to select appropriately targeted therapies (Videnovic et al., 2017).

Although studies have investigated the clinical characteristics of sleep disturbances among patients with PD, this relationship remains unclear and warrants further investigation. In addition, whether PD clinical characteristics, such as disease severity, prescription of dopaminergic medication, and other NMSs, are associated with sleep disorders remains controversial. Therefore, this study aimed to investigate the association between sleep characteristics and motor and non-motor dysfunctions of PD in this single-center observational cross-sectional study. An improved understanding of the common causes of sleep and PD characteristics is crucial and can provide evidence to guide therapeutic interventions and nursing strategies for patients with PD.

# METHODS

## Study Participants

This observational, single-center, cross-sectional study enrolled 551 participants who visited the Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from October 2014 to October 2021. PD was diagnosed based on the most recent clinical criteria (Postuma et al., 2015). Participants were excluded if they (1) were diagnosed with Parkinsonism-plus syndrome, including multiple system atrophy, progressive supranuclear palsy, and Lewy body dementia; (2) had a history of surgery, including stereotactic nerve nuclei lesions and deep brain stimulation; and (3) had a history of psychiatric symptoms, cancer, or any serious cardiovascular complications. This study was approved by the Medical Ethics Committee of Tongji Hospital, Tongji College of Medicine, Huazhong University of Science and Technology (Wuhan, China). Finally, 328 patients with PD were included, whereas 96 participants were excluded due to the above diagnosis and diseases, as well as 127 for incomplete data. Written informed consent was obtained from the participants or their legally acceptable representatives.

## Sleep Characteristics Assessment Headings

Sleep characteristics were measured using the PSQI (Högl et al., 2010). The PSQI is a self-reported questionnaire evaluating sleep quality during the last 1 month, consisting of 19 individual items that generate seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction). Each sleep component had a score of 0–3 and the total score ranged from 0 to 21, corresponding to increasingly impaired sleep. Total scores $\leq$5, 6–10, and $>$10 were defined as good, general, and poor sleep, respectively, whereas each component scores 0–3 as good, general, poor, and poorer, respectively. The questionnaire also included some specific questions, such as bedtime, getup time, total time spent in bed (TIB), and nocturnal sleep duration (NSD); however, approximately one-fourth of the participants did not provide these data.

## Clinical Assessments

Detailed history taking and examination were performed for all subjects, including the evaluations of demographic and clinical characteristics (i.e., age, sex, educational level, body mass index [BMI], disease onset age, disease duration, and daily levodopa equivalent dose [LEDD]), disease severity, motor subtypes, and motor and NMSs. Trained researchers performed all examinations.

Early onset PD (EOPD) and late-onset PD (LOPD) were defined as the disease onset age before and after 50 years of age, respectively. Additionally, tremor dominant (TD) and postural instability gait disorder (PIGD) were divided based on the ratio of

TD to PIGD scores: patients with ratios >1.15 were classified into TD, those with ratios <0.90 to the PIGD, and patients with ratios among 0.90–1.15 were classified as "undetermined" (Stebbins et al., 2013). LEDD was calculated according to the common conversion formulae (Tomlinson et al., 2010), and almost all patients with PD were on the medication for their condition at the time of evaluation.

Motor symptoms were measured using the Hoehn and Yahr (H&Y) stage (Goetz et al., 2004), as well as the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part II and III (Goetz et al., 2008), whereas the quality of life was assessed using the 39-Item Parkinson's Disease Questionnaire (PDQ-39) (Jenkinson et al., 1997). The MDS-UPDRS-IV was applied to assess PD complications, and the Freezing of Gait Questionnaire (FOG-Q) was used to evaluate FOG. In addition, NMSs were evaluated using various scales (van Wamelen et al., 2021). Specifically, the MDS-UPDRS-I and Non-Motor Symptoms Scale (NMSS) were used for NMS burden; cognitive function was examined using the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA); Hamilton Rating Scale for Depression (HAMD) and Anxiety (HAMA) were used to evaluate mood symptoms; Scales for Outcomes in Parkinson's Disease-Autonomic (SCOPA-AUT) was used to assess autonomic dysfunction; and the Apathy Scale (AS) was used for apathy evaluation.

## Statistical Analyses

Differences in demographic characteristics between the good, general, and poor sleep groups were examined using the Mann–Whitney $U$ test (continuous variables) and $\chi^2$ test (categorical variables). Spearman's rank correlations were used to explore the associations between sleep parameters (sleep components and times) and the clinical characteristics of PD. Furthermore, non-linear regression analyses using the quadratic model ($y = \alpha x^2 + \beta x + c$) were employed to investigate the non-linear associations between sleep habits (bed time, getup time, TIB, and NSD) and PD characteristics (Hayes and Preacher, 2010). When the coefficient of the quadratic term was significantly larger (U-shaped) or smaller (reverse U-shaped) than zero, significant non-linear associations were observed. Most scales did not display a normal distribution (Kolmogorov–Smirnov test, $p < 0.05$); therefore, they were log10-transformed to approximate the normal distribution. The Bonferroni method was used for multiple corrections (for seven sleep components), except where specifically noted. Next, mediation analyses were used to evaluate whether the associations between sleep and PD severity were mediated by NMSs (Baron and Kenny, 1986). In each model, sleep characteristics were included as independent variables, and PD motor symptoms and quality of life were the dependent variables. All mediation tests were performed with 10,000 bootstrap replications.

All analyses were conducted using R (version 3.6.3) and GraphPad Prism version 8.0, and the statistical significance threshold was set at two-tailed $p < 0.05$. Bonferroni corrections were applied for multiple corrections of all sleep characteristics or PD scales.

**TABLE 1 |** The characteristics of participants.

| Characteristic | PSQI ($n = 328$) | | | $p$ |
|---|---|---|---|---|
| | Good ($n = 99$) | General ($n = 147$) | Poor ($n = 82$) | |
| **Demographic characteristics** | | | | |
| Age (SD), year | 59.4 (9.6) | 60.9 (10.9) | 61.2 (9.0) | 0.222 |
| Female%, *n* (%) | 35 (35.4) | 67 (45.6) | 43 (52.4) | 0.064 |
| Education (*SD*), year | 9.4 (4.0) | 9.0 (4.7) | 7.9 (4.4) | 0.069 |
| BMI (*SD*), Kg/m$^2$ | 23.5 (3.4) | 23.3 (3.2) | 23.4 (3.3) | 0.611 |
| Disease onset (*SD*), year | 55.4 (10.2) | 55.5 (10.9) | 55.6 (9.6) | 0.970 |
| Disease duration (*SD*), year | 4.0 (3.8) | 5.0 (4.7) | 5.3 (3.9) | **0.005** |
| Hoehn-Yahr stage (*SD*) | 1.9 (0.9) | 2.2 (1.0) | 2.4 (1.0) | **0.001** |
| LEDD (*SD*), mg | 526.9 (201.8) | 578.9 (265.1) | 629.2 (246.4) | **0.009** |
| FOG-Q ($n = 151$) | 4.0 (5.4) | 7.0 (6.6) | 9.0 (7.4) | **0.004** |
| **Subtypes** | | | | |
| Motor (TD/PIGD/Undetermined) | 41/53/5 | 61/79/7 | 26/53/3 | 0.554 |
| Onset age (EOPD/LOPD/Unclear) | 35/62/2 | 50/95/2 | 23/57/2 | 0.822 |
| **MDS-UPDRS** | | | | |
| UPDRS-I | 6.0 (3.8) | 9.3 (4.8) | 14.2 (5.9) | **<0.001** |
| UPDRS-II | 8.8 (6.7) | 10.8 (7.8) | 14.0 (8.4) | **<0.001** |
| UPDRS-III | 30.0 (16.5) | 31.8 (16.9) | 35.7 (18.6) | 0.091 |
| UPDRS-IV ($n = 293$) | 1.0 (2.7) | 1.1 (2.6) | 1.1 (2.7) | 0.697 |
| **Non-motor symptoms assessments** | | | | |
| MMSE | 25.6 (5.0) | 25.7 (4.3) | 23.8 (5.3) | **0.003** |
| MoCA ($n = 300$) | 21.4 (6.0) | 21.4 (5.7) | 20.6 (5.1) | 0.200 |
| HAMD | 9.2 (6.4) | 12.9 (6.9) | 18.0 (7.0) | **<0.001** |
| HAMA | 7.5 (5.2) | 11.3 (6.4) | 17.0 (6.7) | **<0.001** |
| PDQ-39 | 31.8 (21.3) | 42.7 (25.0) | 53.1 (25.4) | **<0.001** |
| SCOPA-AUT | 29.9 (5.6) | 33.7 (7.4) | 36.7 (7.9) | **<0.001** |
| NMSS | 28.3 (19.1) | 43.0 (29.2) | 72.4 (31.4) | **<0.001** |
| AS ($n = 219$) | 11.2 (10.5) | 14.5 (11.2) | 16.9 (13.9) | **0.046** |
| FSS ($n = 141$) | 24.6 (17.7) | 32.3 (18.5) | 37.5 (17.0) | **0.003** |

*AS, Apathy Scale; BMI, body mass index; EOPD, early-onset Parkinson's disease; FOG-Q, Freezing of Gait Questionnaire; FSS, Fatigue Severity Scale; HAMA, Hamilton Anxiety Rating Scale; HAMD, Hamilton Depression Rating Scale; LEDD, levodopa equivalent daily dose; LOPD, late-onset Parkinson's disease; UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental State Examination; n, number; MoCA, Montreal Cognitive Assessment; NMSS, Non-motor symptoms Scales; PDQ-39, 39-item Parkinson's Disease Questionnaire; PIGD, postural instability gait disorder; PSQI, Pittsburgh Sleep Quality Index; SCOPA-AUT, Scale for Outcomes in PD for Autonomic Symptoms; SD, standard deviation; TD, tremor dominant. The bold values were symbolized to statistically significant difference ($p < 0.05$).*

# RESULTS

## Participant Characteristics

The characteristics of the participants included in the present study are summarized in **Table 1**, whereas the detailed sleep characteristics are shown in **Figure 1**. A total of 328 patients with PD aged 60.5 years ($SD = 10.1$; 145 women) were enrolled. There were significant differences in disease duration among the three groups ($p = 0.005$), whereas there were no differences in age, sex, educational level, BMI, disease onset age, and subtypes (all $p >$

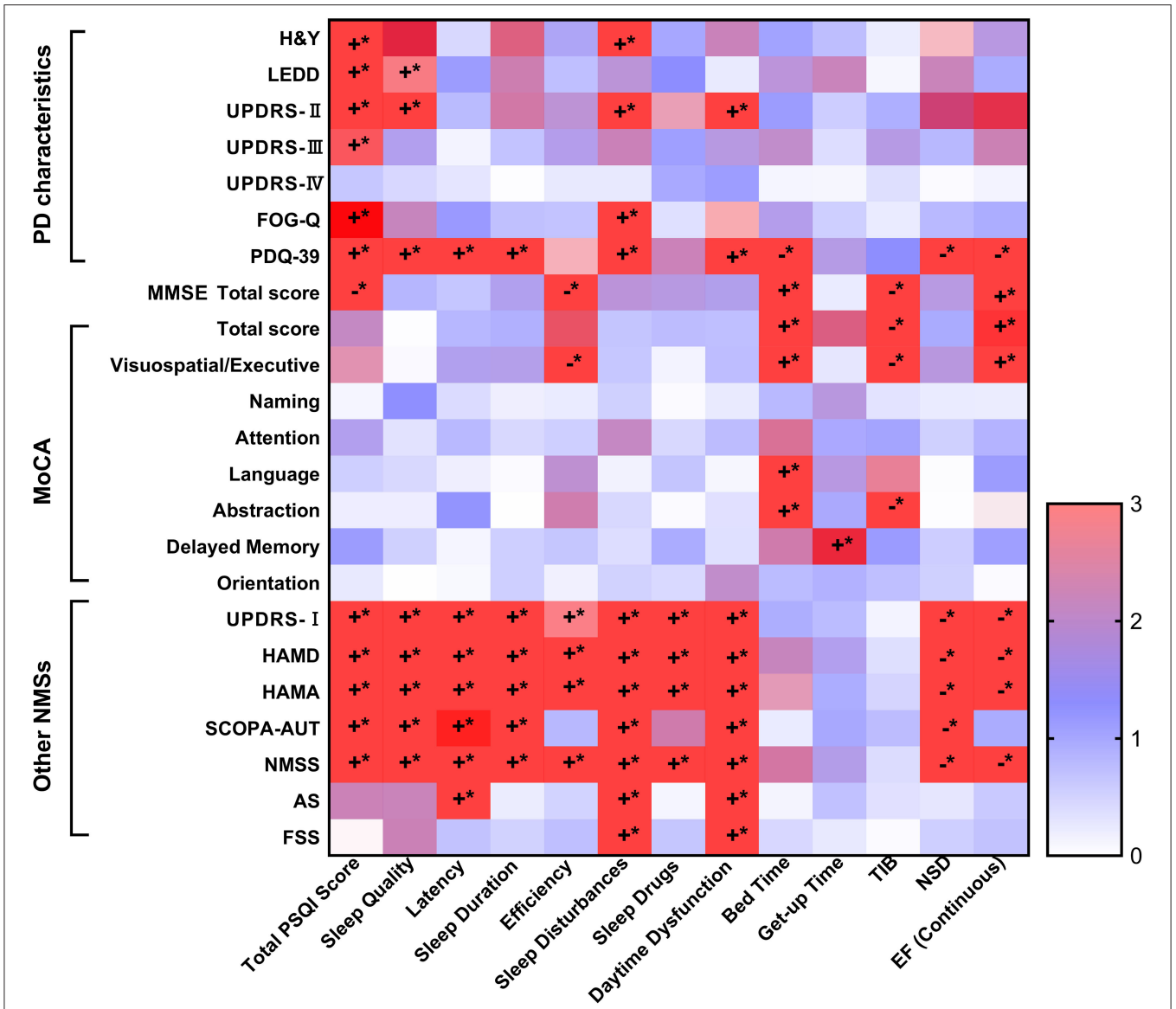


**FIGURE 1 |** The Spearman's correlations of PSQI and PD symptoms. The sleep characteristics and habits showed significant associations with PD characteristics, as well as motor and NMSs. Specifically, the increasing total PSQI scores were related to more severe PD characteristics, including higher scores of H&Y stages, UPDRS-II and III, FOG-Q and PDQ-39, as well as higher doses of LEDD. Poorer sleep levels were also associated to worse cognitive function, though only total MMSE score, orientation, and language and praxis survived after Bonferroni correction ($p < 0.002$). Further, it was illustrated that elevated total PSQI scores correlated with more serious NMSs covered NMS burden, depression, anxiety, autonomic dysfunction, apathy, and fatigue ($p < 0.05$). *The $p$-value was survived after Bonferroni correction ($p < 0.002$). *AS*, Apathy Scale; *EF*, efficiency; *FOG-Q*, Freezing of Gait Questionnaire; *FSS*, Fatigue Severity Scale; *HAMA*, Hamilton Anxiety Rating Scale; *HAMD*, Hamilton Depression Rating Scale; *H&Y,* Hoehn-Yahr stage; *LEDD*, levodopa equivalent daily dose; *MDS-UPDRS*, Movement Disorder Society Unified Parkinson's Disease Rating Scale; *MMSE*, Mini-Mental State Examination; *MoCA*, Montreal Cognitive Assessment; *NMSS*, Non-Motor Symptoms Scales; *NSD*, nocturnal sleep duration; *PD*, Parkinson's disease; *PDQ-39*, 39-item Parkinson's Disease Questionnaire; *PSQI*, Pittsburgh Sleep Quality Index; *SCOPA-AUT*, Scale for Outcomes in PD for Autonomic Symptoms, *TIB*, total time spent in bed. The "+" was symbolized to positive correlated, while the "−" was negative correlated. The "*" was on behalf of statistically significant difference ($p < 0.05$).

0.05). Additionally, poor sleep quality was associated with more severe motor and non-motor symptoms (**Table 1**).

## Spearman's Correlations of PSQI Domains and PD Characteristics

Sleep characteristics and habits were significantly associated with PD characteristics as well as motor and NMSs scores (**Figure 1**). Specifically, the increasing total PSQI scores were related to more severe PD characteristics, including higher H&Y stage scores, UPDRS-II and III, FOG-Q, and PDQ-39, as well as higher doses of LEDD (all Spearman's $p < 0.01$). Poorer sleep levels were also associated with worse cognitive function, although only the total MMSE score, total MoCA score, and executive function survived after Bonferroni correction ($p < 0.002$). Furthermore,

**TABLE 2 |** The associations of PSQI parameters with PD characteristics using nonlinear regression models.

| Characteristic | PSQI | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bed time | | | Get-up time | | | TIB | | | NSD | | |
| | β | α | Extreme point | β | α | Extreme point | β | α | Extreme point | β | α | Extreme point |
| **Motor symptoms** | | | | | | | | | | | | |
| UPDRS-II | **−0.543** | **0.028** | **21:52**[b] | ns | ns | ns | −0.193 | 0.012 | 7.9h[a] | ns | ns | ns |
| UPDRS-III | −0.362 | 0.017 | 22:24[a] | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| UPDRS-IV | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| FOG-Q | ns | ns | ns | ns | ns | ns | ns | ns | ns | **−2.287** | **0.186** | **6.2h**[b] |
| **Non-Motor Symptoms** | | | | | | | | | | | | |
| UPDRS-I | ns | ns | ns | ns | ns | ns | **−0.289** | **0.017** | **8.7h**[c*] | **−0.932** | **0.068** | **6.9h**[b] |
| MMSE | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| MOCA | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| HAMD | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| HAMA | ns | ns | ns | ns | ns | ns | **−0.196** | **0.012** | **8.2h**[b] | ns | ns | ns |
| PDQ-39 | −0.398 | 0.019 | 10:38[a] | ns | ns | ns | **−0.235** | **0.015** | **8.0h**[b] | −0.903 | 0.068 | 6.6h[a] |
| SCOPA-AUT | ns | ns | ns | ns | ns | ns | **−0.083** | **0.004** | **9.2h**[b] | ns | ns | ns |
| NMSS | ns | ns | ns | ns | ns | ns | **−0.309** | **0.018** | **8.4h**[c*] | **−1.741** | **0.133** | **6.6h**[c*] |
| AS | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| FSS | ns | ns | ns | ns | ns | ns | −0.204 | 0.012 | 8.7h[a] | ns | ns | ns |

[a] *p<0.10 (p for trend).*
[b] *p<0.05.*
[c] *p<0.01.*
[*] *The p-value was survived after Bonferroni correction (p < 0.008).*
*AS, Apathy Scale; FOG-Q, Freezing of Gait Questionnaire; FSS, Fatigue Severity Scale; HAMA, Hamilton Anxiety Rating Scale; HAMD, Hamilton Depression Rating Scale; UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NMSS, Non-motor symptoms Scales; NSD, nocturnal sleep duration; PDQ-39, 39-item Parkinson's Disease Questionnaire; PSQI, Pittsburgh Sleep Quality Index; SCOPA-AUT, Scale for Outcomes in PD for Autonomic Symptoms; TIB, total time spent in bed. The bold values were symbolized to statistically significant difference (p < 0.05).*

elevated total PSQI scores correlated with more serious NMS burden, depression, anxiety, autonomic dysfunction, apathy, and fatigue ($p < 0.05$). Additionally, different sleep components were associated with PD characteristics, as shown in **Figure 1** and **Supplementary Tables 1, 2**.

## Non-Linear Associations of Sleep Habits and PD Characteristics

There were significant non-linear relationships between sleep habits and motor and non-motor symptoms in individuals with PD (**Table 2**). The reflection point for bedtime was ∼21:52, associated with UPDRS-II, whereas no significant non-linear relationships were found for the getup time. The recommended TIB was ∼8–9.2 h, indicating that the fitted curve reached its peak (**Figure 2**). Both insufficient and excessive TIB are associated with a higher NMS burden. Conversely, the reflection points were suited 6.2–6.9 h, and either fewer and more were disadvantageous for PD characteristics, suggesting that the proper sleep duration was ∼6–7 h per day.

## Mediation Analyses

Mediation analyses were performed to investigate whether NMSs contributed to the quality of life and severity of PD as assessed by sleep levels (**Figure 3** and **Supplementary Tables 3–6**). We found that various scales of NMSs, consisting of the MDS-UPDRS-I, MMSE, HAMD, HAMA, SCOPA-AUT, NMSS, AS, and FSS, could mediate the effect of quality of life (PDQ-39) and the severity of PD (H&Y stages, MDS-UPDRS II and III) with a range of 6.8–95.4%. Anxiety, depression, and NMS burden showed leading mediated effects, whereas cognition and fatigue did not survive after the Bonferroni correction ($p < 0.001$).

# DISCUSSION

This study comprehensively evaluated the association of sleep characteristics and habits with motor symptoms and NMSs in patients with PD using a Chinese cohort. The primary results showed that poorer sleep levels were linked to more severe motor and non-motor symptoms in individuals with PD. Notably, we found several reflection points that showed the best bedtime, as well as the appropriate TIB and NSD, which were most beneficial to PD symptoms. Equally, we discovered that the associations between sleep quality and PD severity, quality of life, and motor symptoms were mediated by a number of NMSs to varying degrees, which provides new insights for clinical practice to intervene in PD deterioration with sleep disorders. Our findings also highlight the importance of increasing the awareness of sleep problems in patients with PD.

The PSQI was chosen to evaluate global sleep quality because it has been generally used for the occurrence of sleep disturbances in PD populations with good sensitivity and specificity (Pal

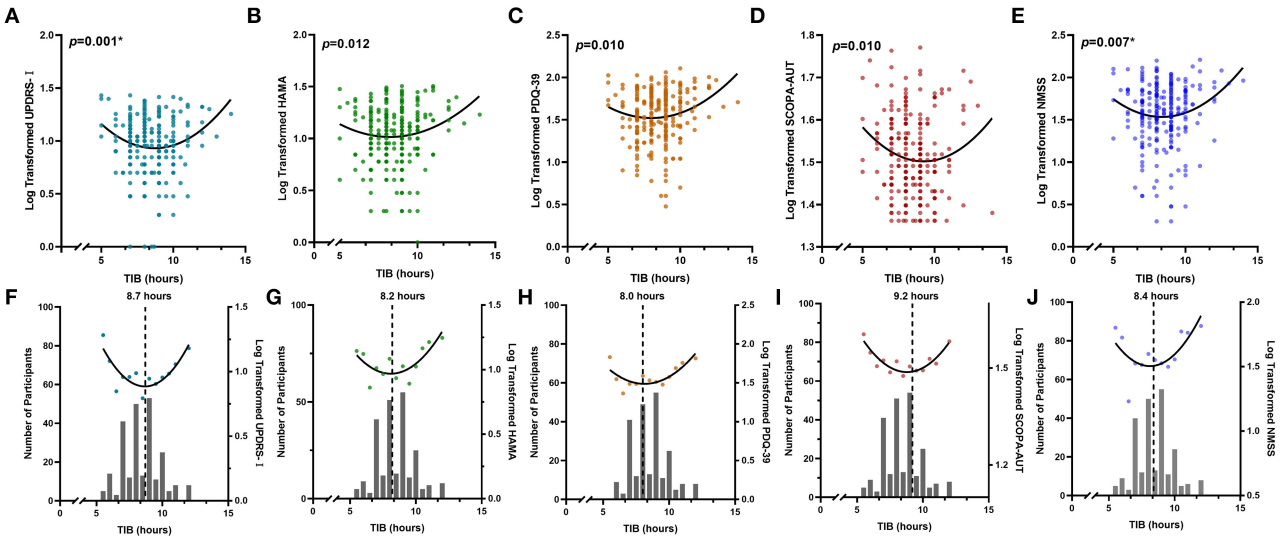


**FIGURE 2 |** Non-linear associations between sleep habits and PD symptoms. Total time spent in bed showed reverse U-shaped associations with different NMSs, including UPDRS-I **(A,F)**, HAMA **(B,G)**, PDQ-39 **(C,H)**, SCOPA-AUT **(D,I)**, and NMSS **(E,J)**. Either insufficient or excessive TIB is associated with worse performances of NMSs. The numbers of individuals with each sleep hour are shown in the bar graph. *The *p*-value was survived after Bonferroni correction ($p < 0.008$). *HAMA*, Hamilton Anxiety Rating Scale; *MDS-UPDRS*, Movement Disorder Society Unified Parkinson's Disease Rating Scale; *NMSS*, Non-Motor Symptoms Scales; *PDQ-39*, 39-item Parkinson's Disease Questionnaire; *SCOPA-AUT*, Scale for Outcomes in PD for Autonomic Symptoms; *TIB*, total time spent in bed.

et al., 2004). The PSQI has been reported to be sensitive to subjective sleep and therefore can be used to screen for the presence of sleep alteration and to rate the severity ulteriorly. Consistent with our results, previous studies have shown that poor sleep quality and efficiency are the promising markers of prodromal PD, and that an increased number of sleep disturbances and poor nighttime sleep are positively correlated with PD-related disability (Mao et al., 2018; Lysen et al., 2019). It is suggested that lesions of the lower brainstem expand and sleep disorders become more pronounced with PD deterioration (Braak et al., 2004). Motor symptoms aggravate the condition of patients, and they often combine with advanced multi-autonomic dysfunctions such as nocturia, nightmares, and restless leg syndrome, which further affect sleep. Since the maintenance of the sleep-wake cycle is closely related to dopamine (Lima, 2013), patients with PD with advanced motor symptoms tend to experience aggravation of sleep disturbances, which may be related to the degeneration of the dopaminergic system in the nigrostriatal pathway. Sleep disturbances may also contribute to the progression of PD symptoms (Lauretti et al., 2017). These nocturnal sleep problems can be aggravated by discomfort resulting from nighttime hypokinesia, particularly impaired bed turning; meanwhile, pain and other sensations caused by PD may also negatively influence sleep.

One novelty of this study is that we observed a U-shaped relationship between sleep habits and symptoms of PD. Bed time has shown positive non-linear associations with motor symptoms in patients with PD, whereas either excessive or insufficient TIB and NSD are unprofitable for the quality of life and NMSs. Several studies have reported that excessive sleep duration increases PD risk (Zhang et al., 2021), and others have found that too little nocturnal sleep duration increases the risk of motor symptoms (Lysen et al., 2019). Therefore, inadequate TIB and NSD could possibly lead to worse motor and NMS burden and even the deterioration of PD, which is consistent with our results. Likewise, disruptions to the sleep–wake cycle have been associated with inefficient metabolic clearance and increased oxidative stress in the central nervous system, which leads to excessive accumulation of alpha-synuclein and induction of neuronal loss, thus accelerating the pathogenesis and progression of PD. A cross-sectional study has proven that poor sleep, such as insufficient or excessive sleep duration and unsuitable sleep time, is associated with lower levels of cerebrospinal fluid alpha-synuclein in non-PD individuals (Wang et al., 2020). These U-shaped associations between sleep duration and PD can be explained from the perspectives of sleep quality and efficiency. As recommended, we claim that the optimum bed time, TIB, and NSD are approximately 22 o'clock, 8–9, and 6–7 h, respectively. Conversely, the overnight sleep for healthy age is often fragmented and lasts for <6.0–7.5 h due to the changes in sleep and circadian rhythms with aging (Gulia and Kumar, 2018). Compared with healthy elders, patients with PD have more serious sleep problems as a disorder of sleep maintenance, but also as a disorder of sleep onset or early morning awakening due to motor and non-motor symptoms. Therefore, it is more difficult, but important, for them to obtain sufficient sleep.

The impairment of sleep rhythm significantly impacts the quality of life in PD, including autonomic, cognitive, and psychiatric functions in patients with PD, in turn worsening motor manifestations and affective symptoms (Videnovic et al., 2014a). Additionally, non-motor problems, such as nocturia,

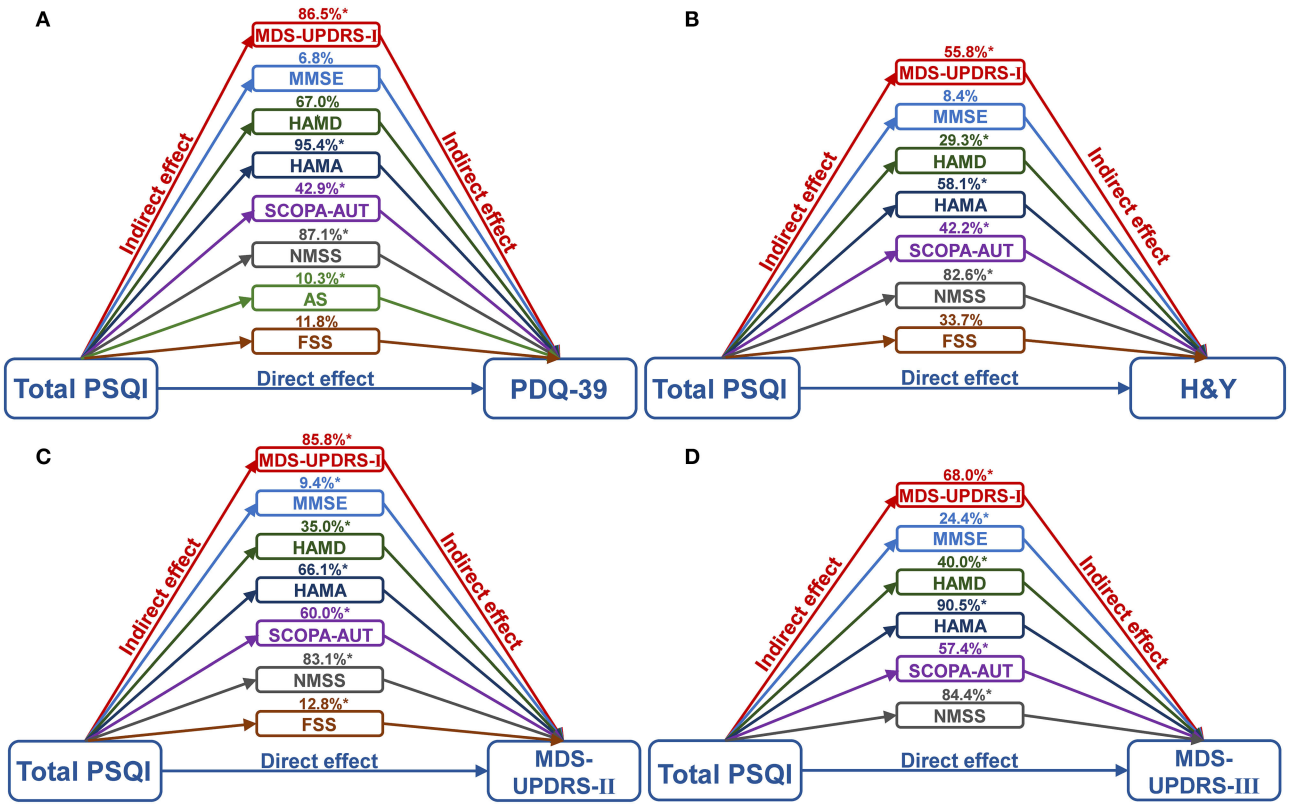


**FIGURE 3 |** Mediation analyses of NMSs with global sleep quality and PD. It was found that various scales of NMSs, consisting of MDS-UPDRS-I, MMSE, HAMD, HAMA, SCOPA-AUT, NMSS, AS, and FSS, could mediate the effect of quality of life **(A)**, and the severity of PD, including H&Y stages **(B)**, MDS-UPDRS II **(C)** and III **(D)** with a range of 6.8–95.4%. ***The** *p*-value was survived after Bonferroni correction ($p < 0.001$). *AS*, Apathy Scale; *FSS*, Fatigue Severity Scale; *HAMA*, Hamilton Anxiety Rating Scale; *HAMD*, Hamilton Depression Rating Scale; *MDS-UPDRS*, Movement Disorder Society Unified Parkinson's Disease Rating Scale; *MMSE*, Mini-Mental State Examination; n, number; *MoCA*, Montreal Cognitive Assessment; *NMSS*, Non-Motor Symptoms Scales; *PD*, Parkinson's disease; *PDQ-39*, 39-item Parkinson's Disease Questionnaire; *PSQI*, Pittsburgh Sleep Quality Index; *SCOPA-AUT*, Scale for Outcomes in PD for Autonomic Symptoms.

related pain, and neuropsychiatric symptoms, often give rise to sleep fragmentation. The generation and maintenance of the sleep–wake cycle depend on the central neurotransmitter systems that mainly involve 5-hydroxytryptamine, norepinephrine, and acetylcholine, and studies have indicated that these monoaminergic neurotransmitters play the important roles in regulating circuits related to emotion, psychology, stress, and cognitive performance (Thobois et al., 2017). The dynamic equilibrium and interaction of neurotransmitters determine normal functions of the circadian rhythm, and the neurons undergo degeneration and death to varying degrees in patients with PD, which can explain the significant correlation between NMSs and sleep disturbances. Another novel finding of this study is that non-motor complications, such as cognitive decline, depression, anxiety, autonomic dysfunction, apathy, and fatigue, partly mediate the impact of global sleep quality on PD severity, quality of life, and motor symptoms. These mediating effects varied from 10 to 90%, suggesting that NMSs and sleep disturbances are not independently linked to PD severity. Therefore, psychiatric symptoms contributed the most. These may be attributed to multi-system impairments, including the nigrostriatal dopaminergic system (Pfeiffer, 2016), and lead to the assumption that multiple coexisting conditions, such as motor and NMSs, contribute to sleep disorders (Kurtis et al., 2013). However, cognition and fatigue have less mediating effects, which suggests the independent roles in sleep.

Cognitive impairment is one of the most epidemic NMSs of PD, with a prevalence of 30–40%, and worse sleep efficiencies in PD subjects tend to be significantly associated with working memory and verbal memory consolidation (Emre, 2003). Our results also correlated with orientation and executive function. Anxiety and depression are the important factors that affect sleep status in patients with PD, and emotion generation and regulation are influenced by alterations in the frontal limbic systems (Palmer and Alfano, 2017). The relationships between sleep disturbances and emotional symptoms are bidirectional and can give rise to vicious circles for PD individuals, in which both reinforce each other (Rutten et al., 2017). A study reported that reduced melatonin secretion results in a 4-fold decrease in circulating melatonin levels in patients with PD (Videnovic et al., 2014b), often associated with cognitive decline and psychiatric disorders. Additionally, premature occurrence of

apathy symptoms may forebode the existence of sleep disorders with procession NMSs, and clinical diagnosis and treatment should pay more attention to apathy. Autonomic failure and fatigue are the pervasive problems associated with PD (Suzuki et al., 2014; Siciliano et al., 2018). Gastrointestinal distress, such as constipation and defecatory dysfunction, causes discomfort and an unpredictable need for toilets at night. Urinary symptoms such as nocturia are common among patients with PD and may also contribute to disrupted sleep as well (Bjørnarå et al., 2014). Sleep disturbances thus seem complex and likely multifactorial, and comorbidities probably play roles. However, sleep disorders in PD have not received sufficient attention and are consequently under-diagnosed and untreated. The treatment of sleep problems will likely improve NMSs but also postpone PD progression. It can potentially reduce the overall disability and thereby improve the lives of patients and their caregivers (Leroi et al., 2010).

Furthermore, sleep disorders were more likely to be positively correlated with higher LEDD in our study and previous studies (Verbaan et al., 2008; Antczak et al., 2013). Levodopa treatment is believed to improve sleep efficiency with reduced sleep latency by improving motor scores (Ferreira et al., 2014) and is also able to improve sleep quality partly by reducing night-time dyskinesia or tremor which severely interferes with normal sleep (Poewe et al., 2007). However, these drugs, particularly at higher doses, may cause insomnia and excessive daytime sleepiness, with a sudden onset of sleep attacks. One hypothesis is that poor sleep complainants receive higher doses of levodopa than good sleepers, rather than higher doses of levodopa, leading to poor sleep (Mao et al., 2017). Additionally, antiparkinsonian drugs do not work at midnight due to the levodopa doses in the blood, leading to various PD symptoms, and can cause vivid dreams, hallucinations, and paranoia, particularly at night. Our results showing that the associations between sleep and LEDD do not exist after Bonferroni correction have verified the complexities of levodopa treatment on sleep, which needs further exploration.

This study has several strengths and limitations. Our analyses systematically evaluated the sleep disorders and global PD characteristics and found worthy results such as optimal sleep habits for patients with PD. Meanwhile, the current findings stress the mediating effect of NMSs between sleep disorders and PD symptoms and quality of life, enabling the development of comprehensive therapeutic approaches. Conversely, the PSQI is a self-reported scale that does not reflect objective sleep quality, and there are different sleep disorders in PD beyond insomnia, such as apnea, rapid eye movement sleep behavior disorder, and excessive daytime sleepiness which could not be disentangled using the PSQI or any single scale that is needed to select objective measures such as polysomnography for sleep monitoring. In addition, this study only sought to propose strong hypotheses about the potential roles of sleep disorders in PD and will be examined in future longitudinal studies.

## CONCLUSIONS

In summary, our cross-sectional study indicated a close relationship between sleep characteristics and the burden of PD symptoms in patients with PD. Poor sleep quality and efficiency as well as insufficient or excessive sleep duration are associated with more severe PD symptoms, including PD stages, motor symptoms, NMSs, and quality of life. Therefore, our findings provide new insights into the monitoring and management of sleep and PD, which need to be further explored in the future studies.

## 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 Tongji Hospital, Tongji College of Medicine, Huazhong University of Science and Technology, Wuhan, China. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

XZ and QY designed and conceptualized study. QY, CY-P, LJ-T, LJ-Y, QQ-X, WD-L, and ZJ-W conducted the study. QY, MZ-J, XY-J, MZ, and XZ analyzed and extracted the data. QY, CY-P, and XZ wrote the first draft of the manuscript. All authors reviewed the manuscript.

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## SUPPLEMENTARY MATERIAL

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## REFERENCES

Al-Qassabi, A., Fereshtehnejad, S. M., and Postuma, R. B. (2017). Sleep disturbances in the prodromal stage of Parkinson disease. *Curr. Treat. Options Neurol.* 19:22. doi: 10.1007/s11940-017-0458-1

Antczak, J. M., Rakowicz, M. J., Banach, M., Derejko, M., Sienkiewicz, J., Zalewska, U., et al. (2013). Negative influence of L-dopa on subjectively assessed sleep but not on nocturnal polysomnography in Parkinson's disease. *Pharmacol. Rep.* 65, 614–623. doi: 10.1016/s1734-1140(13)71038-7

Bargiotas, P., Schuepbach, M. W., and Bassetti, C. L. (2016). Sleep-wake disturbances in the premotor and early stage of Parkinson's disease. *Curr. Opin. Neurol.* 29, 763–772. doi: 10.1097/WCO.0000000000000388

Baron, R. M., and Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J. Pers. Soc. Psychol.* 51, 1173–1182. doi: 10.1037/0022-3514.51.6.1173

Bjørnarå K. A., Dietrichs, E., and Toft, M. (2014). Clinical features associated with sleep disturbances in Parkinson's disease. *Clin. Neurol. Neurosurg.* 124, 37–43. doi: 10.1016/j.clineuro.2014.06.027

Bohnen, N. I., and Hu, M. T. M. (2019). Sleep disturbance as potential risk and progression factor for Parkinson's disease. *J. Parkinsons Dis.* 9, 603–614. doi: 10.3233/JPD-191627

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., and Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res.* 318, 121–134. doi: 10.1007/s00441-004-0956-9

Breen, D. P., Vuono, R., Nawarathna, U., Fisher, K., Shneerson, J. M., Reddy, A. B., et al. (2014). Sleep and circadian rhythm regulation in early Parkinson disease. *JAMA Neurol.* 71, 589–595. doi: 10.1001/jamaneurol.2014.65

Cai, G. E., Luo, S., Chen, L. N., Lu, J. P., Huang, Y. J., and Ye, Q. Y. (2019). Sleep fragmentation as an important clinical characteristic of sleep disorders in Parkinson's disease: a preliminary study. *Chin. Med. J.* 132, 1788–1795. doi: 10.1097/CM9.0000000000000329

Diederich, N. J., Vaillant, M., Mancuso, G., Lyen, P., and Tiete, J. (2005). Progressive sleep 'destructuring' in Parkinson's disease. A polysomnographic study in 46 patients. *Sleep Med.* 6, 313–318. doi: 10.1016/j.sleep.2005.03.011

Emre, M. (2003). What causes mental dysfunction in Parkinson's disease? *Mov. Disord.* 18, S63–S71. doi: 10.1002/mds.10565

Ferreira, T., Prabhakar, S., and Kharbanda, P. S. (2014). Sleep disturbances in drug naïve Parkinson's disease (PD) patients and effect of levodopa on sleep. *Ann. Indian Acad. Neurol.* 17, 416–419. doi: 10.4103/0972-2327.144016

Goetz, C. G., Poewe, W., Rascol, O., Sampaio, C., Stebbins, G. T., Counsell, C., et al. (2004). Movement disorder society task force report on the Hoehn and Yahr staging scale: status and recommendations. *Mov. Disord.* 19, 1020–1028. doi: 10.1002/mds.20213

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin, P., et al. (2008). Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* 23, 2129–2170. doi: 10.1002/mds.22340

Gulia, K. K., and Kumar, V. M. (2018). Sleep disorders in the elderly: a growing challenge. *Psychogeriatrics* 18, 155–165. doi: 10.1111/psyg.12319

Hayes, A. F., and Preacher, K. J. (2010). Quantifying and testing indirect effects in simple mediation models when the constituent paths are nonlinear. *Multivariate Behav. Res.* 45, 627–660. doi: 10.1080/00273171.2010.498290

Högl, B., Arnulf, I., Comella, C., Ferreira, J., Iranzo, A., Tilley, B., et al. (2010). Scales to assess sleep impairment in Parkinson's disease: critique and recommendations. *Mov. Disord.* 25, 2704–2716. doi: 10.1002/mds.23190

Jasti, D. B., Mallipeddi, S., Apparao, A., Vengamma, B., Kolli, S., and Mohan, A. (2018). Quality of sleep and sleep disorders in patients with Parkinsonism: a polysomnography based study from Rural South India. *J. Neurosci. Rural Pract.* 9, 92–99. doi: 10.4103/jnrp.jnrp_189_17

Jenkinson, C., Fitzpatrick, R., Peto, V., Greenhall, R., and Hyman, N. (1997). The Parkinson's Disease Questionnaire (PDQ-39): development and validation of a Parkinson's disease summary index score. *Age Ageing.* 26, 353–357. doi: 10.1093/ageing/26.5.353

Kurtis, M. M., Rodriguez-Blazquez, C., and Martinez-Martin, P. (2013). Relationship between sleep disorders and other non-motor symptoms in Parkinson's disease. *Parkinsonism Relat. Disord.* 19, 1152–1155. doi: 10.1016/j.parkreldis.2013.07.026

Lauretti, E., Di Meco, A., Merali, S., and Praticò D. (2017). Circadian rhythm dysfunction: a novel environmental risk factor for Parkinson's disease. *Mol. Psychiatry* 22, 280–286. doi: 10.1038/mp.2016.47

Leroi, I., Baker, P., Kehoe, P., Daniel, E., and Byrne, E. J. (2010). A pilot randomized controlled trial of sleep therapy in Parkinson's disease: effect on patients and caregivers. *Int. J. Geriatr. Psychiatry* 25, 1073–1079. doi: 10.1002/gps.2472

Lima, M. M. (2013). Sleep disturbances in Parkinson's disease: the contribution of dopamine in REM sleep regulation. *Sleep Med. Rev.* 17, 367–375. doi: 10.1016/j.smrv.2012.10.006

Lysen, T. S., Darweesh, S. K. L., Ikram, M. K., Luik, A. I., and Ikram, M. A. (2019). Sleep and risk of parkinsonism and Parkinson's disease: a population-based study. *Brain.* 142, 2013–2022. doi: 10.1093/brain/awz113

Mao, C. J., Yang, Y. P., Chen, J. P., Wang, F., Chen, J., Zhang, J. R., et al. (2018). Poor nighttime sleep is positively associated with dyskinesia in Parkinson's disease patients. *Parkinsonism Relat. Disord.* 48, 68–73. doi: 10.1016/j.parkreldis.2017.12.022

Mao, Z. J., Liu, C. C., Ji, S. Q., Yang, Q. M., Ye, H. X., Han, H. Y., et al. (2017). Clinical characteristics of sleep disorders in patients with Parkinson's disease. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 37, 100–104. doi: 10.1007/s11596-017-1701-4

Pal, P. K., Thennarasu, K., Fleming, J., Schulzer, M., Brown, T., and Calne, S. M. (2004). Nocturnal sleep disturbances and daytime dysfunction in patients with Parkinson's disease and in their caregivers. *Parkinsonism Relat. Disord.* 10, 157–168. doi: 10.1016/j.parkreldis.2003.11.002

Palmer, C. A., and Alfano, C. A. (2017). Sleep and emotion regulation: an organizing, integrative review. *Sleep Med. Rev.* 31, 6–16. doi: 10.1016/j.smrv.2015.12.006

Pandey, S., Bajaj, B. K., Wadhwa, A., and Anand, K. S. (2016). Impact of sleep quality on the quality of life of patients with Parkinson's disease: a questionnaire based study. *Clin. Neurol. Neurosurg.* 148, 29–34. doi: 10.1016/j.clineuro.2016.06.014

Pfeiffer, R. F. (2016). Non-motor symptoms in Parkinson's disease. *Parkinsonism Relat. Disord.* 22, S119–S122. doi: 10.1016/j.parkreldis.2015.09.004

Poewe, W. H., Rascol, O., Quinn, N., Tolosa, E., Oertel, W. H., Martignoni, E., et al. (2007). Efficacy of pramipexole and transdermal rotigotine in advanced Parkinson's disease: a double-blind, double-dummy, randomised controlled trial. *Lancet Neurol.* 6, 513–520. doi: 10.1016/S1474-4422(07)70108-4

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., et al. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* 30, 1591–1601. doi: 10.1002/mds.26424

Rutten, S., Vriend, C., van der Werf, Y. D., Berendse, H. W., Weintraub, D., and van den Heuvel, O. A. (2017). The bidirectional longitudinal relationship between insomnia, depression and anxiety in patients with early-stage, medication-naïve Parkinson's disease. *Parkinsonism Relat. Disord.* 39, 31–36. doi: 10.1016/j.parkreldis.2017.01.015

Selvaraj, V. K., and Keshavamurthy, B. (2016). Sleep dysfunction in Parkinson's disease. *J. Clin. Diagn. Res.* 10, OC09–OC12. doi: 10.7860/JCDR/2016/16446.7208

Siciliano, M., Trojano, L., Santangelo, G., De Micco, R., Tedeschi, G., and Tessitore, A. (2018). Fatigue in Parkinson's disease: a systematic review and meta-analysis. *Mov. Disord.* 33, 1712–1723. doi: 10.1002/mds.27461

Stebbins, G. T., Goetz, C. G., Burn, D. J., Jankovic, J., Khoo, T. K., and Tilley, B. C. (2013). How to identify tremor dominant and postural instability/gait difficulty groups with the movement disorder society unified Parkinson's disease rating scale: comparison with the unified Parkinson's disease rating scale. *Mov. Disord.* 28, 668–670. doi: 10.1002/mds.25383

Stefani, A., and Högl, B. (2020). Sleep in Parkinson's disease. *Neuropsychopharmacology.* 45, 121–128. doi: 10.1038/s41386-019-0448-y

Suzuki, K., Miyamoto, M., Miyamoto, T., and Hirata, K. (2014). [Sleep and autonomic function: sleep related breathing disorders in Parkinson's disease and related disorders]. *Rinsho Shinkeigaku* 54, 1041–1043. doi: 10.5692/clinicalneurol.54.1041

Sveinbjornsdottir, S. (2016). The clinical symptoms of Parkinson's disease. *J. Neurochem.* 139, 318–324. doi: 10.1111/jnc.13691

Thobois, S., Prange, S., Sgambato-Faure, V., Tremblay, L., and Broussolle, E. (2017). Imaging the etiology of apathy, anxiety, and depression in Parkinson's disease: implication for treatment. *Curr. Neurol. Neurosci. Rep.* 17:76. doi: 10.1007/s11910-017-0788-0

Tomlinson, C. L., Stowe, R., Patel, S., Rick, C., Gray, R., and Clarke, C. E. (2010). Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov. Disord.* 25, 2649–2653. doi: 10.1002/mds.23429

van Wamelen, D. J., Martinez-Martin, P., Weintraub, D., Schrag, A., Antonini, A., Falup-Pecurariu, C., et al. (2021). The Non-Motor Symptoms Scale

in Parkinson's disease: validation and use. *Acta Neurol. Scand.* 143, 3–12. doi: 10.1111/ane.13336

Verbaan, D., van Rooden, S. M., Visser, M., Marinus, J., and van Hilten, J. J. (2008). Nighttime sleep problems and daytime sleepiness in Parkinson's disease. *Mov. Disord.* 23, 35–41. doi: 10.1002/mds.21727

Videnovic, A., Klerman, E. B., Wang, W., Marconi, A., Kuhta, T., and Zee, P. C. (2017). Timed light therapy for sleep and daytime sleepiness associated with Parkinson disease: a randomized clinical trial. *JAMA Neurol.* 74, 411–418. doi: 10.1001/jamaneurol.2016.5192

Videnovic, A., Lazar, A. S., Barker, R. A., and Overeem, S. (2014a). 'The clocks that time us'–circadian rhythms in neurodegenerative disorders. *Nat. Rev. Neurol.* 10, 683–693. doi: 10.1038/nrneurol.2014.206

Videnovic, A., Noble, C., Reid, K. J., Peng, J., Turek, F. W., Marconi, A., et al. (2014b). Circadian melatonin rhythm and excessive daytime sleepiness in Parkinson disease. *JAMA Neurol.* 71, 463–469. doi: 10.1001/jamaneurol.2013.6239

Wang, X. T., Liu, F. T., Bi, Y. L., Shen, X. N., Xu, W., Wang, J., et al. (2020). Associations of sleep characteristics with alpha-synuclein in cerebrospinal fluid in older adults. *Ann. Clin. Transl. Neurol.* 7, 2026–2034. doi: 10.1002/acn3.51204

Ylikoski, A., Martikainen, K., Sieminski, M., and Partinen, M. (2015). Parkinson's disease and insomnia. *Neurol. Sci.* 36, 2003–2010. doi: 10.1007/s10072-015-2288-9

Yong, M. H., Fook-Chong, S., Pavanni, R., Lim, L. L., and Tan, E. K. (2011). Case control polysomnographic studies of sleep disorders in Parkinson's disease. *PloS ONE.* 6:e22511. doi: 10.1371/journal.pone.0022511

Zea-Sevilla, M. A., and Martínez-Martín, P. (2014). Rating scales and questionnaires for assessment of sleep disorders in Parkinson's disease: what they inform about? *J. Neural Transm.* 121, S33–S40. doi: 10.1007/s00702-014-1217-z

Zhang, X., Molsberry, S. A., Pavlova, M., Schwarzschild, M. A., Ascherio, A., and Gao, X. (2021). Association of sleepwalking and REM sleep behavior disorder with Parkinson disease in men. *JAMA Netw. Open* 4:e215713. doi: 10.1001/jamanetworkopen.2021.5713

Zhu, K., van Hilten, J. J., and Marinus, J. (2016). Course and risk factors for excessive daytime sleepiness in Parkinson's disease. *Parkinsonism Relat. Disord.* 24, 34–40. doi: 10.1016/j.parkreldis.2016.01.020

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# The relationship between Parkinson's disease and gastrointestinal diseases

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An increasing number of studies have provided evidence for the hypothesis that the pathogenesis of Parkinson's disease (PD) may derive from the gut. Firstly, Lewy pathology can be induced in the enteric nervous system (ENS) and be transported to the central nervous system (CNS) via the vagal nerve. Secondly, the altered composition of gut microbiota causes an imbalance between beneficial and deleterious microbial metabolites which interacts with the increased gut permeability and the gut inflammation as well as the systemic inflammation. The activated inflammatory status then affects the CNS and promotes the pathology of PD. Given the above-mentioned findings, researchers start to pay attention to the connection between PD and gastrointestinal diseases including irritable bowel syndrome, inflammatory bowel disease (IBD), microscopic colitis (MC), gastrointestinal infections, gastrointestinal neoplasms, and colonic diverticular disease (CDD). This review focuses on the association between PD and gastrointestinal diseases as well as the pathogenesis of PD from the gut.

KEYWORDS

**Parkinson's disease, gastrointestinal diseases, microbiota-gut-brain axis, gut microbiota, microbial metabolites, gut inflammation**

## Introduction

Parkinson's disease (PD) is the second most prevalent progressive neurodegenerative disease after Alzheimer's disease. The incidence of PD, rising with age, is the highest in people aged around 80 (Ascherio and Schwarzschild, 2016). PD has the classical motor symptoms of rest tremor, muscular rigidity, bradykinesia, and postural and gait impairment (Cacabelos, 2017). Non-motor symptoms including impaired olfaction, constipation, depression, excessive daytime sleepiness, and rapid eye movement sleep behavior disorder (RBD) usually occur in the prodromal period of PD, before the onset of the primary motor symptoms. In this period, key features of PD pathology can already be identified (Klingelhoefer and Reichmann, 2015). The pathological hallmarks of PD

are dopaminergic neurons loss in the substantia nigra pars compacta (SNpc) along with other parts of the brain, and the formation of Lewy bodies, mainly composed of an abnormally folded protein called α-synuclein, in the neurons of the central nervous system (CNS) and peripheral nervous system such as the enteric nervous system (ENS). Lewy pathology is hypothesized to develop following 6 stages. In the early stages before the onset of motor symptoms, Lewy bodies can be found in the peripheral nervous system, related to the non-motor symptoms. Moreover, pathological forms of α-synuclein aggregates besides Lewy bodies are also harmful to neurons (Kalia and Lang, 2015). Neuroinflammation also plays an important role in the pathology of PD. Reactive gliosis caused by activated astrocytes and microgliosis resulting from microglial activation is identified in the areas of neurodegeneration in PD (Phani et al., 2012). There is an increasing number of studies supporting that the above-mentioned pathology in the brain originates from the gastrointestinal tract. This article aims to review the roles the gut plays in the pathogenesis of PD and the association between PD and gastrointestinal diseases as well as the possible underlying mechanisms to reveal new insights into the etiology and pathophysiology of PD, explore early peripheral diagnosis of PD for early intervention, and facilitate timely identification and management of gastrointestinal comorbidities.

## Search strategy

We searched PubMed and Embase for articles published in English from Jan 1, 2000, to Jan 31, 2022 using subject words combined with free words. The search terms "parkinson," "PD," "gut microbiota," "gastrointestinal diseases" were used. We also manually searched for additional relevant studies via the reference lists of included articles and review articles.

## The gut-derived pathogenesis of Parkinson's disease

In recent years, the interaction between the gut, gut microbiota, and the brain has been demonstrated and named the microbiota-gut-brain axis (Cryan and Dinan, 2012). By the microbiota-gut-brain axis, gut microbiota can modulate the bidirectional gut-brain signaling pathways by interaction with the host (Cryan et al., 2019).

### Lewy pathology in enteric nervous system

The Lewy pathology has been identified in the gastrointestinal tract on an average of 7 years before the onset of the motor symptoms (Stokholm et al., 2016). BRAAK in 2006 put forward a hypothesis that the pathology of PD may be initiated from the gut and then the pathological substances transported from the gut to the brain. Subsequently, several studies presented evidence to support this hypothesis. Animal studies demonstrated that the intragastrically administered rotenone, a PD inducer, could cause pathological α-synuclein formation in ENS, in the vagal nerve, and then the brainstem. When vagotomy was performed, the pathological α-synuclein stopped transporting to the brain (Phillips et al., 2008; Kim et al., 2019). Furthermore, human α-synuclein injected into the intestinal wall of healthy mice could transport via the vagal nerve and reach the brainstem (Holmqvist et al., 2014). Interestingly, cohort studies found that compared to controls, patients receiving truncal vagotomy had a decreased risk of PD (Svensson et al., 2015; Liu et al., 2017); vagotomy referring to resecting the vagal nerve, is a treatment for peptic ulcers. The above-mentioned studies provide evidence for the hypothesis that Lewy pathology may be initiated in ENS and move toward the SNpc and other parts of the CNS (Braak et al., 2006).

### Gut microbiota

Gut microbiota is composed of a variety of microorganisms including bacteria, archaea, fungi, and viruses in the gastrointestinal tract. Studies presented heterogeneous or conflicting results of changes in the gut microbiota of PD patients and healthy controls. Nevertheless, taking 21 studies together, we summarized the gut microbiota with altered abundance in the PD group compared to the healthy control group in **Table 1** (Scheperjans et al., 2015; Kuai et al., 2021; Vascellari et al., 2021; Yan et al., 2021; Zheng et al., 2021). The heterogeneity of results may be due to different sequencing methods of gut microbiota and confounding factors such as diets, age, and comorbidity. Despite the heterogeneity, these results provide candidates for further exploration of mechanisms.

The above-mentioned studies suggested a possible role of the altered composition of microbiota in the pathogenesis of PD, and a more direct relationship should be established by intervention studies. In a validated mouse model of PD (Sampson et al., 2016), antibiotic treatment alleviated, while microbial re-colonization promoted, the pathophysiology of PD in mice. Furthermore, mice colonized with fecal microbiota from PD patients had worse motor performance than that colonized with fecal microbiota from healthy controls. It indicates that the altered composition of microbiota represents a risk factor for PD.

The altered composition of microbiota in PD causes increased gut permeability and systemic inflammation via the translocation of bacteria and proinflammatory microbial metabolites (Forsyth et al., 2011; Fang, 2016; Felice et al., 2016).

TABLE 1 Altered abundance of gut microbiota in PD patients compared to controls.

| | Bacteria | n↑* | n↓* |
|---|---|---|---|
| Phylum | Bacteroidetes | | 2 |
| | Firmicutes | 2 | |
| Family | Prevotellaceae | | 7 |
| | Lachnospiraceae | | 8 |
| | Enterococcaceae | 2 | 1 |
| | Lactobacillaceae | 6 | 2 |
| | Blautia | | 3 |
| | Akkermansia | 7 | |
| | Enterobacteriaceae | 3 | |
| Genus | Faecalibacterium | | 4 |
| | Bacteroides | 1 | 3 |
| | Prevotella | 1 | 7 |
| | Clostridium | 1 | 3 |
| | Verrucomicrobiacea Faecalibacteriume | 4 | 1 |
| | Ruminococcus | | 2 |
| | Lactobacillus | 4 | 2 |
| | Roseburia | | 3 |
| | Oscillospira | 4 | |
| | Bifidobacterium | 5 | |

*n↑ refers to number of studies reporting increased abundance of gut microbiota in PD patients compared to controls. n↓ refers to number of studies reporting decreased abundance.

For example, a reduced Provotella population can result in decreased levels of protective short-chain fatty acids (SCFAs) and vitamins, such as thiamine and folate; moreover, Provotella can produce hydrogen sulfide which has a protective effect on dopaminergic neurons (Cakmak, 2015). The decreased SCFAs–producing Roseburia and Faecalibacterium may also facilitate neuroinflammation in PD. Akkermansia has been reported to have the ability to degrade the mucus layer of the gut. Then the increased gut permeability exposes the intestinal neural plexus to oxidative stress and/or pesticide/herbicide, causing abnormal aggregation of α-synuclein fibrils in the intestine (Nishiwaki et al., 2020). The abundances of Verrucomicrobia and Bacteroides were associated with elevated plasma levels of TNF-α and IFN-γ in PD patients, suggesting the role of Verrucomicrobia and Bacteroides in systemic inflammation (Lin et al., 2019). Besides, gut microbiota acts as an activator of immune cells in the brain, including brain-resident microglia (Erny et al., 2015). Activated microglia can release reactive oxygen, nitrogen species, and pro-inflammatory cytokines to induce neuroinflammation (Phani et al., 2012). Moreover, the α-synuclein-dependent motor dysfunction and gastrointestinal function could be significantly improved in antibiotics-treated animals (Sampson et al., 2016), suggesting the critical role of gut microbiota in the pathogenesis of PD. A preliminary study (Xue et al., 2020) including 15 PD patients reported relieved motor and non-motor symptoms after fecal microbiota transplantation (FMT), and compared with nasointestinal FMT, colonic FMT appears to be more effective. However, the follow-up period was 3 months, unable to evaluate the long-term effect of FMT on PD and it was a small sample-size self-controlled study that lacked a rigorous design. Hence, more randomized controlled trials are needed to further evaluate the efficacy and safety of FMT in treating PD.

## Microbial metabolites

It has been widely accepted that gut microbiota and their metabolites play a pivotal role in the pathogenesis of PD. The gut microbiota may contribute to the neurodegeneration of PD via the cumulative effects of microbial metabolites throughout the whole disease course before the onset of PD. The microbial metabolites in the gut can affect CNS via endocrine and neural pathways. In turn, the CNS alters gut movement, gut microbiota composition, secretion, permeability, and inflammation via autonomic interaction with the ENS (Zheng et al., 2021). SCFAs, including acetate, propionate, and butyrate are putative neuroprotective microbial metabolites by fermentation of fibers. Studies found that the levels of both serum and fecal SCFAs were differentially abundant between patients with PD and controls and the levels of SCFAs were significantly lower in PD patients (Unger et al., 2016; Russo et al., 2018; Wu et al., 2022). Additionally, butyrate was found to have a negative correlation with postural instability-gait disorder score and constipation severity (Tan et al., 2021). Bacteria-derived butyrate modulates hypoxia-inducible factor (HIF), which coordinates gut barrier protection (Kelly et al., 2015). The impaired gut barrier and gut inflammation have been suggested to be involved in the pathogenesis of PD, which will be discussed in the next section. However, an animal study demonstrated that SCFAs alone could activate microglia, induce increased α-synuclein aggregation in the brain and aggravate motor deficits (Sampson et al., 2016) by yet unknown pathways. For this, clinical studies are needed to further explore whether there is a positive relationship between PD and elevated SCFAs.

In addition to SCFAs, gut microbiota can also modulate the metabolism of amino acids. The levels of fecal branched-chain amino acids and aromatic amino acids were decreased in PD patients. Such amino acids were associated with an increased abundance of Alistipes, Rikenellaceae_RC9_gut_group, Bifidobacterium, Parabacteroides, and a decreased abundance of Faecalibacterium (Yan et al., 2021). Compared to healthy controls, the analysis of fecal metabolites showed higher levels of cadaverine, ethanolamine, hydroxypropionic acid, isoleucine and leucine, and phenylalanine. In contrast, glutamic acid, pyroglutamic acid, and succinic acid were significantly decreased in PD patients (Vascellari et al., 2020). The increased molecules may promote inflammatory responses and α-synuclein aggregation in the ENS via inducing oxidative

stress and binding the N-terminal region of the amyloid beta peptide (Vascellari et al., 2021). Another study also revealed increased production of deleterious amino acids of protein degradation by gut microbiota, including p-cresol and phenylacetylglutamine in PD patients (Cirstea et al., 2020). The toxic amino acids have a harmful effect on the colonocytes (Diether and Willing, 2019) and may contribute to the disorder in the gut microenvironment.

Furthermore, gut microbiota plays a key role in metabolizing dietary constituents such as choline or L-carnitine into Trimethylamine N-oxide (TMAO), which is detectable in the cerebrospinal fluid. A study demonstrated that compared to healthy controls, plasma TMAO levels in patients with drug-naïve early stage PD were lower. Lower TMAO levels were related to more rapid longitudinal increases in doses of dopamine-based medications and higher risks for dementia (Chung et al., 2021). Another study also revealed reduced fecal levels of TMAO in PD, and fecal choline levels were higher in patients with motor response complications (occurring in the advanced stage of PD) (Tan et al., 2021). Studies have shown the protective effects of TMAO. TMAO could transform α-synuclein from one conformation to another, which may prevent the α-synuclein aggregation and formation of insoluble fibrils leading to PD (Jamal et al., 2017). When TMAO is at a high concentration level, α-synuclein forms oligomers, probably representing the physiologically folded form of the protein (Uversky et al., 2001). However, other studies reported a positive correlation between levels of TMAO and poor prognosis of various diseases, including neurological disorders and non-neurological disorders (Chung et al., 2021). TMAO has been reported to accelerate Aβ aggregation of Alzheimer's disease and has been suggested to disrupt the blood-brain barrier (BBB) by reducing the expression of tight junction proteins like claudin-5 and tight junction protein-1. One of the mechanisms underlying the harmful effects of TMAO is demonstrated to be inflammation (Janeiro et al., 2018). Another study in 2020 demonstrated that plasma TMAO levels were elevated in patients with PD and correlated with disease severity and motor symptom progression (Chen et al., 2020). Likewise, a study revealed that plasma TMAO levels of patients with PD (with an average disease course of 8.1 years) were higher than that of the controls, and the levels were even higher in patients with motor fluctuation (Sankowski et al., 2020). The conflicting results may arise from the fact that the subjects in these studies were on medication and had a longer course of disease. The function of the above-mentioned microbial metabolites is summarized in **Table 2**.

## Gut inflammation

Gut inflammation has been suggested to be associated with PD. In a study with small sample size, compared with controls, biopsies from the ascending colon showed increased levels of pro-inflammatory cytokines including TNF-α, IF-γ, IL-6, and IL-1β (Devos et al., 2013). A Systematic review summarized the peripheral blood inflammatory cytokine data and concluded with higher levels of IL-6, tumor necrosis factor, IL-1β, IL-2, IL-10, C-reactive protein, and RANTES in patients with PD (Qin et al., 2016). Additionally, lipopolysaccharide (LPS), an inflammatory microbial metabolite, has been proven to accelerate α-synuclein aggregation. Some speculated that LPS initiated the pathology of PD in the gut via binding to α-synuclein (Bhattacharyya et al., 2019). Moreover, LPS is involved in the activation of inflammation in the colon and SN via the TLR4/MyD88/NF-κB signaling pathway (Zhao et al., 2021). Gastrointestinal inflammation may be responsible for increased intestinal permeability. Traversed gut microbiota and their metabolites then induce proinflammatory cytokine production in the ENS and aggregation of pathological α- synuclein. Pathological α-synuclein activates $CD4^+$T-cells into Th1 and Th17. Th1 and Th17 then enter the brain via damaged BBB and activate microglia which induces neuroinflammation (Chen et al., 2019). Li et al. (2021) found that enriched Actinobacteria and Firmicutes phylum-associated epitopes were positively associated with inflammatory indicators such as neutrophil percentage, monocyte count/percentage, and white blood cell count in PD patients. Additionally, they were also positively associated with histidine degradation and proline biosynthesis pathways (Li et al., 2021). The relationship between gut microbiota, SCFAs, inflammation indicators, and the gut barrier in PD has been demonstrated. A study showed higher levels of calprotectin, lower levels of SCFA in stool, and lower levels of CXCL8 in plasma in PD patients than those of controls. The inflammatory markers, neutrophil gelatinase-associated lipocalin (NGAL) and calprotectin, and the gut permeability marker zonulin were also positively correlated. Stool SCFAs were negatively correlated with PD onset and symptom severity. PD patients had an increased abundance of Firmicutes and a decreased abundance of Prevotella. Prevotella is associated with higher levels of butyric acid and lower levels of NGAL and zonulin in stool. Moreover, the abundances of Butyricicoccus, Clostridium sensustricto, Roseburia, Lachnospiraceae, were positively correlated with levels of SCFAs, whereas the abundances of Enterobacteriaceae and Enterobacteriaceae, Akkermansia, Escherichia/Shigella, Flavonifractor, Intestinimonas, Phascolarctobacterium, and Sporobacter were negatively correlated with levels of SCFAs (Aho et al., 2021), in accordance with changes of gut microbiota in PD patients. In summary, the altered composition of gut microbiota may cause a decrease in protective SCFAs and gut inflammation. Gut inflammation may increase intestinal permeability, allowing the leakage of bacteria, and promoting the pathogenesis of PD. The mechanisms are summarized in **Figure 1**.

TABLE 2 Potential roles of microbial metabolites in PD.

| Microbial metabolites | Function | Potential roles in PD | References |
|---|---|---|---|
| SCFAs | Modulate HIF | Coordinate gut barrier protection and regulate inflammatory responses | Kelly et al., 2015 |
| | Activate microglia | Induce increased α-synuclein aggregation in the brain and aggravate motor deficits | Sampson et al., 2016 |
| Deleterious amino acids | Induce oxidative stress, bind the N-terminal region of the amyloid beta peptide and damage colonocytes | Promote inflammatory responses and α-synuclein aggregation in the CNS, and damage the gut barrier | Diether and Willing, 2019; Vascellari et al., 2021 |
| TMAO | Transform the conformation of α-synuclein | Prevent the α-synuclein aggregation | Jamal et al., 2017 |
| | Reducing the expression of tight junction proteins and cause inflammation | Disrupt the blood-brain barrier | Janeiro et al., 2018 |

SCFAs, Short-chain fatty acids; HIF, Hypoxia-inducible factor; CNS, Central nervous system; TMAO, Trimethylamine N-oxide.

Based on the evidence illustrating the relationship between the gut and PD, several observational studies worldwide have started to focus on the association between gastrointestinal diseases and PD which is reviewed in the next part and collated in **Table 3**.

# Gastrointestinal diseases and Parkinson's disease

## Irritable bowel syndrome

IBS is a functional gastrointestinal disorder, characterized by chronic abdominal pain and altered bowel habits (including diarrhea, constipation, or alternating diarrhea and constipation). Several studies have revealed that patients with IBS had a higher risk of developing PD than those without IBS (Lai et al., 2014; Mertsalmi et al., 2017a; Liu et al., 2021; Zhang et al., 2021). Subsequently, a variety of studies were performed to investigate the prevalence of IBS in patients with PD and concluded that patients with PD had a higher prevalence of IBS than the general population (Mishima et al., 2017). Patients with IBS have an altered intestinal microenvironment and the subtypes of IBS may differ in the composition of gut microbiota. The subtypes of IBS include IBS with predominant constipation (IBS-C), IBS with predominant diarrhea (IBS-D), IBS with mixed bowel habits (IBS-M), and unclassified IBS. Another study in Finland further demonstrated that IBS-M was significantly more prevalent in PD patients than in healthy controls, while IBS-C and IBS-D were of no statistically significant difference from controls. And the fecal analysis showed a significantly lower abundance of the genus Prevotella and Bacteroides and the family Prevotellaceae in PD patients with IBS-like symptoms than that in PD patients without IBS-like symptoms (Mertsalmi et al., 2017b). Besides, abnormal gastrointestinal motility, including increased frequency and irregularity of luminal contractions, plays an important role in the pathogenesis of IBS. Increased gut motility can alleviate symptoms (Caldarella et al., 2002). Additionally, the link between ENS and CNS may also exist in the pathogenesis of IBS. Visceral hypersensitivity plays a role in IBS. The external stimuli are transported via receptors of the gut wall and afferent neural pathways toward the CNS, causing the perception of abdominal pain, gastrointestinal discomfort, and other symptoms. Furthermore, small intestinal bacterial overgrowth (SIBO) is frequently reported in IBS. SIBO is associated with an increase in the number and/or species of bacteria in the upper gastrointestinal tract. Of all, Methanobrevibacter smithii, contributing to a positive methane breath test, is associated with IBS-C. SIBO may lead to increased intestinal permeability, dysmotility, chronic inflammation, autoimmunity, decreased absorption of bile salts, and altered enteral and central neuronal activity in patients with IBS (Takakura and Pimentel, 2020). SIBO has also been reported to be prevalent in PD patients. And the eradication of SIBO in PD may improve motor dysfunctions. The underlying mechanisms may involve increased intestinal permeability, bacterial translocation, and systemic inflammation (Losurdo et al., 2020). Overall, IBS and PD may share the common multiple pathogenesis pathways as stated, thus linking to each other.

## Inflammatory bowel disease

Gastrointestinal inflammation has been suggested to be involved in the pathogenesis of PD. Microscopic colitis (MC) and inflammatory bowel disease (IBD), featuring the inflammation in the gut, may share a similar pathology. Thus, studies started to focus on the association between these diseases.

IBD consists of two major disorders: ulcerative colitis (UC) and Crohn's disease (CD). UC affects the colon, while CD can affect the whole gastrointestinal tract from the mouth to the perianal area. The pathology and clinical characteristics of UC and CD overlap significantly with each other. Five cohort studies reported that IBD patients had an increased risk of developing PD (Lin et al., 2016; Peter et al., 2018; Park et al., 2019;

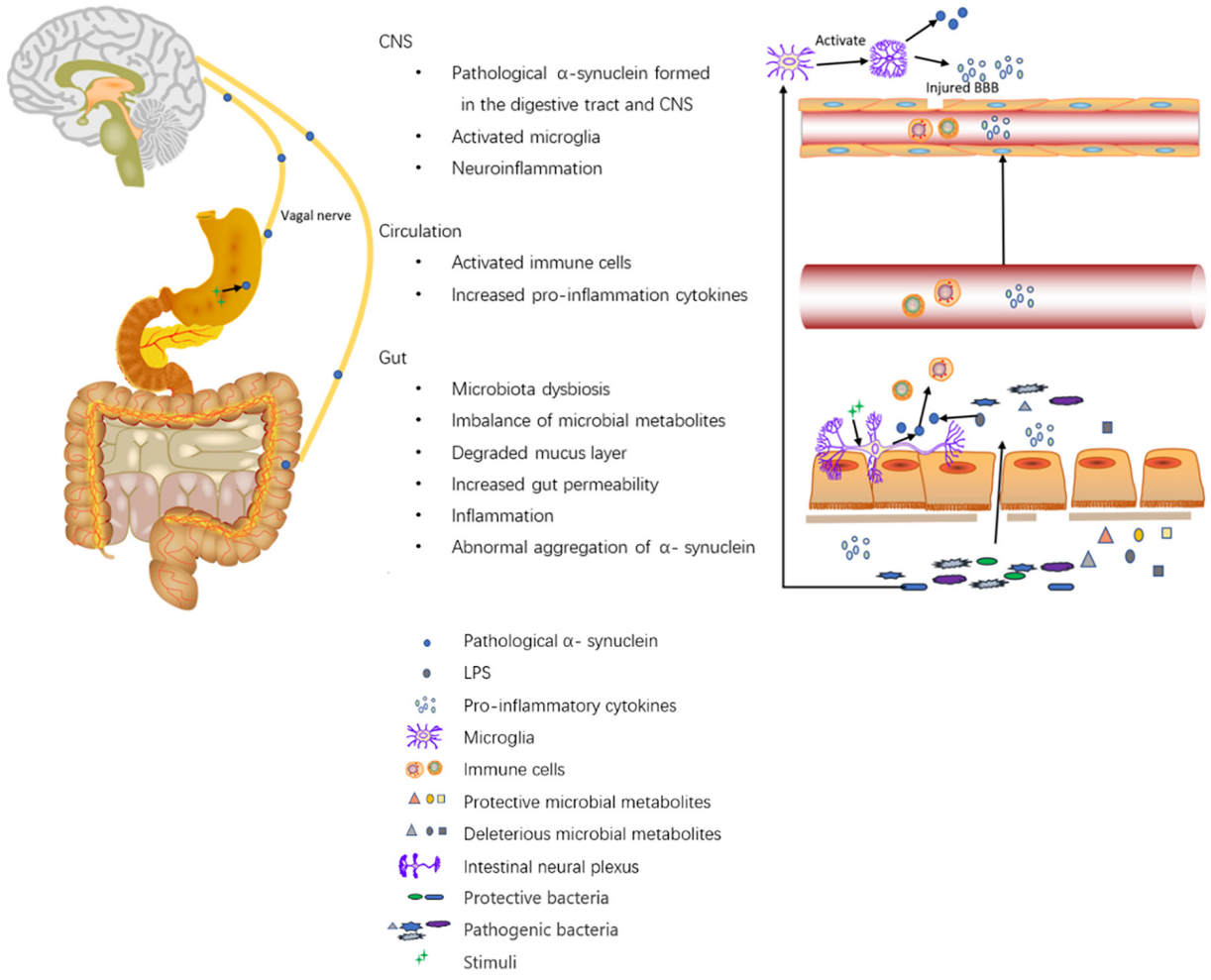


FIGURE 1
Roles of the digestive tract in the pathogenesis of PD. The altered composition of gut microbiota leads to altered levels of microbial metabolites such as SCFAs, vitamins, hydrogen sulfide, LPS, amino acid, and TMAO. First, on the one hand, SCFAs modulate gut barrier protection via HIF and modulate inflammation by inhibiting NF-kappaB activation; on the other hand, SCFAs alone may activate microglia and induce increased α-synuclein aggregation in the brain. Second, hydrogen sulfide has a protective effect on dopaminergic neurons. Third, LPS promotes intestinal α-synuclein aggregation. Forth, the imbalance of microbial metabolites can cause increased gut permeability and increased levels of inflammatory cytokines like TNF-α, IF-γ, IL-6, IL-1β, calprotectin, and NGAL. Moreover, Akkermansia can degrade the mucus layer of the gut. Then, the increased gut permeability exposes intestinal neural plexus to stimuli such as oxidative stress, pesticide/herbicide, traversed gut microbiota, and microbial metabolites, causing abnormal aggregation of α-synuclein fibrils in the intestine and inducing inflammation. Pathological α-synuclein promotes the activation of Th1 and Th17 in the circulation (accompanied by elevated levels of IL-6, TNF-α, IFN-γ, IL-1β, IL-2, IL-10, C-reactive protein, and RANTES), which activate microglia in the CNS through damaged BBB. Furthermore, gut microbiota dysbiosis can also activate microglia in the CNS. The activated microglia promote the aggregation of α-synuclein and releases reactive oxygen, nitrogen species, and pro-inflammatory cytokines to induce neuroinflammation. Additionally, Intra-gastric stimulation causes the formation of pathological α-synuclein within ENS, and the α-synuclein can directly be transmitted to the brain via the vagal trunk.

Villumsen et al., 2019; Weimers et al., 2019). Of them, one study reported the risk was the highest among patients with CD (Lin et al., 2015), and another study reported that patients with UC had an increased risk of parkinsonism while patients with CD did not (Villumsen et al., 2019). However, 2 studies did not find a higher risk of PD in individuals with IBD. Besides, Camacho-Soto et al. (2018) showed that PD was inversely associated with IBD (OR = 0.85, 95% CI 0.80–0.91), both CD and UC. Another study also reported that in PD patients, no significant difference between the prevalence of CD and that of the general population (Fujioka et al., 2017). IBD and PD may share the following risk factors. First, the onset of IBD usually occurs in 2 age range. The second one is from 50 to 80 years old, which corresponds with the onset age of PD. Second, the leucine-rich repeat kinase 2 (LRRK2) gene is one of the major gene loci related to increased susceptibility for CD (Franke et al., 2010). LRRK2 mutations are also commonly observed in patients with PD (Tolosa et al., 2020). Third, although there is

TABLE 3 The link between Parkinson's disease and gastrointestinal diseases.

| References | Diseases | Association | Possible mechanisms |
|---|---|---|---|
| Lai et al. (2014) | Irritable bowel syndrome | Positive | Abnormal gastrointestinal motility, small intestinal bacterial overgrowth |
| Liu et al. (2021) | | Positive | |
| Mertsalmi et al. (2017a) | | Positive | |
| Mishima et al. (2017) | | Positive# | |
| Mertsalmi et al. (2017b) | | Positive# | |
| Zhang et al. (2021) | | Positive | |
| Lin et al. (2016) | Inflammatory bowel disease | Positive | Age, gene mutations, gut microbiota dysbiosis, intestinal inflammation |
| Peter et al. (2018) | | Positive | |
| Weimers et al. (2019) | | Positive | |
| Villumsen et al. (2019) | | Positive | |
| Park et al. (2019) | | Positive | |
| Camacho-Soto et al. (2018) | | Negative | |
| Fujioka et al. (2017) | | Insignificant# | |
| Kang et al. (2021) | Microscopic colitis | Positive* | Intestinal inflammation or drug side effects |
| | | Insignificant | |
| Tian et al. (2021) | Gastrointestinal neoplasms | Positive | Gut microbiota dysbiosis, gene mutations |
| Lin et al. (2015) and Fang et al. (2021) | | Negative* | |
| | | Positive* | |
| Macerollo et al. (2017) | Colonic diverticular disease | Positive | Abnormal gut motility, gut microbiota dysbiosis, intestinal inflammation |

Positive refers to patients with gastrointestinal diseases having higher risks of PD. Negative refers to patients with gastrointestinal diseases having lower risks of PD. Positive# refers to PD patients having a higher prevalence of gastrointestinal diseases. Positive* refers to PD patients having higher risks of gastrointestinal diseases. Negative* refers to PD patients having lower risks of gastrointestinal diseases. Insignificant refers to patients with gastrointestinal diseases not having higher risks of PD. Insignificant# refers to PD patients not having a higher prevalence of gastrointestinal diseases.

no consistent conclusion on the changes in gut microbiota in IBD patients, studies have reported an elevated abundance of Enterobacteriaceae, a decreased abundance of Lachnospiraceae, and SCFAs-producing bacteria Butyricicoccus in IBD patients, sharing a similar signature of altered microbiota with that of PD patients (Yang et al., 2021). Additionally, gut microbiota dysbiosis promotes the onset of IBD and the abundance of SCFAs-producing bacteria is reduced in IBD patients. Forth, intestinal inflammation is a hallmark of IBD. IBD and PD share the above-mentioned mechanisms of pathogenesis and this may account for the increased risk of PD in IBD patients.

Overall, these studies suggest a potential risk of PD in patients with IBD and indicate the effect of IBD treatment on the prevention of PD. The conflicting results may be due to some critical covariates not being considered, misclassification of IBD, and masked symptoms of PD. Moreover, there exist such limitations as ascertainment bias, biases related to confounder adjustments, and inaccessibility of data related to the severity of IBD and PD.

## Microscopic colitis

MC is a chronic inflammatory colonic disease, characterized by chronic, watery, non-bloody diarrhea. It typically affects middle-aged patients. It has been hypothesized that an impaired epithelial barrier may cause an increase in the transmucosal permeability of antigens and bacteria, resulting in immune dysregulation and intestinal inflammation (Andersen et al., 1993). A positive association between MC and IBD has been established. Patients with MC were reported to have a higher cc of IBD (Khalili et al., 2020). However, an assumed association between PD and MC does not exist. A Swedish cohort study showed that patients with MC did not have an increased risk of PD, but found that a previous diagnosis of PD was strongly associated with MC (Kang et al., 2021). Another study reported an increased risk of MC induced by levodopa/dopa-decarboxylase inhibitor (Ong et al., 2021). Therefore, the observed increased incidence of MC in PD patients may be due to the side effects of levodopa/dopa-decarboxylase inhibitor.

So far, no evidence has been presented to support a higher risk of PD in MC patients, and more studies should be conducted to explore it in the future.

## Gastrointestinal infections

Gastrointestinal infections (GIIs) can cause gut microbiota dysbiosis on the one hand, and gut inflammation on the

other hand, which suggests a potential link between GIIs and PD. A prospective cohort study involving 228,485 individuals revealed an increased risk of PD in patients with GIIs compared with the control group with hazard ratio adjusted for multivariates. The pathogens included unspecified origin, viruses and bacteria (Nerius et al., 2020). There exists the question of whether there is a specific pathogen as a risk factor for PD. Population-based studies showed an increased risk of PD in chronic HP infection (Nielsen et al., 2012) and Clostridium difficile infection (CDI) (Kang et al., 2020). Interestingly, PD patients receiving Helicobacter pylori (HP) eradication therapy had significant clinical improvement in the symptoms of PD (Lolekha et al., 2021). However, whether it is due to HP eradication or gut microbiota restoration needs a further investigation in randomized controlled trials. The pathogens may exert effects via microbial products. Microbial products LPS could activate TLR4/MyD88/NF-κB signaling pathway in the SN and the colon. FMT administration could restore the gut microbial community and ameliorate the motor deficits in PD mice via the TLR4 signaling pathway (Zhao et al., 2021), suggesting a promising therapeutic target at the microbiota-gut-brain axis.

## Gastrointestinal neoplasms

A study using gene expression barcode algorithm and bioinformatics methods revealed that PD and gastric cancer have an association (Tian et al., 2021). This study indicates a positive association between PD and gastric cancer, but association does not equal causation. Therefore, clinical studies should be carried out to investigate the causal relationship. Despite the limitation, the underlying mechanisms of the association between PD and gastric cancer still deserve exploration. Gastric cancer is characterized by the aberrant expression of microRNAs (miRNAs). MiR-148a was down-regulated in gastric cancer tissues (Xia et al., 2014), corresponding with the finding that miR-148a expression levels were lower in PD patients than those in controls. MiR148a can affect cell proliferation, apoptosis, cell invasion, and metastasis. It is known to inhibit gastric cancer metastasis. Furthermore, miR148a-regulated proteins, such as ENAH (Isoform 2 of Protein enabled homolog), STX3 (Isoform B of Syntaxin-3), and TMED2 (Transmembrane emp24 domain-containing protein 2) have been reported to influence neurological development and function. Thus, decreased miR-148a expression may be the underlying link between PD and gastric cancer (Hu et al., 2013).

A systematic review including 17 studies concluded with a lower risk of colorectal cancer in PD patients in the Western population (Fang et al., 2021). In contrast, a cohort study in Taiwan reported a higher risk of developing subsequent cancers in PD patients, cancers including gastric cancer and colorectal cancer. Genetic, environmental, and dietary differences may account for the opposite results (Lin et al., 2015). Moreover, studies found that when ORs were adjusted for physician visits, odds of cancer in the total population after PD was reduce. The research by Freedman and Pfeiffer (2016) did not report ORs adjusted for physician visits, which may cause a bias. Nevertheless, we then discussed the possible mechanisms of these 2 conflicting results.

The lower risk of colorectal cancer in PD patients may result from altered microbiota composition and dysregulated molecular pathways. Dysfunction of the ubiquitin-proteasome system (UPS) can cause formation of the Lewy bodies, a pathological hallmark of PD (Sherman and Goldberg, 2001). While the function of UPS is usually up-regulated in CRC (Manasanch and Orlowski, 2017). Furthermore, studies revealed the activated PI3K /AKT/mTOR pathway in patients with CRC (Bahrami et al., 2018), which, however, may prevent the loss of dopaminergic neurons by inhibiting apoptosis, thus lowering the risk of PD (Leikas et al., 2017).

The higher risk of colorectal cancer in PD patients may be due to the following mechanisms. PD patients carrying the LRRK2 G2019S mutation have been reported to have an increased risk of colon cancer when compared with idiopathic PD patients (Agalliu et al., 2019). Moreover, PARK2 is a tumor suppressor gene, the mutation of which contributes significantly to the onset of PD. And PARK2 is inactivated and mutated in colon cancer (Veeriah et al., 2010). It indicates that genetic mutations in PD may be attributed to a higher risk of colorectal cancer. Though there is no uniform conclusion of CRC-associated microbiota structure, studies have reported enriched abundances of Streptococcus bovis, Enterotoxigenic Bacteroides fragilis, Fusobacterium nucleatum, Enterococcus faecalis, Escherichia coli, Peptostreptococcus anaerobius, and decreased abundances or depletion of butyrate-producing Clostridium butyicum and lactate-producing *S. thermophilus*. Studies also suggested the link between inflammation, genotoxins, oxidative stress, metabolites, biofilm underlying colorectal carcinogenesis, and gut microbiota (Veeriah et al., 2010). Hence, the altered microbiota may be attributed to the higher risk of colorectal cancer in PD.

The bidirectional association between gastrointestinal neoplasms and PD is in line with the hypothesis proposed in recent years that PD may be composed of two subtypes, "brain-first" and "body-first" types. Studies found that the brainstem-predominant type with stronger α-syn accumulation in the brainstem probably belonged to the "body-first" type, and a limbic/amygdala-predominant type, with stronger midbrain pathology, probably belonged to the "brain-first" type (Borghammer et al., 2021; Van Den Berge and Ulusoy, 2022). A recent study using multimodal imaging technique identified that compared to RBD-negative PD cases, the imaging data of RBD-positive PD patients and isolated RBD patients were characterized by initial loss of peripheral autonomic nervous system signal followed by loss of central signal, suggesting a "body-first" trajectory. By contrast, RBD-negative PD patient

data showed an inverse order (Horsager et al., 2020). These findings support the two-subtype hypothesis of PD. Indeed, pathology in the gastrointestinal tract may be an initial cause for PD in some cases whereas a consequence of PD in other. Whether the subtype is related to the clinical type of PD and whether the different gut microbiome signatures can predict the subtype deserve further studies.

## Colonic diverticular disease

Diverticulosis is defined by the presence of diverticula, which refers to a sac-like protrusion of the colonic wall. A nationwide population-based cohort study revealed a significantly higher risk of PD in the colonic diverticular disease (CDD) cohort than in the control cohort (Macerollo et al., 2017). The underlying mechanisms of the link should be further explained. The pathological mechanisms of CDD remain to be elucidated, but altered gut microbiota is thought to be one of the mechanisms leading to CDD. Moreover, the abnormal gut motility results in an increase in the intraluminal pressure. Then, the intestinal mucosa and submucosa are probably more prone to herniation due to the increased intraluminal pressure (Tursi, 2016). The ENS plays a critical role in the regulation of gut motility (Sharma and Rao, 2017). A study found that patients with diverticular disease showed enteric neuropathy in the ENS characterized by myenteric and submucosal oligo-neuronal hypoganglionosis (Wedel et al., 2010). The pathology in the ENS suggests a possible link between CDD and PD. Future studies are needed to further detect pathological α-synuclein formation in the ENS of patients with CDD to confirm the association between PD and CDD. In addition, CDD can develop into diverticulitis, the macroscopic inflammation of diverticula which corresponds with the gut inflammation in PD. The above-mentioned underlying link between PD and CDD may explain the higher risk of PD in CDD patients.

# Conclusion

Since Braak et al. (2006) put forward the hypothesis that the pathogenesis of PD may start from the gut, accumulating evidence has been presented to support the hypothesis. Firstly, Lewy pathology can be induced in the ENS and transported to the CNS via the vagal nerve. Secondly, the altered composition of gut microbiota causes an imbalance between beneficial and deleterious microbial metabolites which interacts with the increased gut permeability and gut inflammation as well as systemic inflammation. The activated inflammatory status then affects the CNS and promotes the pathology of PD. Higher risks of subsequent PD were observed in IBS, IBD, GIIs, gastrointestinal neoplasms, and CCD. Although the underlying mechanisms of the link between PD and gastrointestinal diseases remain unclear, such gastrointestinal dysfunction or diseases may have common pathogenesis derived from the gut with PD as mentioned before. Interestingly, PD in turn could cause altered risks of subsequent gastrointestinal neoplasms, the bidirectional association suggested "brain-first" and "body-first" subtypes of PD. Previous studies focused on the prevalence of IBS, IBD, and CCD in PD patients, and whether PD is a risk factor for IBS, IBD, and CCD needs further investigation, which will play an important role in fully understanding the association between PD and gastrointestinal diseases. What is more, there exists the limitation that there are differences in ethnology, cultural background, and lifestyles that can cause heterogeneous results between studies. Hence, more studies from different nations and regions are needed to explore the relationship between PD and gastrointestinal diseases in the future. In conclusion, this review provides a new direction for pathophysiological research on PD, reasonable targets and grounds for peripheral diagnosis and treatment of early PD, as well as timely identification and management of gastrointestinal comorbidities.

# Author contributions

JZ discussed the contents and wrote the manuscript. XW searched for relevant studies and assessed the quality of them. FP and ZM reviewed the manuscript. All authors read and approved the final manuscript.

# 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.

# Publisher's note

# References


Agalliu, I. A.-O., Ortega, R. A., Luciano, M. S., Mirelman, A., Pont-Sunyer, C., Brockmann, K., et al. (2019). Cancer outcomes among Parkinson's disease patients with leucine rich repeat kinase 2 mutations, idiopathic Parkinson's disease patients, and nonaffected controls. *Mov. Disord.* 34, 1392–1398. doi: 10.1002/mds.27807

Aho, V. A.-O., Houser, M. A.-O., Pereira, P. A.-O., Chang, J., Rudi, K., Paulin, L. A.-O., et al. (2021). Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. *Mol. Neurodegener.* 16:6.

Andersen, T., Andersen, J. R., Tvede, M., and Franzmann, M. B. (1993). Collagenous colitis: Are bacterial cytotoxins responsible? *Am. J. Gastroenterol.* 88, 375–377.

Ascherio, A., and Schwarzschild, M. A. (2016). The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* 15, 1257–1272. doi: 10.1016/s1474-4422(16)30230-7

Bahrami, A., Khazaei, M., Hasanzadeh, M., ShahidSales, S., Joudi Mashhad, M., Farazestanian, M., et al. (2018). Therapeutic Potential of Targeting PI3K/AKT Pathway in Treatment of Colorectal Cancer: Rational and Progress. *J. Cell Biochem.* 119, 2460–2469. doi: 10.1002/jcb.25950

Bhattacharyya, D., Mohite, G. M., Krishnamoorthy, J., Gayen, N., Mehra, S., Navalkar, A., et al. (2019). Lipopolysaccharide from gut microbiota modulates α-synuclein aggregation and alters its biological function. *ACS Chem. Neurosci.* 10, 2229–2236. doi: 10.1021/acschemneuro.8b00733

Borghammer, P., Horsager, J., Andersen, K., Van Den Berge, N., Raunio, A., Murayama, S., et al. (2021). Neuropathological evidence of body-first vs. brain-first Lewy body disease. *Neurobiol. Dis.* 161:105557. doi: 10.1016/j.nbd.2021.105557

Braak, H., de Vos, R. A., Bohl, J., and Del Tredici, K. (2006). Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci. Lett.* 396, 67–72. doi: 10.1016/j.neulet.2005.11.012

Cacabelos, R. (2017). Parkinson's disease: from pathogenesis to pharmacogenomics. *Int. J. Mol. Sci.* 18:551. doi: 10.3390/ijms18030551

Cakmak, Y. O. (2015). Provotella-derived hydrogen sulfide, constipation, and neuroprotection in Parkinson's disease. *Mov. Disord.* 30:1151. doi: 10.1002/mds.26258

Caldarella, M. P., Serra, J., Azpiroz, F., and Malagelada, J. R. (2002). Prokinetic effects in patients with intestinal gas retention. *Gastroenterology* 122, 1748–1755. doi: 10.1053/gast.2002.33658

Camacho-Soto, A., Gross, A., Searles Nielsen, S., Dey, N., and Racette, B. A. (2018). Inflammatory bowel disease and risk of Parkinson's disease in Medicare beneficiaries. *Parkinsonism Relat. Disord.* 50, 23–28.

Chen, Q. Q., Haikal, C., Li, W., and Li, J. Y. (2019). Gut inflammation in association with pathogenesis of parkinson's disease. *Front. Mol. Neurosci.* 12:218. doi: 10.3389/fnmol.2019.00218

Chen, S. J., Kuo, C. H., Kuo, H. C., Chen, C. C., Wu, W. K., Liou, J. M., et al. (2020). The gut metabolite trimethylamine N-oxide is associated with parkinson's disease severity and progression. *Mov. Disord.* 35, 2115–2116. doi: 10.1002/mds.28246

Chung, S. J., Rim, J. H., Ji, D., Lee, S., Yoo, H. S., Jung, J. H., et al. (2021). Gut microbiota-derived metabolite trimethylamine N-oxide as a biomarker in early Parkinson's disease. *Nutrition* 83:111090. doi: 10.1016/j.nut.2020.111090

Cirstea, M. S., Yu, A. C., Golz, E., Sundvick, K., Kliger, D., Radisavljevic, N., et al. (2020). Microbiota composition and metabolism are associated with gut function in parkinson's disease. *Mov. Disord.* 35, 1208–1217. doi: 10.1002/mds.28052

Cryan, J. F., and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712. doi: 10.1038/nrn3346

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., et al. (2019). The microbiota-gut-brain axis. *Physiol. Rev.* 99, 1877–2013. doi: 10.1152/physrev.00018.2018

Devos, D., Lebouvier, T., Lardeux, B., Biraud, M., Rouaud, T., Pouclet, H., et al. (2013). Colonic inflammation in Parkinson's disease. *Neurobiol. Dis.* 50, 42–48.

Diether, N. E., and Willing, B. P. (2019). Microbial fermentation of dietary protein: an important factor in diet¯microbe¯host interaction. *Microorganisms* 7:19. doi: 10.3390/microorganisms7010019

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., et al. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 18, 965–977. doi: 10.1038/nn.4030

Fang, H., Du, Y., Pan, S., Zhong, M., and Tang, J. (2021). Patients with Parkinson's disease predict a lower incidence of colorectal cancer. *BMC Geriatr.* 21:564. doi: 10.1186/s12877-021-02497-z

Fang, X. (2016). Potential role of gut microbiota and tissue barriers in Parkinson's disease and amyotrophic lateral sclerosis. *Int. J. Neurosci.* 126, 771–776. doi: 10.3109/00207454.2015.1096271

Felice, V. D., Quigley, E. M., Sullivan, A. M., O'Keeffe, G. W., and O'Mahony, S. M. (2016). Microbiota-gut-brain signalling in Parkinson's disease: Implications for non-motor symptoms. *Parkinsonism Relat. Disord.* 27, 1–8. doi: 10.1016/j.parkreldis.2016.03.012

Forsyth, C. B., Shannon, K. M., Kordower, J. H., Voigt, R. M., Shaikh, M., Jaglin, J. A., et al. (2011). Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS One* 6:e28032. doi: 10.1371/journal.pone.0028032

Franke, A., McGovern, D. P., Barrett, J. C., Wang, K., Radford-Smith, G. L., Ahmad, T., et al. (2010). Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat. Genet.* 42, 1118–1125.

Freedman, D. M., and Pfeiffer, R. M. (2016). Associations Between Parkinson Disease and Cancer in US Asian Americans. *JAMA Oncol.* 2, 1093–1094. doi: 10.1001/jamaoncol.2016.0729

Fujioka, S., Curry, S. E., Kennelly, K. D., Tacik, P., Heckman, M. G., Tsuboi, Y., et al. (2017). Occurrence of Crohn's disease with Parkinson's disease. *Parkinsonism Relat. Disord.* 37, 116–117.

Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., et al. (2014). Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* 128, 805–820. doi: 10.1007/s00401-014-1343-6

Horsager, J., Andersen, K. B., Knudsen, K., Skjærbæk, C., Fedorova, T. D., Okkels, N., et al. (2020). Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain* 143, 3077–3088. doi: 10.1093/brain/awaa238

Hu, C. W., Tseng, C. W., Chien, C. W., Huang, H. C., Ku, W. C., Lee, S. J., et al. (2013). Quantitative proteomics reveals diverse roles of miR-148a from gastric cancer progression to neurological development. *J. Proteome Res.* 12, 3993–4004. doi: 10.1021/pr400302w

Jamal, S., Kumari, A., Singh, A., Goyal, S., and Grover, A. (2017). Conformational ensembles of α-synuclein derived peptide with different osmolytes from temperature replica exchange sampling. *Front. Neurosci.* 11:684. doi: 10.3389/fnins.2017.00684

Janeiro, M. H., Ramírez, M. J., Milagro, F. I., Martínez, J. A., and Solas, M. (2018). Implication of trimethylamine N-Oxide (TMAO) in disease: potential biomarker or new therapeutic target. *Nutrients* 10:1398. doi: 10.3390/nu10101398

Kalia, L. V., and Lang, A. E. (2015). Parkinson's disease. *Lancet* 386, 896–912. doi: 10.1016/s0140-6736(14)61393-3

Kang, X., Ploner, A., Ludvigsson, J. F., Williams, D. M., Larsson, H., Pedersen, N. L., et al. (2020). Clostridium difficile infection and risk of Parkinson's disease: a Swedish population-based cohort study. *Eur. J. Neurol.* 27, 2134–2141. doi: 10.1111/ene.14400

Kang, X. A.-O., Ploner, A., Roelstraete, B., Khalili, H., Williams, D. M., Pedersen, N. L., et al. (2021). Association between microscopic colitis and parkinson's disease in a swedish population. *Mov. Disord.* 36, 1919–1926.

Kelly, C. J., Zheng, L., Campbell, E. L., Saeedi, B., Scholz, C. C., Bayless, A. J., et al. (2015). Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 17, 662–671. doi: 10.1016/j.chom.2015.03.005

Khalili, H., Burke, K. E., Roelstraete, B., Sachs, M. C., Olén, O., and Ludvigsson, J. F. (2020). Microscopic colitis and risk of inflammatory bowel disease in a nationwide cohort study. *Gastroenterology* 158, 1574–1583.e2. doi: 10.1053/j.gastro.2019.12.028

Kim, S., Kwon, S. H., Kam, T. I., Panicker, N., Karuppagounder, S. S., Lee, S., et al. (2019). Transneuronal propagation of pathologic α-synuclein from the gut to the brain models parkinson's disease. *Neuron* 103, 627–641.e7. doi: 10.1016/j.neuron.2019.05.035

Klingelhoefer, L., and Reichmann, H. (2015). Pathogenesis of Parkinson disease–the gut-brain axis and environmental factors. *Nat. Rev. Neurol.* 11, 625–636. doi: 10.1038/nrneurol.2015.197

Kuai, X. Y., Yao, X. H., Xu, L. J., Zhou, Y. Q., Zhang, L. P., Liu, Y., et al. (2021). Evaluation of fecal microbiota transplantation in Parkinson's disease patients with constipation. *Microb. Cell Fact.* 20:98. doi: 10.1186/s12934-021-01589-0

Lai, S. W., Liao, K. F., Lin, C. L., and Sung, F. C. (2014). Irritable bowel syndrome correlates with increased risk of Parkinson's disease in Taiwan. *Eur. J. Epidemiol.* 29, 57–62. doi: 10.1007/s10654-014-9878-3

Leikas, J. V., Kohtala, S., Theilmann, W., Jalkanen, A. J., Forsberg, M. M., and Rantamäki, T. (2017). Brief isoflurane anesthesia regulates striatal AKT-GSK3β signaling and ameliorates motor deficits in a rat model of early-stage Parkinson's disease. *J. Neurochem.* 142, 456–463. doi: 10.1111/jnc.14066

Li, Z., Lu, G., Li, Z., Wu, B., Luo, E., Qiu, X., et al. (2021). Altered actinobacteria and firmicutes phylum associated epitopes in patients with parkinson's disease. *Front. Immunol.* 12:632482. doi: 10.3389/fimmu.2021.632482

Lin, C. H., Chen, C. C., Chiang, H. L., Liou, J. M., Chang, C. M., Lu, T. P., et al. (2019). Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J. Neuroinflammation* 16:129. doi: 10.1186/s12974-019-1528-y

Lin, J. C., Lin, C. S., Hsu, C. W., Lin, C. L., and Kao, C. H. (2016). Association between parkinson's disease and inflammatory bowel disease: a nationwide taiwanese retrospective cohort study. *Inflamm. Bowel Dis.* 22, 1049–1055. doi: 10.1097/MIB.0000000000000735

Lin, P. Y., Chang, S. N., Hsiao, T. H., Huang, B. T., Lin, C. H., and Yang, P. C. (2015). Association between parkinson disease and risk of cancer in Taiwan. *JAMA Oncol.* 1, 633–640. doi: 10.1001/jamaoncol.2015.1752

Liu, B., Fang, F., Pedersen, N. L., Tillander, A., Ludvigsson, J. F., Ekbom, A., et al. (2017). Vagotomy and Parkinson disease: a swedish register-based matched-cohort study. *Neurology* 88, 1996–2002. doi: 10.1212/WNL.0000000000003961

Liu, B., Sjölander, A., Pedersen, N. L., Ludvigsson, J. F., Chen, H., Fang, F., et al. (2021). Irritable bowel syndrome and Parkinson's disease risk: register-based studies. *NPJ Parkinsons. Dis.* 7:5. doi: 10.1038/s41531-020-00145-8

Lolekha, P., Sriphanom, T., and Vilaichone, R. K. (2021). *Helicobacter* pylori eradication improves motor fluctuations in advanced Parkinson's disease patients: a prospective cohort study (HP-PD trial). *PLoS One* 16:e0251042. doi: 10.1371/journal.pone.0251042

Losurdo, G., Salvatore, D., Abramo, F., Indellicati, G., Lillo, C., Ierardi, E., et al. (2020). The influence of small intestinal bacterial overgrowth in digestive and extra-intestinal disorders. *Int. J. Mol. Sci.* 21:3531. doi: 10.3390/ijms21103531

Macerollo, A., Lu, M. K., Huang, H. C., Chen, H. J., Lin, C. C., Kao, C. H., et al. (2017). Colonic diverticular disease: a new risk factor for Parkinson's disease? *Parkinsonism Relat. Disord.* 42, 61–65. doi: 10.1016/j.parkreldis.2017.06.011

Manasanch, E. E., and Orlowski, R. Z. (2017). Proteasome inhibitors in cancer therapy. *Nat. Rev. Clin. Oncol.* 14, 417–433. doi: 10.1038/nrclinonc.2016.206

Mertsalmi, T., Aho, V., Pereira, P. A. B., Paulin, L., Pekkonen, E., Auvinen, P., et al. (2016). Irritable bowel syndrome is more prevalent than functional constipation in Parkinson's disease: Clinical spectrum and changes in gut microbiota. *Move. Disord.* 31, S111–S112. doi: 10.1002/mds.26688

Mertsalmi, T., Pekkonen, E., and Scheperjans, F. (2017a). Diagnosis of irritable bowel syndrome increases risk of Parkinson's disease in the Finnish population. *Move. Disord.* 32, 54–55. doi: 10.1002/mds.27087

Mertsalmi, T. H., Aho, V. T. E., Pereira, P. A. B., Paulin, L., Pekkonen, E., Auvinen, P., et al. (2017b). More than constipation - bowel symptoms in Parkinson's disease and their connection to gut microbiota. *Eur. J. Neurol.* 24, 1375–1383. doi: 10.1111/ene.13398

Mishima, T., Fukae, J., Fujioka, S., Inoue, K., and Tsuboi, Y. (2017). The prevalence of constipation and irritable bowel syndrome in parkinson's disease patients according to Rome III diagnostic criteria. *J. Parkinsons Dis.* 7, 353–357. doi: 10.3233/jpd-160982

Nerius, M., Doblhammer, G., and Tamgüney, G. (2020). GI infections are associated with an increased risk of Parkinson's disease. *Gut* 69, 1154–1156. doi: 10.1136/gutjnl-2019-318822

Nielsen, H. H., Qiu, J., Friis, S., Wermuth, L., and Ritz, B. (2012). Treatment for *Helicobacter* pylori infection and risk of Parkinson's disease in Denmark. *Eur. J. Neurol.* 19, 864–869. doi: 10.1111/j.1468-1331.2011.03643.x

Nishiwaki, H., Ito, M., Ishida, T., Hamaguchi, T., Maeda, T., Kashihara, K., et al. (2020). Meta-Analysis of Gut Dysbiosis in Parkinson's Disease. *Mov. Disord.* 35, 1626–1635. doi: 10.1002/mds.28119

Ong, T. L., Dal, S., Martin, A. J., Chang, F. C., Williams, L. J., Babu, S., et al. (2021). Levodopa/dopa decarboxylase inhibitor associated microscopic colitis: An under-recognized drug reaction. *Parkinson. Relat. Disord.* 86, 84–90. doi: 10.1016/j.parkreldis.2021.03.031

Park, S. A.-O., Kim, J., Chun, J. A.-O., Han, K., Soh, H. A.-O., Kang, E. A., et al. (2019). Patients with inflammatory bowel disease are at an increased risk of parkinson's disease: a south korean nationwide population-based study. *J. Clin. Med.* 8:1191.

Peter, I., Dubinsky, M., Bressman, S., Park, A., Lu, C., Chen, N., et al. (2018). Anti-Tumor necrosis factor therapy and incidence of parkinson disease among patients with inflammatory bowel disease. *JAMA Neurol.* 75, 939–946.

Phani, S., Loike, J. D., and Przedborski, S. (2012). Neurodegeneration and inflammation in Parkinson's disease. *Parkinsonism Relat. Disord.* 18(Suppl. 1), S207–S209.

Phillips, R. J., Walter, G. C., Wilder, S. L., Baronowsky, E. A., and Powley, T. L. (2008). Alpha-synuclein-immunopositive myenteric neurons and vagal preganglionic terminals: autonomic pathway implicated in Parkinson's disease? *Neuroscience* 153, 733–750. doi: 10.1016/j.neuroscience.2008.02.074

Qin, X. Y., Zhang, S. P., Cao, C., Loh, Y. P., and Cheng, Y. (2016). Aberrations in peripheral inflammatory cytokine levels in parkinson disease: a systematic review and meta-analysis. *JAMA Neurol.* 73, 1316–1324. doi: 10.1001/jamaneurol.2016.2742

Russo, R., Cristiano, C., Avagliano, C., De Caro, C., La Rana, G., Raso, G. M., et al. (2018). Gut-brain axis: role of lipids in the regulation of inflammation, pain and CNS diseases. *Curr. Med. Chem.* 25, 3930–3952. doi: 10.2174/0929867324666170216113756

Sampson, T. R., Debelius, J. W., Thron, T., Janssen, S., Shastri, G. G., Ilhan, Z. E., et al. (2016). Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's Disease. *Cell* 167, 1469.e–1480.e. doi: 10.1016/j.cell.2016.11.018

Sankowski, B., Księżarczyk, K., Raćkowska, E., Szlufik, S., Koziorowski, D., and Giebułtowicz, J. (2020). Higher cerebrospinal fluid to plasma ratio of p-cresol sulfate and indoxyl sulfate in patients with Parkinson's disease. *Clin. Chim. Acta* 501, 165–173. doi: 10.1016/j.cca.2019.10.038

Scheperjans, F., Aho, V., Pereira, P. A., Koskinen, K., Paulin, L., Pekkonen, E., et al. (2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov. Disord.* 30, 350–358.

Sharma, A., and Rao, S. (2017). Constipation: pathophysiology and current therapeutic approaches. *Handb. Exp. Pharmacol.* 239, 59–74. doi: 10.1007/164_2016_111

Sherman, M. Y., and Goldberg, A. L. (2001). Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* 29, 15–32. doi: 10.1016/s0896-6273(01)00177-5

Stokholm, M. G., Danielsen, E. H., Hamilton-Dutoit, S. J., and Borghammer, P. (2016). Pathological α-synuclein in gastrointestinal tissues from prodromal Parkinson disease patients. *Ann. Neurol.* 79, 940–949. doi: 10.1002/ana.24648

Svensson, E., Horváth-Puhó, E., Thomsen, R. W., Djurhuus, J. C., Pedersen, L., Borghammer, P., et al. (2015). Vagotomy and subsequent risk of Parkinson's disease. *Ann. Neurol.* 78, 522–529.

Takakura, W., and Pimentel, M. (2020). Small intestinal bacterial overgrowth and irritable bowel syndrome - an update. *Front. Psychiatry* 11:664. doi: 10.3389/fpsyt.2020.00664

Tan, A. H., Chong, C. W., Lim, S. Y., Yap, I. K. S., Teh, C. S. J., Loke, M. F., et al. (2021). Gut microbial ecosystem in Parkinson Disease: new clinicobiological insights from multi-omics. *Ann. Neurol.* 89, 546–559. doi: 10.1002/ana.25982

Tian, S., Zhao, S., Tang, M., and Wang, C. (2021). Gene expression barcode values reveal a potential link between Parkinson's disease and gastric cancer. *Aging* 13, 6171–6181. doi: 10.18632/aging.202623

Tolosa, E. A.-O., Vila, M., Klein, C., and Rascol, O. (2020). LRRK2 in Parkinson disease: challenges of clinical trials. *Nat. Rev. Neurol.* 16, 97–107.

Tursi, A. (2016). Diverticulosis today: unfashionable and still under-researched. *Therap. Adv. Gastroenterol.* 9, 213–228. doi: 10.1177/1756283x15621228

Unger, M. M., Spiegel, J., Dillmann, K.-U., Grundmann, D., Philippeit, H., Bürmann, J., et al. (2016). Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat. Disord.* 32, 66–72. doi: 10.1016/j.parkreldis.2016.08.019

Uversky, V. N., Li, J., and Fink, A. L. (2001). Trimethylamine-N-oxide-induced folding of alpha-synuclein. *FEBS Lett.* 509, 31–35. doi: 10.1016/s0014-5793(01)03121-0

Van Den Berge, N., and Ulusoy, A. (2022). Animal models of brain-first and body-first Parkinson's disease. *Neurobiol. Dis.* 163:105599. doi: 10.1016/j.nbd.2021.105599

Vascellari, S., Melis, M., Palmas, V., Pisanu, S., Serra, A., Perra, D., et al. (2021). Clinical Phenotypes of Parkinson's disease associate with distinct gut microbiota and metabolome enterotypes. *Biomolecules* 11:144. doi: 10.3390/biom11020144

Vascellari, S., Palmas, V., Melis, M., Pisanu, S., Cusano, R., Uva, P., et al. (2020). Gut microbiota and metabolome alterations associated with parkinson's disease. *mSystems* 5:e00561-20.

Veeriah, S., Taylor, B. S., Meng, S., Fang, F., Yilmaz, E., Vivanco, I., et al. (2010). Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. *Nat. Genet.* 42, 77–82. doi: 10.1038/ng.491

Villumsen, M., Aznar, S., Pakkenberg, B., Jess, T., and Brudek, T. A.-O. (2019). Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014. *Gut.* 68, 18–24. doi: 10.1136/gutjnl-2017-315666

Wedel, T., Büsing, V., Heinrichs, G., Nohroudi, K., Bruch, H. P., Roblick, U. J., et al. (2010). Diverticular disease is associated with an enteric neuropathy as revealed by morphometric analysis. *Neurogastroenterol. Motil.* 40, 407–414. doi: 10.1111/j.1365-2982.2009.01445.x

Weimers, P., Halfvarson, J., Sachs, M. C., Saunders-Pullman, R., Ludvigsson, J. F., Peter, I., et al. (2019). Inflammatory bowel disease and parkinson's disease: a nationwide swedish cohort study. *Inflamm. Bowel Dis.* 25, 111–123. doi: 10.1093/ibd/izy190

Wu, G., Jiang, Z., Pu, Y., Chen, S., Wang, T., Wang, Y., et al. (2022). Serum short-chain fatty acids and its correlation with motor and non-motor symptoms in Parkinson's disease patients. *BMC Neurol.* 22:13. doi: 10.1186/s12883-021-02544-7

Xia, J., Guo, X., Yan, J., and Deng, K. (2014). The role of miR-148a in gastric cancer. *J. Cancer Res. Clin. Oncol.* 140, 1451–1456.

Xue, L. J., Yang, X. Z., Tong, Q., Shen, P., Ma, S. J., Wu, S. N., et al. (2020). Fecal microbiota transplantation therapy for Parkinson's disease: A preliminary study. *Medicine* 99:e22035. doi: 10.1097/md.0000000000022035

Yan, Z., Yang, F., Cao, J., Ding, W., Yan, S., Shi, W., et al. (2021). Alterations of gut microbiota and metabolome with Parkinson's disease. *Microb. Pathog.* 160:105187.

Yang, M., Gu, Y., Li, L., Liu, T., Song, X., Sun, Y., et al. (2021). Bile Acid-Gut Microbiota Axis in Inflammatory Bowel Disease: From Bench to Bedside. *Nutrients* 13, doi: 10.3390/nu13093143

Zhang, X., Svn, Z., Liv, M., Yang, Y., Zeng, R., Huang, Q., et al. (2021). Association between irritable bowel syndrome and risk of parkinson's disease: a systematic review and meta-analysis. *Front. Neurol.* 12:720958. doi: 10.3389/fneur.2021.720958

Zhao, Z., Ning, J., Bao, X. Q., Shang, M., Ma, J., Li, G., et al. (2021). Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis. *Microbiome* 9:226. doi: 10.1186/s40168-021-01107-9

Zheng, S. Y., Li, H. X., Xu, R. C., Miao, W. T., Dai, M. Y., Ding, S. T., et al. (2021). Potential roles of gut microbiota and microbial metabolites in Parkinson's disease. *Ageing Res. Rev.* 69:101347. doi: 10.1016/j.arr.2021.101347

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# *Pitx3* deficiency promotes age-dependent alterations in striatal medium spiny neurons

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**Background:** The classical motor symptoms of Parkinson's disease (PD) are tightly linked to the gradual loss of dopamine within the striatum. Concomitantly, medium spiny neurons (MSNs) also experience morphological changes, such as reduced dendritic complexity and spine density, which may be potentially associated with motor dysfunction as well. Thus, MSNs may serve as the emerging targets for PD therapy besides the midbrain dopaminergic neurons.

**Results:** To comprehensively examine pathological alterations of MSNs longitudinally, we established a $TH^{Cre/}$ $Pitx3^{fl/fl}$ ($Pitx3^{cKO}$) mouse model that developed canonical PD features, including a significant loss of SNc DAergic neurons and motor deficits. During aging, the targeted neurotransmitter, MSNs morphology and DNA methylation profile were significantly altered upon *Pitx3* deficiency. Specifically, dopamine, GABA and glutamate decreased in the model at the early stage. While nuclear, soma and dendritic atrophy, as well as nuclear invaginations increased in the aged MSNs of $Pitx3^{cko}$ mice. Furthermore, more nuclear DNA damages were characterized in MSNs during aging, and *Pitx3* deficiency aggravated this phenomenon, together with alterations of DNA methylation profiling associated with lipoprotein and nucleus pathway at the late stage.

**Conclusion:** The early perturbations of the neurotransmitters within MSNs may potentially contribute to the alterations of metabolism, morphology and epigenetics within the striatum at the late stage, which may provide new perspectives on the diagnosis and pathogenesis of PD.

KEYWORDS

**medium spiny neurons, neuronal morphology, DNA methylation, aging, Parkinson's disease**

Abbreviations: PD, Parkinson's disease; MSNs, spiny projection neurons; DA, dopamine; TH, tyrosine hydroxylase; SNc, substantia nigra; GABA, γ-aminobutyric acid; *ak*, aphakia; VTA, ventral tegmental area; GFP, green fluorescent protein; DMGs, differentially methylated genes.

## Introduction

The striatum is the largest integrative component of the basal ganglia and plays an essential role in modulating complex behaviors, such as facilitation or inhibition of actions and reward learning (Du and Graves, 2019; Prager and Plotkin, 2019). It receives the glutamatergic afferents from the cerebral cortex and thalamus as well as DAergic afferents from the SNc. These massive neurochemical inputs from corticostriatal, thalamostriatal, and nigrostriatal projections are largely processed by striatal MSNs, together with the interneurons in a topographically organized manner (Bariselli et al., 2019; Filipovic et al., 2019; Chen et al., 2020). MSNs use γ-aminobutyric acid (GABA) as a neurotransmitter and constitute 90–95% of the striatal neuronal population (He et al., 2021). Despite high homogeneity, MSNs can be divided into two distinct subpopulations based on their output projection pathways and neurochemical content (Gerfen et al., 1990; Gong et al., 2003; He et al., 2021). Meanwhile, the MSNs are the only striatal neurons with dendritic spines that are highly specialized structures of neuronal connectivity for the regulation of synaptic strength (Bolam et al., 2000; Parisiadou et al., 2014).

In PD patients, the striatum undergoes progressive DA depletion (Wang et al., 2021), consequently leading to cardinal motor symptoms, such as resting tremor, bradykinesia, postural instability, and rigidity. Meanwhile, MSNs as the predominant striatal neuron population, also experience the morphologic alterations, i.e., the dendritic alterations have been observed in postmortem studies of PD brains (McNeill et al., 1988; Stephens et al., 2005; Zaja-Milatovic et al., 2005). Previously, the toxic PD animal models showed the reduced dendritic length and spine density of MSNs, resembling the findings identified in PD patients (Day et al., 2006; Villalba et al., 2009; Zhang et al., 2013; Suarez et al., 2014; Toy et al., 2014). However, these observations may be due in part to the toxic effects and independent of the PD-induced pathology. Later on, Suarez et al. (2018) represented that the MSNs in *Pitx3* knockout mice do appear similar morphologic abnormalities to that of toxic PD animal models, excluding the toxic effects and suggesting a close association between PD and MSNs. Pitx3 is a transcription factor mainly expressed in midbrain DAergic neurons and plays an essential role in DAergic neuronal development (Smidt et al., 1997, 2004). Later on, our studies showed that Pitx3 is also involved in maintaining the normal physiological functions in the postnatal DAergic neurons (Wang et al., 2021). During aging, the vulnerability of SNc DAergic neurons increased with an early decline in glial cell line-derived neurotrophic factor (GDNF) and aldehyde dehydrogenase 1a1 (Aldh1a1) levels (Wang et al., 2021). Since the deficiency of *Pitx3* triggered a profound loss of SNc DAergic neurons even at the embryonic stage (Hwang et al., 2003; Filali and Lalonde, 2016), few DAergic innervations project onto the striatum and thereby most MSNs are exposed to little DA throughout life, rather than to the progressive depletion of DA during aging. To overcome this drawback and comprehensively examine the pathological alterations of MSNs longitudinally, we generated a $TH^{Cre}/Pitx3^{fl/fl}$ ($Pitx3^{cKO}$), a conditional knockout mouse model, where a progressive reduction of striatal DA occurs in the fully developed MSNs. Meanwhile, the mice showed a significant loss of SNc DAergic neurons and movement abnormalities. Thus, the utilization of the $Pitx3^{cKO}$ model may offer great potential for systematically examining the neurochemistry, morphology and epigenetics of MSNs during aging. Our preliminary studies revealed that multiple neurotransmitters were deducted first in the young $Pitx3^{cKO}$ mice. The early disruption of neurochemistry may thereby contribute to the remodeling of MSNs late—reduced dendritic complexity of MSNs, and shrinkage of soma and nuclear size, together with altered epigenetic profiling in $Pitx3^{cKO}$ mice.

## Materials and methods

### The generation of conditional knockout *Pitx3* mouse model

The heterozygous mice $Pitx3^{Flox/wt}$ with C57BL/6J background were generated by ViewSolid Biotech Co., Ltd. (Beijing, China) and the TH-Cre driver mice with C57BL/6J background were generated by Shanghai Model Organisms (Shanghai, China); both mouse lines are available upon request. The RioTag mice were purchased from JAX lab (#011029) (Shigeoka et al., 2016). To achieve the conditional knockout *Pitx3* mouse model in the DA neuronal system, $Pitx3^{cKO}$ mice were produced by breeding mice carrying an *Cre* recombinase under the *TH* promoter with the homozygous mice $Pitx3^{Flox/Flox}$. All experimental mice were maintained under specific-pathogen-free (SPF) conditions (temperature, 22°C ± 2°C; air exchange, per 20 min; 12 h/12 h light–dark cycle) with free access to food and water. Animal care and procedures were carried out in accordance with the Laboratory Animal Care Guidelines approved by the Institutional Animal Care Committee at Dalian Medical University. The protocol was approved by the Institutional Animal Care Committee at Dalian Medical University.

For $Pitx3^{Flox/wt}$ mice, CRISPR technology was applied to cut the DNA of the intron of the *Pitx3* gene, providing the homologous template donor. The sequences of floxp were inserted at both ends of the specific exons (exon 2 and exon 3) through homologous recombination. When mated with tissue-specific expression Cre mice, the specific exon 2 and exon 3 of *Pitx3* were deleted, thereby achieving the purpose of conditional knockout of the *Pitx3* gene. For *TH-Cre* knock-in mouse driver line, the Cre recombinase was cloned following an

IRES sequence. An frt-flanked neomycin selection cassette was added, and the construct was cloned in the 3′ untranslated end of the TH gene (as described by Althini et al., 2003). The coding sequence of TH is not affected, nor are the expression levels, so both the TH and Cre recombinase proteins are produced in Th-expressing cells of this mouse line.

## Behavioral test

The open field test was performed in a quiet testing room. To measure the locomotor activity, mice were placed into an Activity Monitor instrument (25 cm × 25 cm × 30 cm, Med Associates Inc., St. Albans, United States) equipped with computer-controlled photocells. Locomotor activity was automatically recorded for 6 min, and different elements of open field test were calculated by the Med system.

Rotarod motor skill learning test was performed as described previously (Wu et al., 2019). Mice were placed onto a rotating rod with auto acceleration from 0 to 40 rpm in 5 min (Model 755, IITC Life Science). The length of time the mouse stayed on the rotating rod was recorded across 10 trials. Such experiments were performed on six continuous days.

## Immunostaining

Mouse brains were collected at indicated time points. The brains were rapidly isolated and postfixed in ice-cold 4% paraformaldehyde and subsequently dehydrated for 24 h in 15% and 30% sucrose at 4°C, as described previously (Dong et al., 2020). Sections were incubated for 1 h in blocking solution (5% normal goat serum, 0.2% Triton-X 100, and 0.05% $NaN_3$ in PBS). The primary antibodies were used as follows: anti-TH (1:1,000, AB152; Millipore, United States), anti-TH (1:2,000, T1299; Sigma-Aldrich, United States), anti-TH (1:1,000, TYH, Aves Labs, United States), anti-NeuN (1:1,000, MAB377; Millipore, United States), anti-LaminB1 (1:1,000, 12987-1-AP; Proteintech, United States), anti-Darpp32 (1:1,000, 2306; CST, United States), anti-phospho-Histone H2AX (1:1,000, 2577; CST, United States), anti-GFP (1:1,000, ab6662; Abcam, United States), anti-nuclear pore complex (1:1,000, ab24609; Abcam, United States), anti-TOM20 (1:1,000, 42406; CST, United States), anti-Ctip2 (1:500, ab18465; Abcam, United States), anti-HA (1:500, ab9110; Abcam, United States) and anti-Pitx3 (provided by Dr. Marten P. Smidt's lab at the University of Amsterdam, Netherlands). The section images were visualized and photographed directly with a confocal microscope (A1 confocal, Nikon Instruments [Shanghai] Co., Ltd.) and a DP80 CCD brightfield microscope (Olympus, Japan). The outlines of the SNc and VTA were delimited according to anatomical landmarks (Fu et al., 2012).

## Image analysis

For neuron counting, a series of coronal sections (40 μm per section, every third section from Bregma –2.70 to –3.88 mm) were selected and stained with anti-TH and anti-NeuN antibodies for quantification. Usually, 10–11 sections were collected per animal. The entire midbrain regions were scanned under a 10X objective (A1 confocal, Nikon Instruments [Shanghai] Co., Ltd.). We multiplied the total calculated from 10 to 11 sections by 3 to obtain the final number of $TH^+$ or $NeuN^+$ neurons (Dong et al., 2020; Wang et al., 2021). The IFC intensity of the striatum was analyzed using ImageJ software. Typically, the data were collected from 2 to 3 slices per animal.

## Stereotaxic viral injection

The stereotaxic AAV injections (AAV-hSyn1-eGFP, GeneCopoeia) were conducted on 6- and 12-month-old $Pitx3^{cWT}$ and $Pitx3^{cKO}$ mice. Before surgery, mice were deeply anesthetized by intraperitoneal injection of ketamine (100 mg/kg)/xylazine (10 mg/kg) solution. To achieve sparse labeling, $1.1 \times 10^{12}$ viral particles with a total volume of 500 nl were injected into dorsal striatum (coordinates used, AP: 0.98 mm, ML: ± 2.2 mm from bregma, DV: –3.0 mm from exposed dura mater). Virus solution was injected at an infusion rate of 100 nl/min and the needle was withdrawn 10 min after the end of injection. Following virus injection, the scalp was sutured, and the mice were returned to their home cages. The virus-injected mice were used for experiment at least 4 weeks after the virus infusion.

## Stereology for neuronal tracing

Based on the previous study (Lu and Yang, 2017), the AAV-infused mouse brains were sectioned at 60 μm of thickness. The sections were stained with GFP antibody (1:1000, ab6662; Abcam, Cambridge, UK) and Ctip2 antibody (1:500, ab18465, Abcam, Cambridge, UK). Afterward, the stained sections were imaged using a laser scanning confocal microscope (A1 confocal, Nikon Instruments [Shanghai]Co., Ltd.) under 40× objective lens. The MSNs were identified based on the positive staining of Ctip2. Neuronal structure reconstruction with neuTube (Feng et al., 2015) and Sholl analysis were performed with ImageJ (Longair et al., 2011).

## Neurotransmitter identification

First, we added 400 μl solution 1 (methanol/water, 1:1, v/v, with 0.1% formic acid) with succinic acid as interior label into the striatum tissue, and the mixture were homogenized.

The homogenate was ultrasounded in the ice bath for 10 min, and then was incubated in the ice bath (–20°C) for 30 min. The samples were centrifuged at 12,000 rpm × 10 min under 4°C and 300 μl supernatant was preserved. The pellet was incubated with 200 μl solution 1, vortex for 30 s, and then was ultrasounded in the ice bath for 5 min. The samples were centrifuged at 12,000 rpm × 10 min under 4°C and 200 μl supernatant was preserved. We combined 200 μl supernatant with 300 μl preserved supernatant above and obtained 500 μl supernatant totally. All these 500 μl supernatant was transferred into a glass vial to conduct vacuum-drying. After dissolving and centrifuging, the obtained supernatant was used to identify dopamine, GABA and glutamate using LC-MS system.

## MethylRAD sequencing and DNA methylation data analysis

Genomic DNA was extracted from striatum tissues per mouse at indicated timepoints (QIAamp DNA Micro kit Qiagen, German). MethylRAD library preparation and sequencing were conducted according to the protocol described by Wang et al. (2015). PE sequencing was performed on the Illumina HiSeq X-Ten platform. After QC and filtering of the original reads and removal of the sequences with linkers, low-quality sequences (more than five bases with a quality lower than 10), and those with Ns (unidentified bases), the high-quality clean reads containing the methylated CG/CWG sites were mapped to the reference sequence (signatures with CG/CWG sites) of the mouse genome GENCODE V38 by the SOAP program (version 2.21). Sites covered by at least three reads were regarded as reliable DNA methylation sites. Then, the number of methylated sites and the depth of signature coverage of each sample were calculated. The methylation levels of a site (CG/CWG) could be reflected by the sequencing depth of the methylated signature. The unit of the quantitative value of site methylation was RPM (reads per million), which means that the quantitative value of the methylation level of a site was equal to the coverage at that site in number of reads/the number of high-quality reads in the library multiplied by 1,000,000. Furthermore, the distributions of the methylated CG/CWG sites on different elements of the genome, especially on the different regions of genes, were evaluated by SnpEff software (version: 4.1g) (Cingolani et al., 2012) and bed tools software (v2.25.0) (Quinlan and Hall, 2010). Then, the DNA methylation levels of the genes were evaluated by summing the methylation levels of sites that were localized in the gene region. The differential DNA methylation levels of the sites and genes were identified by using the R package DESeq (Anders and Huber, 2010). Finally, the genes with different methylation levels in different samples were further analyzed based on Gene Ontology (GO) enrichment by DAVID and Kyoto Encyclopedia of Genes and Genomes (KEGG)[1] enrichment.

## Statistics

Graph Pad Prism 8 and R were used for statistical analysis. The data were collected and processed randomly. No statistical methods were used to predetermine sample size, but our sample sizes are similar to those reported in previous publications. The statistical significance was determined using Student's *t*- test, 2way ANOVA with Sidak's multiple comparisons test, conditional logistic regression, and multiple *t*-test with Benjamini and Hochberg test.

# Results

## DAergic neuronal degeneration in *Pitx3*$^{cKO}$ mice

Our *Pitx3*$^{cKO}$ mouse model utilized a *Cre*-mediated recombination system driven by the *TH* promoter, resulting in the removal of the second and third coding exons of *Pitx3* (**Supplementary Figure 1**). After multiple generations of breeding, homozygous *Pitx3*-floxed mice harboring (*Pitx3*$^{cKO}$) or not harboring (*Pitx3*$^{cWT}$) the *Cre* gene were achieved, and the genotypes were characterized by PCR analysis (**Supplementary Figure 1**). The expression of Pitx3 was hardly detected in 2-month-old *Pitx3*$^{cKO}$ mice by IFC staining (**Supplementary Figure 1**), indicating the success of *Pitx3* deletion. Unlike *ak* and traditional Pitx3 knockout mice, *Pitx3*$^{cKO}$ mice rarely showed eye defects. Additionally, to confirm the TH-driven Cre expressions, we crossed the RiboTag mice with TH-Cre line (**Supplementary Figure 2**).

Also, the number of DAergic neurons (**Figure 1**) and the dendritic complexity of MSN cells (**Supplementary Figure 3**) were comparable between *Pitx3*$^{cKO}$ and *Pitx3*$^{cWT}$ mice at the age of 6 months. These results indicated that the development of retina cells, DAergic neurons and MSNs was not disrupted. However, a significant loss of DAergic neurons was characterized in 12-month-old *Pitx3*$^{cKO}$ mice, where about 20% of SNc DAergic neurons died (**Figure 1**). Moreover, such deficit was exaggerated at 18 months with around 31.5% neuronal loss in *Pitx3*$^{cKO}$ mice compared to *Pitx3*$^{cWT}$ mice (**Figure 1**). Interestingly, VTA DAergic neurons were less affected by *Pitx3*-deficiency and remained intact during aging (**Figure 1**), resembling the neuropathological phenotype observed in *ak*

1 http://www.genome.jp/kegg/

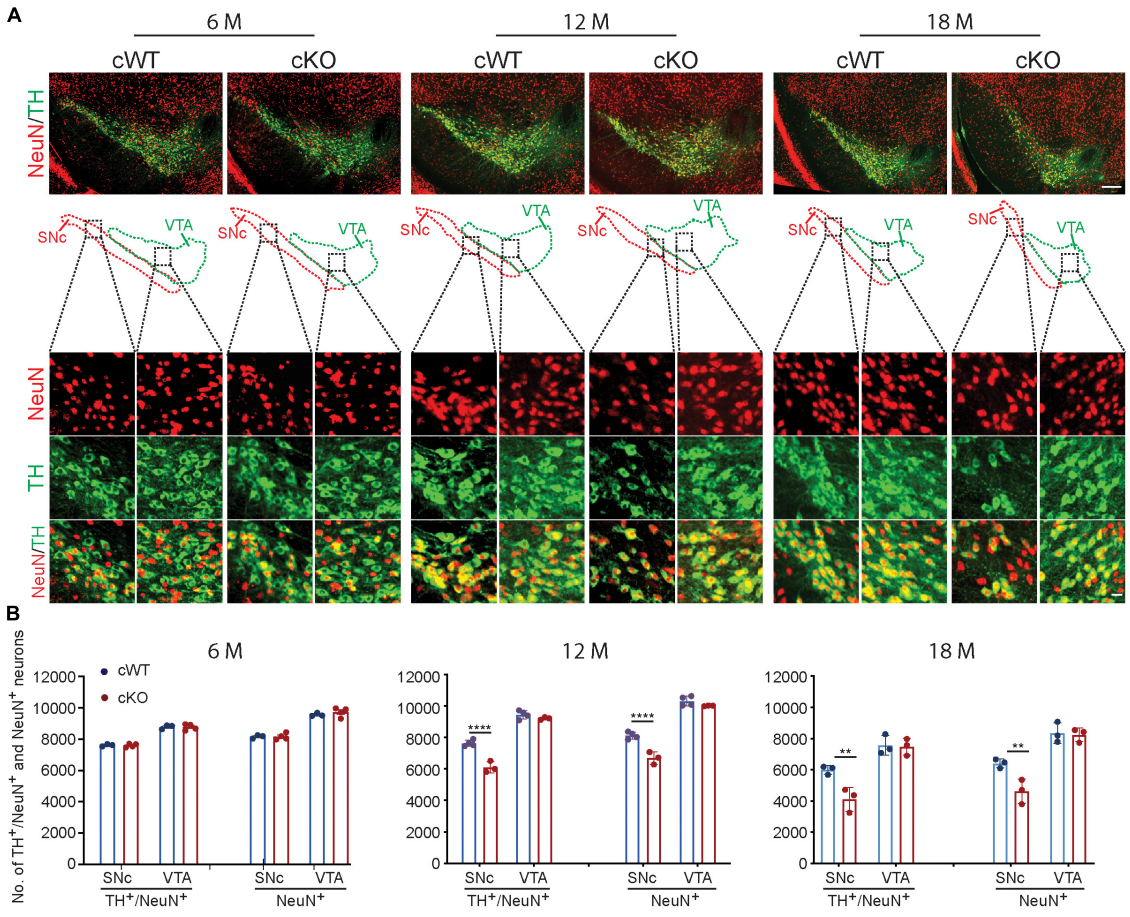


FIGURE 1
Neurodegeneration in 12- and 18-month-old *Pitx3*$^{cKO}$ mice. **(A)** IFC co-staining of TH and NeuN in the ventral midbrain sections from 6-, 12-, and 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice. SNc and VTA were outlined, respectively (scale bar: 200 μm; high-magnification, 20 μm). **(B)** Quantification of TH$^+$/NeuN$^+$ and NeuN$^+$ neurons in the SNc and VTA from 6-, 12-, and 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice ($N$ = 3–4 mice per genotype; all males except for two females in 6-month-old *Pitx3*$^{cKO}$ and 12-month-old *Pitx3*$^{cWT}$). 2way ANOVA analysis with Sidak's multiple comparisons test, ****$p < 0.0001$ (12 months for TH$^+$/NeuN$^+$ co-staining), ****$p < 0.0001$ (12 months for NeuN$^+$ staining), **$p = 0.0041$ (18 months for TH$^+$/NeuN$^+$ co-staining), **$p = 0.0064$ (18 months for NeuN$^+$ staining).

and traditional knockout mice. Also, during aging, there is a natural decline in the number of DAergic neurons of 18-month-old *Pitx3*$^{cWT}$ mice compared to 12-month-old ones (**Supplementary Figure 4**). Taken collectively, these results demonstrated the importance of the *Pitx3* gene in adult neuronal survival (Wang et al., 2021).

## Striatal pathology and movement abnormalities in *Pitx3*$^{cKO}$ mice

Besides neuronal loss, our findings further elucidated that the striatal TH expression levels were decreased by 46% in 18-month-old *Pitx3*$^{cKO}$ mice (**Figures 2A,B**), and the reduction of striatal DA levels in *Pitx3*$^{cKO}$ mice was identified as early as 6 months of age (**Figure 2C**). Specifically, a 36% reduction of DA levels was identified in 12-month-old *Pitx3*$^{cKO}$ mice, compared to the age-matched *Pitx3*$^{cWT}$ mice (**Figure 2C**). Besides dopamine, we also analyzed the contents of GABA and glutamate within the striatum of mice at the age of 6 and 12 months, respectively. GABA as the primary neurotransmitter of MSNs was substantially decreased in 6-month-old *Pitx3*$^{cKO}$, at which point glutamate also showed a significant reduction in the model (**Figure 2C**), but there were no significant changes in these two neurotransmitters between the two genotypes at the late stage, suggesting that the striatal GABA and glutamate displayed age-dependent alterations in our model.

The remarkable striatal pathology may contribute to motor behavioral abnormalities (Valentin et al., 2016). We applied a well-adopted repeated accelerating rotarod test (Yin et al., 2009) to evaluate motor skill learning of mice. 6-month-old *Pitx3*$^{cKO}$ mice performed equally well with age-matched *Pitx3*$^{cWT}$ mice during the 6-day trials, while 12-month-old *Pitx3*$^{cKO}$ mice showed markedly fewer improvements after

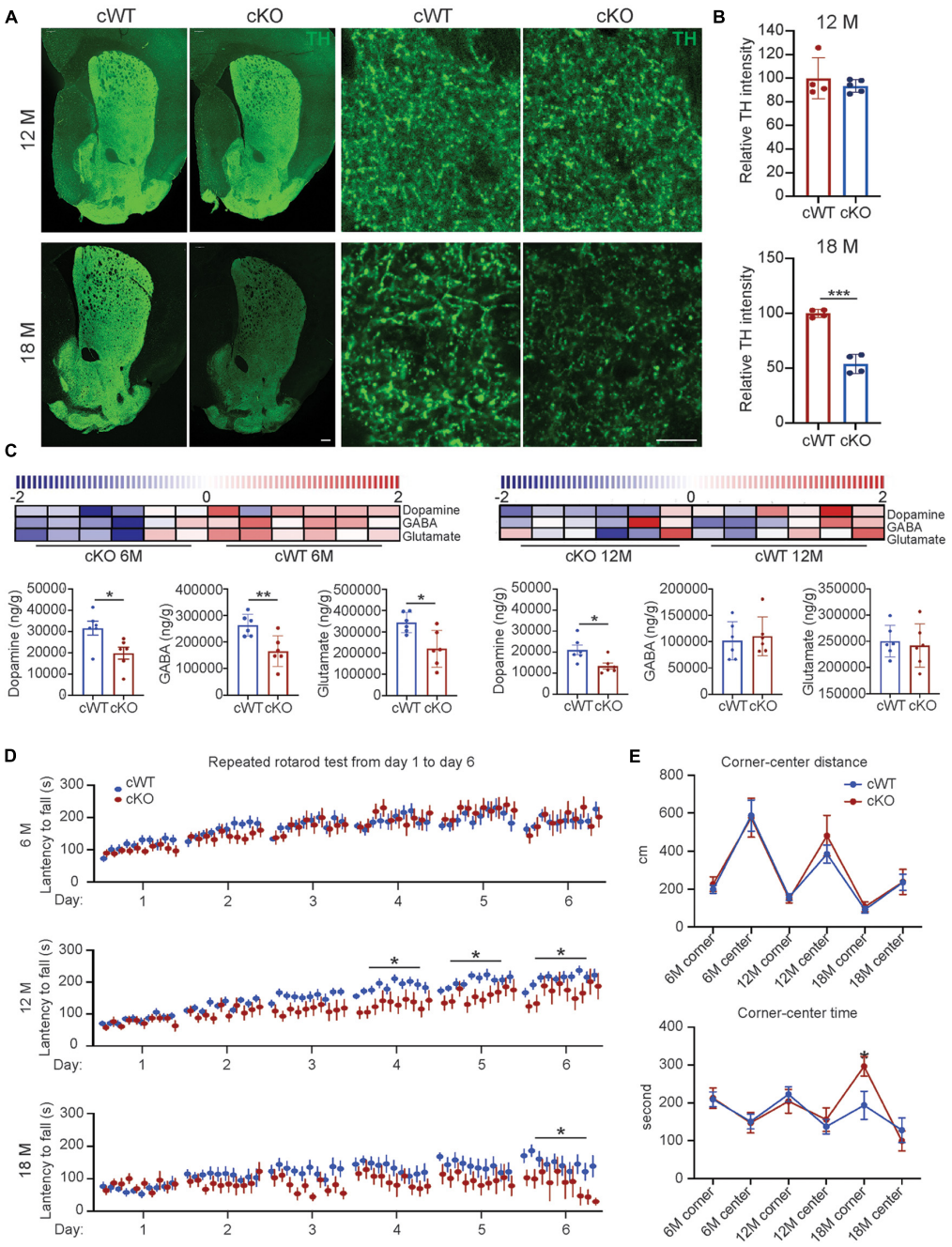


FIGURE 2
Striatal pathology triggered movement abnormalities in *Pitx3*$^{cKO}$ mice. **(A)** IFC staining of TH in the striatal sections from 12- to 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice (scale bar: 200 μm; high-magnification, 10 μm). **(B)** Quantification of relative TH intensity in the striatum from 12- to 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice ($N$ = 4 mice per genotype; all males except for two females in 6-month-old *Pitx3*$^{cKO}$ and 12-month-old *Pitx3*$^{cWT}$). Unpaired $t$-test, ***$p$ = 0.0006 (18 months). **(C)** Levels of neurotransmitter in 6- ($N$ = 6 mice per genotype; all males) and 12-month-old *Pitx3*$^{cKO}$ and *Pitx3*$^{cWT}$ mice ($N$ = 6 mice per genotype; all males). The scaled intensity of three metabolites is relatively depicted according to the color key shown on the above. Red indicates high intensity levels; blue, low intensity levels. Unpaired $t$-test, *$p$ = 0.026 (Dopamine, 6M), **$p$ = 0.0083 (GABA, 6M), *$p$ = 0.012 (Glutamate, 6M), *$p$ = 0.0202 (Dopamine, 12M). **(D)** The latency to fall from rotarod was recorded from *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 6 ($N$ = 11–13 mice per genotype; all males), 12 ($N$ = 11–15 per genotype; all males) and 18 months of age ($N$ = 9–11 mice per genotype; all males). 2way ANOVA analysis with Sidak's multiple comparisons test at 12 and 18 months, *$p$ = 0.0171 (day 4, 12 months), *$p$ = 0.0202 (day 5, 12 months), *$p$ = 0.0376 (day 6, 12 months), *$p$ = 0.0326 (day 6, 18 months). **(E)** Center-corner preference analyses for *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 6 ($N$ = 11–13 mice per genotype; all males), 12 ($N$ = 12–14 per genotype; all males), and 18 ($N$ = 9–10 mice per genotype; all males) months of age. 2way ANOVA analysis with Sidak's multiple comparisons test, *$p$ = 0.05 (time in corner at 18 months).

the first 3 days' training (Figure 2D). Surprisingly, a severely disrupted motor learning phenotype was observed in 18-month-old *Pitx3*$^{cWT}$ mice, and *Pitx3*-deficiency can further affect the last day's training at this advanced stage (Figure 2D). Furthermore, we monitored the voluntary movement of *Pitx3*$^{cKO}$ mice in an open-field test at the age of 6, 12, and 18 months. Overall, multiple elements of locomotor activity were strongly age-dependent rather than genotype-dependent, such as distance, rearing and walking speed (Supplementary Figure 5). Additionally, in center-corner behavioral tests, 18-month-old *Pitx3*$^{cKO}$ mice prefer to stay in the corner for a longer time, compared to age-matched *Pitx3*$^{cWT}$ mice (Figure 2E), suggesting that anxiety levels may be increased. However, the distance traveled in the central zone did not vary between the two genotypes and further investigation may be required, such as an elevated plus-maze test (Rodgers and Dalvi, 1997).

## Morphologic aberrations in medium spiny neurons of *Pitx3*$^{cKO}$ mice during aging

To examine individual MSN morphology, we stereotactically injected AAV1 vectors into the striatum of 6- and 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice, which express a green fluorescent protein (GFP) under the control of synapsin1 promoter. Due to the low viral titer managed, only a few MSNs with GFP signals could be identified in each hemisphere. We subsequently analyze the dendritic complexity by using 3D reconstruction of individual MSN dendritic trees (Figure 3A and Supplementary Figure 3). The results revealed that a profound reduction in the cumulative length of all dendrites was observed in the MSNs of 12-month-old *Pitx3*$^{cKO}$ mice compared to *Pitx3*$^{cWT}$ mice (Figures 3A–E). Additionally, dendritic atrophy was age-dependent, since we did not detect any apparent alterations in MSN dendritic length in *Pitx3*$^{cKO}$ mice at the age of 6 months (Supplementary Figure 3).

We further explored the soma morphology of MSNs in 6-, 12-, and 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice and found a marked reduction of the soma and nuclear size in *Pitx3*$^{cKO}$ during aging (Figures 3F–I). Our longitudinal data demonstrated that the soma and nuclear size of MSNs were steadily increased in *Pitx3*$^{cWT}$ mice from 6 to 18 months of age (Figure 3I). In contrast, the decreased sizes of soma and nucleus in *Pitx3*$^{cKO}$ mice were observed from 6 to 18 months of age (Figure 3I). Despite the alterations in the soma and nuclear size, the nucleus to soma ratio (N/C ratio) remained unchanged (Figure 3J). Besides nuclear size, the nuclear shape was altered as well during aging (Figures 4A,B). The increased nuclear invaginations were characterized in 18-month-old *Pitx3*$^{cKO}$ mice by immunofluorescent staining, where the nuclear envelope marker LaminB and MSN-specific nuclear marker Ctip2 were used (Figures 4A,B). Additionally, the nuclear pore structures were also identified in the enfolded nuclear envelope (Figure 4C), showing the presence of type II nuclear invagination in the MSNs, i.e., both outer and inner nuclear membranes were enfolded. Furthermore, we identified clusters of mitochondria near the mouth of nuclear invagination within striatal neurons (Figure 4D), which may provide extra ATP and/or calcium buffering capacity to protect against the further deformation of nuclear structures (Drozdz and Vaux, 2017). Nuclear invagination was reported to be closely associated with the expressions of γH2AX, a marker for DNA double-strand breaks and damage (Valdiglesias et al., 2013). We thereby examined the DNA stability in the MSNs of *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 12 and 18 months of age. Our results showed a substantial increase in the percentage of MSNs with 10 or more γH2AX-positive foci in the nuclei of 18-month-old *Pitx3*$^{cKO}$ mice compared to *Pitx3*$^{cWT}$ (Figures 4E,F), corresponding to the observed alterations of nuclear shape. Together, these data suggest that the early neurotransmitters' disruptions may be involved in regulating nuclear and soma morphology during aging.

## DNA methylation dynamics in *Pitx3*$^{cKO}$ mice during aging

To further investigate the role of epigenetics on the whole genome within striatal cells, we used MethylRAD sequencing to analyze the DNA methylation at CG and CWG (W for A or T) sites of *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice' genome at the age of 12 and 18 months. Overall, the total DNA methylation ratios on CG (methylated CG/total CG sites) were greatly decreased in 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ (Supplementary Figure 6 and Supplementary Table 1), indicating that aging plays an important role in the global DNA methylation changes. We further examined the distribution patterns of CG methylation sites at the different elements of the genome in all 12 samples (Supplementary Figure 6). The CG methylated sites were concentrated in the introns, followed by the exon and intergenic regions (Supplementary Figure 6). Since CG is more predominant within DNA methylation, we only considered the data from CG sites for the subsequent analyses.

Besides the global genome examination, the DNA methylation levels of individual genes were also evaluated by summing the methylation levels of sites localized in the gene regions. An analysis of differentially methylated genes (DMGs) was conducted for all the samples. We found 182 DMGs (96 hypermethylated and 86 hypomethylated genes) at 12 months and 262 DMGs (154 hypermethylated and 108 hypomethylated genes) at 18 months, respectively (Figure 5A). Further gene-network studies indicated that the DMGs at 12 months are involved in olfaction and mitochondria transportation pathway, whereas the DMGs at 18 months participate in lipoprotein and nucleus pathway, which may be associated with nuclear

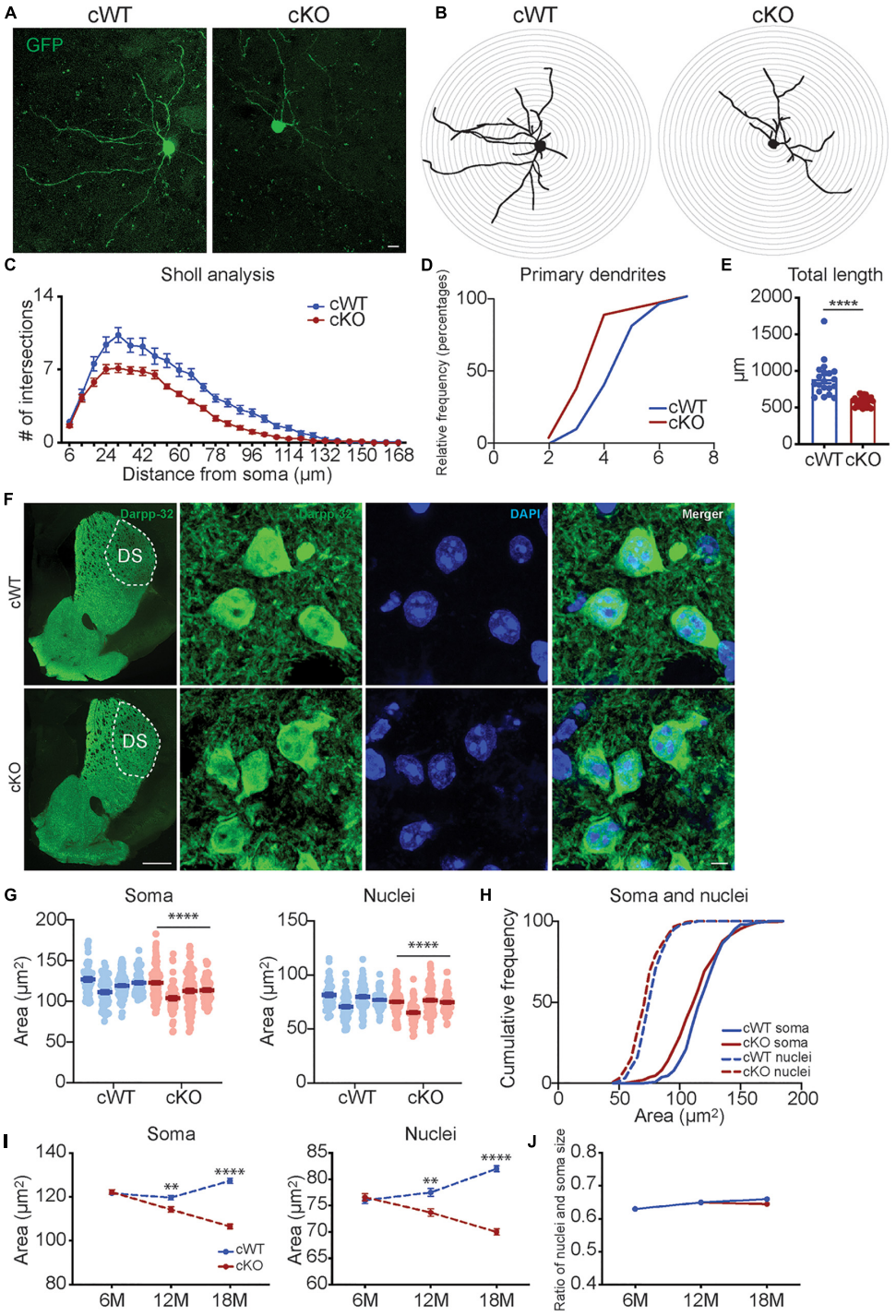


FIGURE 3

Analyses of neuronal morphology in *Pitx3*$^{cKO}$ mice during aging. **(A)** The GFP-labeled individual MSN in 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice (scale bar: 10 μm). **(B,C)** Sholl analysis of dendritic complexity of GFP-labeled MSNs in 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice ($N$ = 3 mice per genotype; 5–8 neurons per mouse were counted). Benjamin- Hochberg multiple comparison test of dendritic complexity at 18, 24, 30, 36, 42, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, and 114 μm from soma, $q \leq 0.05$. **(D)** Analyses of primary dendrites ($N$ = 3 mice per genotype; 5–8 neurons per mouse were counted; all males). **(E)** Dendritic length of GFP-labeled MSNs in 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice ($N$ = 3 mice per genotype; 5–8 neurons per mouse were counted) unpaired $t$-test, $^{****}p < 0.0001$. **(F)** Co-staining of Darpp32 and DAPI in MSNs of 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice (scale bar: 500 μm; high-magnification, 5 μm). **(G)** The soma and nucleus size of MSNs in 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice ($N$ = 4 mice per genotype; all males). Conditional logistic regression test, $^{****}p < 0.0001$. **(H)** Cumulative frequency of the soma and nuclear size distribution in MSNs of 12-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice. **(I)** The soma and

*(Continued)*

FIGURE 3 (Continued)
nucleus size of MSNs in *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 6 ($N$ = 4 mice per genotype; all males), 12 ($N$ = 4 mice per genotype; all males) and 18 months ($N$ = 3 mice per genotype; all males) of age. 2way ANOVA analysis with Sidak's multiple comparisons test, $^{**}p$ = 0.0075 (soma, 12 months), $^{****}p < 0.0001$ (soma, 18 months), $^{**}p$ = 0.0022 (nuclei, 12 months), $^{****}p < 0.0001$ (nuclei, 18 months). **(J)** The nuclear size and soma size ratio (N/C ratio) of MSNs in *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice. DS, dorsal striatum.

morphological changes at this advanced stage (**Figure 5C**). We further identified the DMGs related to normal aging, characterized from 12- to 18-month-old *Pitx3*$^{cWT}$ mice. These genes are involved in multiple cellular process, including aging, glycolytic process, synapse assembly, regulation of translation and mitochondrion organization, consistent with the previous findings (**Figure 5B**). After crossing these DMGs with the ones characterized from 12- to 18-month-old *Pitx3*$^{cKO}$ mice, we totally identified 448 genes, and the alterations of their methylation levels were independent of genotype during aging (**Figure 5D**). Of them, hypermethylated genes are mainly involved in presynaptic membrane assembly and neural tube development, and hypomethylated genes preferentially participate in xenobiotic glucuronidation and regulation of transcription (**Figure 5D**). Notably, in the metabolic pathway analysis, retinol metabolism was affected largely, suggesting that retinoic acid within striatal cells could be regulated specifically by the epigenetic way during aging (**Figure 5E**). Together, these data imply that the DNA methylation modes alters with aging and genotype and may affect multiple cellular process, including retinol metabolism and nuclear pathway, which are potentially associated with the striatal pathology at the advanced stage.

## Discussion

Here we demonstrated that *Pitx3*$^{cKO}$ mice showing PD-related features, such as reduced DA and DAergic neuronal degeneration. Meanwhile, besides DA, the homeostasis of GABA and glutamate was impaired at the early stage in the model, potentially contributing to the striatal pathologies at the late stage. Furthermore, we novelly characterized nuclear atrophy and nuclear invagination increase in MSNs during aging, and these aberrant nuclear phenotypes may be associated with epigenetic alterations at the advanced stage.

In PD, the deficit of midbrain DAergic neurons produces the reduction of DA in the basal ganglia (Weintraub et al., 2022). Our *Pitx3*$^{cKO}$ mice showed a significant reduction of DA at 6 months, and the deficit aggravated at the late stage. Thus, the model provided great potential for studying the age-dependent striatal pathologies under progressive DA depletion. First, we examined the levels of two main neurotransmitters, GABA and glutamate, since they may be involved in remodeling the plasticity of MSNs, synergistically with DA. Interestingly, GABA and glutamate were both decreased at the early stage of our model, compared to controls. Previously the remarkable alterations of GABAergic neurotransmission within the basal ganglia circuit were reported in PD (Jamwal and Kumar, 2019). Moreover, in MPTP mice, the decreased levels of GABA in the striatum have been characterized, where about 75–80% of SNc were lost (Singh et al., 2017). However, in our studies, the significantly altered GABA levels were only characterized in the young *Pitx3*$^{cKO}$ mice, i.e., there were no changes in GABA levels between the two genotypes at the advanced stage, indicating that an adaptive system may respond to restoring the GABA levels in our model during aging. On the other hand, like GABA, the perturbation of glutamate homeostasis also altered in our model in an age-dependent way, i.e., the notably changed glutamate levels in *Pitx3*$^{cKO}$ mice were only identified in the early stage, but not in the late stage. However, our glutamate results were inconsistent with the previous studies that the enhancement of glutamate content was associated with the robust MSNs hyperactivity in the PD-related animal models (Calabresi et al., 1993; Singh et al., 2015; Tozzi et al., 2021). Noticeably, these models were majorly administrated with 6-OHDA, MPTP, or α-syn-PFF, rather than genetic models. One of the outstanding features of these models is the occurrence of the severe loss of DAergic neurons, usually reaching 70–80%, whereas, in genetic models, the death rate of DAergic neurons typically reached 40–50% (Konnova and Swanberg, 2018). Thus, the differences in DAergic neuron loss among the animal models may affect the glutamate release in the striatum. Additionally, when determining neurotransmitter levels, we used whole striatum tissue extracts for HPLC analysis, which may compromise the subtle changes occurring at the extracellular/synaptic levels. In the future, the use of microdialysis will be another better choice. Taken together, the neurotransmitter levels were altered at 6 months, while the MSNs of *Pitx3*$^{cKO}$ mice remained integrity at that time. During aging, the atrophy of dendritic complexity, soma and nuclei was identified in the MSNs of 12-month-old *Pitx3*$^{cKO}$ mice, concomitated with the significant loss of SNc neurons and movement abnormalities. We thereby suggested that the early perturbation of neurotransmitters may progressively trigger the striatal pathologies.

MSNs in our model have shorter dendritic lengths and lower maximal branch order of the dendrites at 12 months. This phenotype is similar to what is observed in patients and the animal models of PD (McNeill et al., 1988; Toy et al., 2014). Unlike *ak* and traditional *Pitx3* knockout mice, MSNs develop and mature with abundant DA afferents in our genetic model. Thus, the observed defects of tree complexity exclude the cause of developmental defects. Besides dendritic

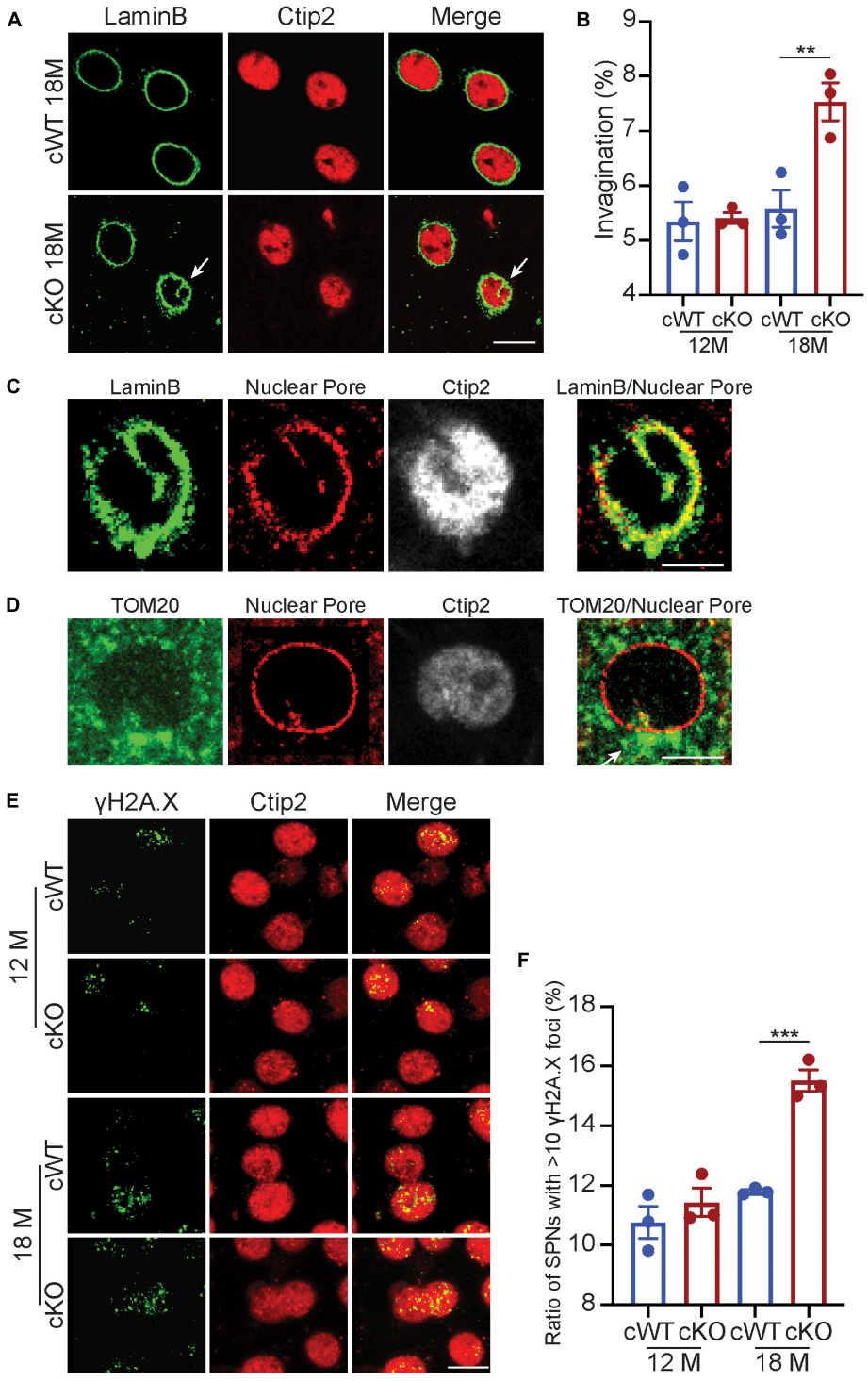


FIGURE 4
Nuclear invaginations increase accompanied with genomic instability in *Pitx3*$^{cKO}$ mice. **(A)** Co-staining of LaminB and Ctip2 in MSNs of 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice (scale bar: 10 μm) white arrow points to nuclear invagination. **(B)** Ratio of MSN nuclei containing ≥ 1 invagination in *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 12 ($N$ = 3 mice per genotype; all males) and 18 ($N$ = 3 mice per genotype; all males) month of age. Unpaired $t$-test, $^{**}p$ = 0.0041. **(C)** Co-staining of Nuclear Pore and LaminB (scale bar: 5 μm). **(D)** Costaining of Nuclear Pore and TOM20 (scale bar: 5 μm). White arrow points to a cluster of mitochondria. **(E)** Co- staining of γH2A.X and Ctip2 in the striatal sections of 12- and 18-month-old *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice (scale bar: 10 μm). **(F)** The ratios of MSNs with 10 or more γH2A.X-positive foci in the nuclei ($N$ = 3 mice per genotype). Unpaired $t$-test, $^{***}p$ = 0.0005.

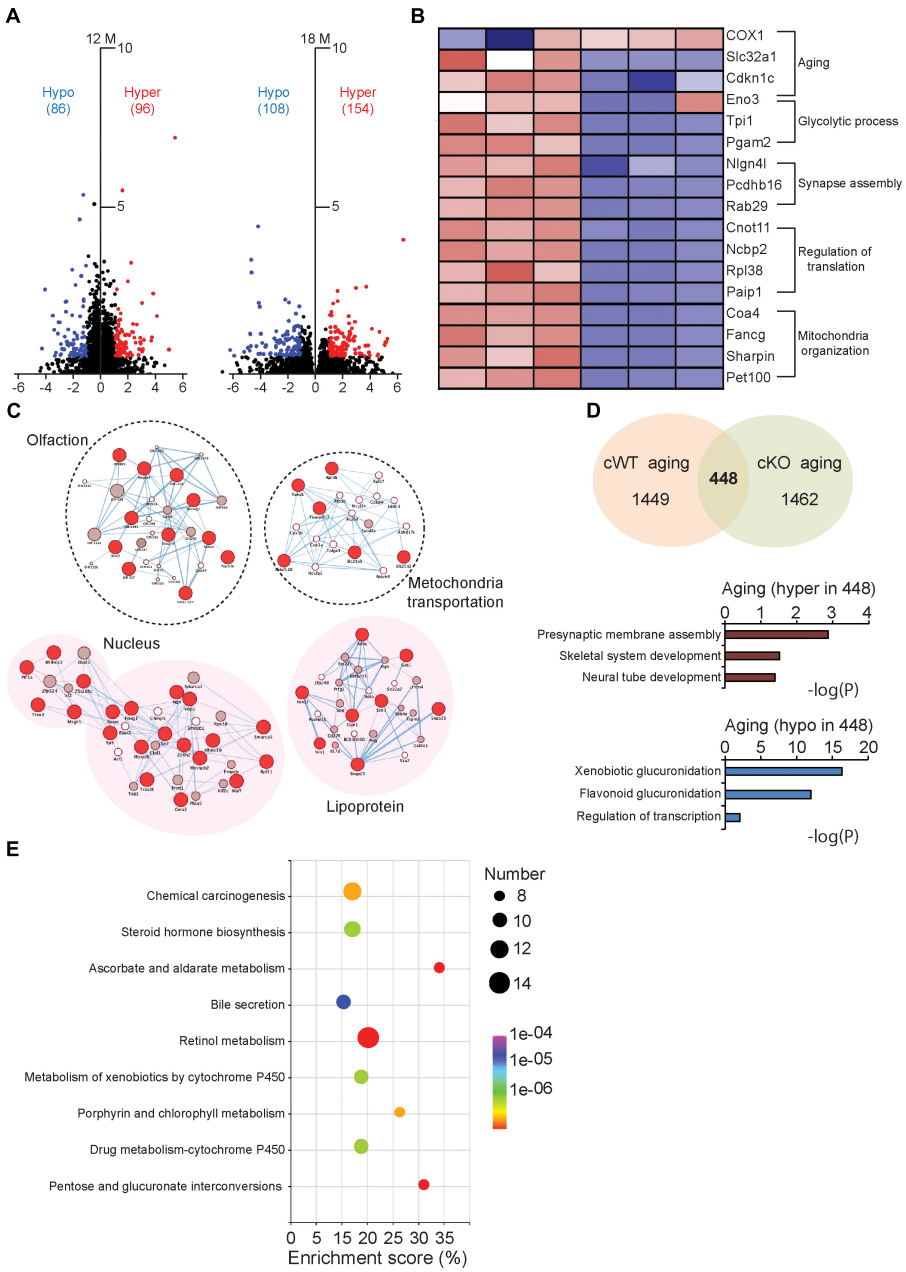


FIGURE 5
Comparative analysis of the DMGs in *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ during aging. **(A)** The volcano plots of DNA methylation data collected from the striatum of *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 12 (*N* = 3 mice per genotype; all males) and 18 months of age (*N* = 3 mice per genotype; all males). **(B)** Supervised clustering for the DMGs data collected from the 12- and 18-month-old *Pitx3*$^{cWT}$ mice. **(C)** Integrated map of GO terms enriched among the DMGs of *Pitx3*$^{cWT}$ and *Pitx3*$^{cKO}$ mice at 12 (background with white color) and 18 months of age (background with pink color). Red circles represented DMGs identified from our data. **(D,E)** 448 DMGs were identified during aging independent of genotype. They were analyzed with GO terms **(D)** and metabolic pathway **(E)**, respectively.

shortage, we identified nuclear morphological alterations in MSNs. Irregular shapes of nuclei have been reported in the neurons of PD patients with LRRK2-related G2019S (Liu et al., 2012; Shani et al., 2019), transgenic mice carrying R1441C mutations (Tsika et al., 2014) and LRRK2 knockout mice (Chen et al., 2020). As an extension of these findings, our present studies demonstrated that MSNs showed reduced nuclear size and increased nuclear invagination during aging. The decreased size of the nucleus likely reflects lower biosynthetic activities of DNA repair/synthesis, transcription, and translation in cells

(Koda et al., 2006), which potentially contributed to the striatal neuronal dysfunction at the advanced stage. On the other hand, in our mouse model, increased nuclear invagination was characterized at the advanced stage, reflecting that the higher neuronal excitability might occur over there (Chen et al., 2020). Comparably it was reported that the depolarizing current significantly evoked more action potentials in MSNs of *Pitx3* knockout mice (Suarez et al., 2018). Thus, our results re-emphasize that increased neural activity facilitates nuclear invagination formation. Together, not only the dendritic complexity but also the nuclear morphology alters in MSNs upon PD-like stress during aging. Especially, the aberrant nuclear phenotype may bring out severe effects on genomics, and further impact the downstream cellular progresses.

Age-related remodeling of DNA methylation comprises events of both hypo- and hypermethylation (Miranda-Morales et al., 2017; Yang et al., 2022). These epigenetic alterations mediated heritable changes in the gene activity and contributed to genomic instability. Our data showed that increased γH2AX was observed in the MSNs of 18-month-old $Pitx3^{cKO}$ mice, indicating that DNA damage might be persistent and accumulative during the aging process. To further elucidate the whole-genome epigenetic changes during aging, we performed DNA methylation studies between $Pitx3^{cKO}$ and $Pitx3^{cWT}$ mice at 12 and 18 months. Our data showed that the total DNA methylation ratios were greatly decreased in 18-month-old mice compared to 12-month-old ones, consistent with previous studies that DNA hypomethylation occurs globally over time (Miranda-Morales et al., 2017). For methylation onto individual genes, the previous studies reported that the MAPT gene was hypomethylated in the putamen of PD patients' post-mortem brains (Coupland et al., 2014). Whereas in our longitudinal epigenetic data, many DMGs involved in the nucleus pathway were characterized in $Pitx3^{cKO}$ mice at the advanced stage, potentially associating with their nuclear morphological alterations. However, what factor contributed to the changes in DNA methylation profile? Recent studies indicated that DA could modify histone H3 glutamine 5 (H3Q5dop) to regulate cocaine-induced transcriptional plasticity in the midbrain (Lepack et al., 2020). The non-neurotransmission roles of neurotransmitters in the epigenetic process have attracted more attention recently. In our model, the progressive reduction of DA and temporally altered neurotransmitters have been identified, thus the perturbation of neurochemicals might affect the epigenetic changes and further remodel the neuronal plasticity. More detailed biochemical and molecular mechanisms of how neurotransmitters regulate neuronal plasticity are also needed in the future.

Previously, we reported another Pitx3 conditional knockout model, $Pitx3^{fl/fl/}DAT^{CreERT2}$ mice (Wang et al., 2021). Similar to the *DAT* model, our model has progressive DAergic neuron degeneration and DA reduction during aging, greatly different from *ak* and $Pitx3^{-/-}$ mice that showed about 60% SNc neuron loss and 80% striatal DA reduction as early as the age of 2 months. One of the reasons why our model facilitates the development of DAergic neurons may be that Pitx3 in SNc is expressed earlier than TH during development (Maxwell et al., 2005). Thus, prior to Cre recombination, some Pitx3 proteins are already present and exert the biological function, i.e., a serials of downstream development events have been triggered. Furthermore, Pitx3 as a transcription factor is considered to modulate TH expressions (Cazorla et al., 2000). Therefore, decreased Pitx3 expressions may reduce the efficiency of the TH-driven Cre-loxp system. Taken together, these contributing factors may aid in the development of DAergic neurons in our model. However, whether the development of DAergic neurons is intrinsically affected in our model needs to be further investigated by examining the expression levels of development-related molecules, such as DAT, Vmat2, Aadc, and so on.

## Conclusion

The striatum is the key player in facilitating voluntary movement. In PD, the striatal neurons undergo the progressive depletion of DA, resulting in impaired physiological function and contributing to the motor symptom of PD. To further explore the striatal pathologies during aging, we generated the $Pitx3^{cKO}$ mice, where a progressive reduction of striatal DA was identified. In this model, the levels of GABA and glutamate decreased at the early stage besides DA. Such early disturbance of neurochemical homeostasis may contribute to the longitudinal plasticity remodeling of neurons, including morphology and movement abnormalities as well as aberrant epigenetic modifications. Our studies may expand the overview of PD treatments and provide a new potential window for therapeutic strategies.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://submit.ncbi.nlm.nih.gov/subs/bioproject/SUB11723472/overview.

## Ethics statement

This animal study was reviewed and approved by the Institutional Animal Care Committee at Dalian Medical University.

## Author contributions

XC and WL designed the experiments, wrote, and edited the manuscript. ZY contributed to stereotactic injection. XC and YS contributed to metabolic analyses. XC and YiW contributed to imaging experiments and data analysis. KK, YuW, and HW contributed to behavior test and data analysis. XC and XX contributed to sample preparation and data analysis for DNA methylation. All authors read and approved the final manuscript.

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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.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.960479/full#supplementary-material

**SUPPLEMENTARY FIGURE 1**
Conditional knockout of *Pitx3* in DA neurons. **(A)** The basic strategy for the generation of $TH^{Cre}/Pitx3^{fl/fl}$ knockout mice. **(B)** PCR detection of *Cre* transgene (upper) and *Pitx3* floxed allele (lower). **(C)** IFC staining for Pitx3 expression in DA neurons was performed using an antibody against Pitx3 (green) together with TH (red) in 2-month-old $Pitx3^{cWT}$ and $Pitx3^{cKO}$ mice (scale bar: 100 μm; high-magnification, 5 μm).

**SUPPLEMENTARY FIGURE 2**
Specific labeling of DAergic neurons using RiboTag mice (Scale bar: 100 μm).

**SUPPLEMENTARY FIGURE 3**
Analyses of dendritic complexity in 6-month-old $Pitx3^{cKO}$ mice. **(A)** The GFP-labeled individual MSN (scale bar: 10 μm). **(B,C)** Sholl analysis of dendritic complexity of GFP-labeled MSNs ($N$ = 3 mice per genotype; 5–8 neurons per mouse were counted; all males). **(D)** Analyses of primary dendrites ($N$ = 3 mice per genotype; 5–8 neurons per mouse were counted; all males). **(E)** Dendritic length of GFP-labeled MSNs ($N$ = 3 mice per genotype; 5–8 neurons per mouse were counted; all males).

**SUPPLEMENTARY FIGURE 4**
$TH^+$ neurons in 12 and 18-month-old $Pitx3^{cWT}$ mice (scale bar: 200 μm).

**SUPPLEMENTARY FIGURE 5**
Open field test for $Pitx3^{cWT}$ and $Pitx3^{cKO}$ mice at 6 ($N$ = 11–13 mice per genotype; all males), 12 ($N$ = 12–14 mice per genotype; all males), and 18 months of age ($N$ = 9–10 mice per genotype; all males).

**SUPPLEMENTARY FIGURE 6**
Overview of the DNA methylation ratio and distribution of CG sites in $Pitx3^{cWT}$ and $Pitx3^{cKO}$ mice. **(A)** The genomic DNA methylation ratio at CG and CWG sites in 12- and 18-month-old $Pitx3^{cWT}$ and $Pitx3^{cKO}$. **(B)** The distribution of CG sites on different functional genic components. Upstream, downstream, exon, and intron indicate the regions 2,000 bp upstream of the transcription start site, the regions 2,000 bp downstream of the transcription terminal site, the whole exons of genes, and the whole introns of genes, respectively. 3′UTR and 5′UTR indicate the regions at the 3′ end and 5′ end of a mature transcript that are not translated into a protein. Intergenic indicates the intergenic regions.

**SUPPLEMENTARY TABLE 1**
DNA methylation ratios in 12- and 18-month-old $Pitx3^{cWT}$ and $Pitx3^{cKO}$ mice.

## References

Althini, S., Bengtsson, H., Usoskin, D., Söderström, S., Kylberg, A., Lindqvist, E., et al. (2003). Normal nigrostriatal innervation but dopamine dysfunction in mice carrying hypomorphic tyrosine hydroxylase alleles. *J. Neurosci. Res.* 15, 444–453. doi: 10.1002/jnr.10606

Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol.* 11:R106. doi: 10.1186/gb-2010-11-10-r106

Bariselli, S., Fobbs, W. C., Creed, M. C., and Kravitz, A. V. (2019). A competitive model for striatal action selection. *Brain Res.* 1713, 70–79. doi: 10.1016/j.brainres.2018.10.009

Bolam, J. P., Hanley, J. J., Booth, P. A., and Bevan, M. D. (2000). Synaptic organisation of the basal ganglia. *J. Anat.* 196, 527–542. doi: 10.1046/j.1469-7580.2000.19640527.x

Calabresi, P., Mercuri, N. B., Sancesario, G., Bernardi, G. (1993). Electrophysiology of dopaminedenervated striatal neurons. Implications for Parkinson's disease. *Brain* 116, 433–52.

Cazorla, P., Smidt, M. P., O'Malley, K. L., and Burbach, J. P. (2000). A response element for the homeodomain transcription factor Ptx3 in the tyrosine hydroxylase gene promoter. *J. Neurochem.* 74, 1829–1837. doi: 10.1046/j.1471-4159.2000.0741829.x

Chen, X., Xie, C., Tian, W., Sun, L., Zheng, W., Hawes, S., et al. (2020). Parkinson's disease-related Leucine-rich repeat kinase 2 modulates nuclear morphology and genomic stability in striatal projection neurons during aging. *Mol. Neurodegener.* 15:12. doi: 10.1186/s13024-020-00360-0

Cingolani, P., Platts, A., Wang le, L., Coon, M., Nguyen, T., Wang, L., et al. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly* 6, 80–92. doi: 10.4161/fly.19695

Coupland, K. G., Mellick, G. D., Silburn, P. A., Mather, K., Armstrong, N. J., Sachdev, P. S., et al. (2014). DNA methylation of the MAPT gene in Parkinson's disease cohorts and modulation by vitamin E *in vitro*. *Mov. Disord.* 29, 1606–1614. doi: 10.1002/mds.25784

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., et al. (2006). Selective elimination of glutamatergic synapses on stratopallidal neurons in Parkinson disease models. *Nat. Neurosci.* 9, 251–259. doi: 10.1038/nn1632

Dong, J., Liu, X., Wang, Y., Cai, H., and Le, W. (2020). Nurr1(Cd11bcre) conditional knockout mice display inflammatory injury to nigrostriatal dopaminergic neurons. *Glia* 68, 2057–2069. doi: 10.1002/glia.23826

Drozdz, M. M., and Vaux, D. J. (2017). Shared mechanisms in physiological and pathological nucleoplasmic reticulum formation. *Nucleus* 8, 34–45. doi: 10.1080/19491034.2016.1252893

Du, Y., and Graves, S. M. (2019). Spiny Projection Neuron Dynamics in Toxin and Transgenic Models of Parkinson's Disease. *Front. Neural Circuits* 13:17. doi: 10.3389/fncir.2019.00017

Feng, L., Zhao, T., and Kim, J. (2015). neuTube 1.0: A New Design for Efficient Neuron Reconstruction Software Based on the SWC Format. *eNeuro* 2:ENEURO.0049–14.2014. doi: 10.1523/ENEURO.0049-14.2014

Filali, M., and Lalonde, R. (2016). Neurobehavioral Anomalies in the Pitx3/ak Murine Model of Parkinson's Disease and MPTP. *Behav. Genet.* 46, 228–241. doi: 10.1007/s10519-015-9753-3

Filipovic, M., Ketzef, M., Reig, R., Aertsen, A., Silberberg, G., and Kumar, A. (2019). Direct pathway neurons in mouse dorsolateral striatum *in vivo* receive stronger synaptic input than indirect pathway neurons. *J. Neurophysiol.* 122, 2294–2303. doi: 10.1152/jn.00481.2019

Fu, Y., Yuan, Y., Halliday, G., Rusznak, Z., Watson, C., and Paxinos, G. (2012). A cytoarchitectonic and chemoarchitectonic analysis of the dopamine cell groups in the substantia nigra, ventral tegmental area, and retrorubral field in the mouse. *Brain Struct. Funct.* 217, 591–612. doi: 10.1007/s00429-011-0349-2

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. Jr., et al. (1990). D1 and D2 dopamine receptor-regulated gene expression of stratonigral and striatopallidal neurons. *Science* 250, 1429–1432. doi: 10.1126/science.2147780

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., et al. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925. doi: 10.1038/nature02033

He, J., Kleyman, M., Chen, J., Alikaya, A., Rothenhoefer, K. M., Ozturk, B. E., et al. (2021). Transcriptional and anatomical diversity of medium spiny neurons in the primate striatum. *Curr. Biol.* 31, 5473–5486.e6. doi: 10.1016/j.cub.2021.10.015

Hwang, D. Y., Ardayfio, P., Kang, U. J., Semina, E. V., and Kim, K. S. (2003). Selective loss of dopaminergic neurons in the substantia nigra of Pitx3-deficient aphakia mice. *Brain Res. Mol. Brain Res.* 114, 123–131. doi: 10.1016/S0169-328X(03)00162-1

Jamwal, S., and Kumar, P. (2019). Insight Into the Emerging Role of Striatal Neurotransmitters in the Pathophysiology of Parkinson's Disease and Huntington's Disease: A Review. *Curr. Neuropharmacol.* 17, 165–175. doi: 10.2174/1570159X16666180302115032

Koda, M., Takemura, G., Okada, H., Kanoh, M., Maruyama, R., Esaki, M., et al. (2006). Nuclear hypertrophy reflects increased biosynthetic activities in myocytes of human hypertrophic hearts. *Circ. J.* 70, 710–718. doi: 10.1253/circj.70.710

Konnova, E., A., and Swanberg, M. (2018). "Chapter 5: Animal models of Parkinson's disease," in *Parkinson's disease: Pathogenesis and clinical aspects [Internet]*, ed. T. B. Stoker and J. C. Greenland (Brisbane, AU: Codon Publications).

Lepack, A. E., Werner, C. T., Stewart, A. F., Fulton, S. L., Zhong, P., Farrelly, L. A., et al. (2020). Dopaminylation of histone H3 in ventral tegmental area regulates cocaine seeking. *Science* 368, 197–201. doi: 10.1126/science.aaw8806

Liu, G. H., Qu, J., Suzuki, K., Nivet, E., Li, M., Montserrat, N., et al. (2012). Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. *Nature* 491, 603–607. doi: 10.1038/nature11557

Longair, M. H., Baker, D. A., and Armstrong, J. D. (2011). Simple Neurite Tracer: Open source software for reconstruction, visualization and analysis of neuronal processes. *Bioinformatics* 27, 2453–2454. doi: 10.1093/bioinformatics/btr390

Lu, X. H., and Yang, X. W. (2017). Genetically-directed Sparse Neuronal Labeling in BAC Transgenic Mice through Mononucleotide Repeat Frameshift. *Sci. Rep.* 7:43915. doi: 10.1038/srep43915

Maxwell, S. L., Ho, H. Y., Kuehner, E., Zhao, S., and Li, M. (2005). Pitx3 regulates tyrosine hydroxylase expression in the substantia nigra and identifies a subgroup of mesencephalic dopaminergic progenitor neurons during mouse development. *Dev. Biol.* 282, 467–479. doi: 10.1016/j.ydbio.2005.03.028

McNeill, T. H., Brown, S. A., Rafols, J. A., and Shoulson, I. (1988). Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. *Brain Res.* 455, 148–152. doi: 10.1016/0006-8993(88)90124-2

Miranda-Morales, E., Meier, K., Sandoval-Carrillo, A., Salas-Pacheco, J., Vazquez-Cardenas, P., and Arias-Carrion, O. (2017). Implications of DNA Methylation in Parkinson's Disease. *Front. Mol. Neurosci.* 10:225. doi: 10.3389/fnmol.2017.00225

Parisiadou, L., Yu, J., Sgobio, C., Xie, C., Liu, G., Sun, L., et al. (2014). LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. *Nat. Neurosci.* 17, 367–376. doi: 10.1038/nn.3636

Prager, E. M., and Plotkin, J. L. (2019). Compartmental function and modulation of the striatum. *J. Neurosci. Res.* 97, 1503–1514.

Quinlan, A. R., and Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842. doi: 10.1093/bioinformatics/btq033

Rodgers, R. J., and Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev.* 21, 801–810.

Shani, V., Safory, H., Szargel, R., Wang, N., Cohen, T., Elghani, F. A., et al. (2019). Physiological and pathological roles of LRRK2 in the nuclear envelope integrity. *Hum. Mol. Genet.* 28, 3982–3996. doi: 10.1093/hmg/ddz245

Shigeoka, T., Jung, H., Jung, J., Turner-Bridger, B., Ohk, J., Lin, J. Q., et al. (2016). Dynamic axonal translation in developing and mature visual circuits. *Cell* 166, 181–192.

Singh, A., Liang, L., Kaneoke, Y., Cao, X., Papa, S., M. (2015). Dopamine regulates distinctively the activity patterns of striatal ouput neurons in advanced parkinsonian primates. *J. Neurophysiol.* 113, 1533–1544.

Singh, S., Jamwal, S., and Kumar, P. (2017). Neuroprotective potential of Quercetin in combination with piperine against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *Neural Regen. Res.* 12, 1137–1144. doi: 10.4103/1673-5374.211194

Smidt, M. P., Smits, S. M., and Burbach, J. P. (2004). Homeobox gene Pitx3 and its role in the development of dopamine neurons of the substantia nigra. *Cell Tissue Res.* 318, 35–43.

Smidt, M. P., van Schaick, H. S., Lanctot, C., Tremblay, J. J., Cox, J. J., van der Kleij, A. A., et al. (1997). A homeodomain gene Ptx3 has highly restricted brain expression in mesencephalic dopaminergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13305–13310. doi: 10.1073/pnas.94.24.13305

Stephens, B., Mueller, A. J., Shering, A. F., Hood, S. H., Taggart, P., Arbuthnott, G. W., et al. (2005). Evidence of a breakdown of corticostriatal connections in Parkinson's disease. *Neuroscience* 132, 741–754.

Suarez, L. M., Alberquilla, S., Garcia-Montes, J. R., and Moratalla, R. (2018). Differential Synaptic Remodeling by Dopamine in Direct and Indirect Striatal Projection Neurons in Pitx3(–/–) Mice, a Genetic Model of Parkinson's Disease. *J. Neurosci.* 38, 3619–3630. doi: 10.1523/JNEUROSCI.3184-17.2018

Suarez, L. M., Solis, O., Carames, J. M., Taravini, I. R., Solis, J. M., Murer, M. G., et al. (2014). L-DOPA treatment selectively restores spine density in dopamine receptor D2-expressing projection neurons in dyskinetic mice. *Biol. Psychiatry* 75, 711–722. doi: 10.1016/j.biopsych.2013.05.006

Toy, W. A., Petzinger, G. M., Leyshon, B. J., Akopian, G. K., Walsh, J. P., Hoffman, M. V., et al. (2014). Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiol. Dis.* 63, 201–209. doi: 10.1016/j.nbd.2013.11.017

Tozzi, A., Sciaccaluga, M., Loffredo, V., Megaro, A., Ledonne, A., Cardinale, A.,et al. (2021). Dopaminedependent early synaptic and motor dysfunctions induced by α-synuclein in the nigrostriatal circuit. *Brain* 144, 3477–3791.

Tsika, E., Kannan, M., Foo, C. S., Dikeman, D., Glauser, L., Gellhaar, S., et al. (2014). Conditional expression of Parkinson's disease-related R1441C LRRK2 in midbrain dopaminergic neurons of mice causes nuclear abnormalities without neurodegeneration. *Neurobiol. Dis.* 71, 345–358. doi: 10.1016/j.nbd.2014.08.027

Valdiglesias, V., Giunta, S., Fenech, M., Neri, M., and Bonassi, S. (2013). gammaH2AX as a marker of DNA double strand breaks and genomic instability in human population studies. *Mutat. Res.* 753, 24–40.

Valentin, V. V., Maddox, W. T., and Ashby, F. G. (2016). Dopamine dependence in aggregate feedback learning: A computational cognitive neuroscience approach. *Brain Cogn.* 109, 1–18. doi: 10.1016/j.bandc.2016.06.002

Villalba, R. M., Lee, H., and Smith, Y. (2009). Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys. *Exp. Neurol.* 215, 220–227.

Wang, S., Lv, J., Zhang, L., Dou, J., Sun, Y., Li, X., et al. (2015). MethylRAD: A simple and scalable method for genome-wide DNA methylation profiling using methylation-dependent restriction enzymes. *Open Biol.* 5:150130. doi: 10.1098/rsob.150130

Wang, Y., Chen, X., Wang, Y., Li, S., Cai, H., and Le, W. (2021). The essential role of transcription factor Pitx3 in preventing mesodiencephalic dopaminergic neurodegeneration and maintaining neuronal subtype identities during aging. *Cell Death Dis.* 12:1008. doi: 10.1038/s41419-021-04319-x

Weintraub, D., Aarsland, D., Chaudhuri, K. R., Dobkin, R. D., Leentjens, A. F., Rodriguez-Violante, M., et al. (2022). The neuropsychiatry of Parkinson's disease: Advances and challenges. *Lancet Neurol.* 21, 89–102.

Wu, J., Kung, J., Dong, J., Chang, L., Xie, C., Habib, A., et al. (2019). Distinct Connectivity and Functionality of Aldehyde Dehydrogenase 1a1-Positive Nigrostriatal Dopaminergic Neurons in Motor Learning. *Cell Rep.* 28, 1167–1181.e7. doi: 10.1016/j.celrep.2019.06.095

Yang, J., Qu, J., and Ma, H. (2022). Recent developments in understanding brain aging: Sex differences, mechanisms, and implications in diseases. *Aging Neur. Dis.* 2:3. doi: 10.1016/B978-0-444-53630-3.00006-3

Yin, H. H., Mulcare, S. P., Hilario, M. R., Clouse, E., Holloway, T., Davis, M. I., et al. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat. Neurosci.* 12, 333–341. doi: 10.1038/nn.2261

Zaja-Milatovic, S., Milatovic, D., Schantz, A. M., Zhang, J., Montine, K. S., Samii, A., et al. (2005). Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. *Neurology* 64, 545–547.

Zhang, Y., Meredith, G. E., Mendoza-Elias, N., Rademacher, D. J., Tseng, K. Y., and Steece-Collier, K. (2013). Aberrant restoration of spines and their synapses in L-DOPA-induced dyskinesia: Involvement of corticostriatal but not thalamostriatal synapses. *J. Neurosci.* 33, 11655–11667. doi: 10.1523/JNEUROSCI.0288-13.2013

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# Amyotrophic lateral sclerosis in seven provinces of Chinese mainland: A cross-sectional survey from 2015 to 2016

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**Background:** The large-scale survey about amyotrophic lateral sclerosis (ALS) based on both population and hospitals in the Chinese mainland has been deficient at present. To this end, we conducted a cross-sectional survey about ALS based on the population and hospitals in seven provinces of the Chinese mainland in 2015–2016.

**Methods:** We surveyed patients with ALS in seven provinces in eastern, middle, and western China. Among them, 13 prefecture-level cities, 13 municipal districts, 13 counties, 26 streets, 52 communities, 39 towns, and 78 administrative villages were selected for the population-based survey. Totally, 13 class-3 general hospitals, 13 class-2 general hospitals, and 26 street health centers or community health service centers in urban districts, and 13 county-level general hospitals, 39 township health centers, and 78 village clinics in rural districts were recruited for the hospital-based survey.

**Results:** Among the Chinese mainland, the total prevalence of ALS was slightly lower than that of the world's other nations or districts. The male patients were more than female patients. The prevalence of rural residents was more than that of urban residents. The prevalence of farmers was higher than that of other professions. The majority of ALS was not accompanied by other chronic diseases. The peak onset age of ALS was higher, familial ALS (fALS) cases were slightly more, and the average survival duration of sporadic ALS (sALS) was slightly lower compared with the previous investigation data. The hospitalization expenses of almost 60% of ALS were not more than 10,000 Chinese Yuan.

**Conclusion:** Hospitalization expenses in our survey objectives were the lowest in the current reported countries and districts. A farmer was a possible higher risk profession for ALS, the majority of ALS were not accompanied by other chronic diseases. Our survey provided more information on the epidemiology of ALS worldwide and supplied the deficiency of epidemiology survey about ALS from the Chinese mainland.

KEYWORDS

**epidemiology, amyotrophic lateral sclerosis, Chinese mainland, population, hospital**

## Highlights

- ALS epidemiological characteristics are in seven provinces of the Chinese mainland in 2015–2016.
- This study is the currently largest and most comprehensive survey about ALS on Chinese mainland.
- Farmer is a possible higher risk profession of ALS in the Chinese mainland.
- This survey is an important addition to both population-based and hospital epidemiology.

## Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease in America, is a disaster disease, which is characterized by the selective and progressive death of the motor neurons in the cerebrum, brain stem, and spinal cord (Zarei et al., 2015). In some countries such as the United Kingdom, India, and Australia, the term motor neuron disease (MND) is commonly used to replace ALS (Zarei et al., 2015), while in other countries including America and China often use the MND term to call for a disease group of four subtypes including ALS, medullae (bulbar) palsy, progressive spinal muscular atrophy, and primary lateral sclerosis, and among them, ALS is the most common subtype (Ludolph et al., 2012). The early symptoms of ALS are mild and easy to be confused with other diseases. Patients may just feel weakness, muscle subsultus (beat or jump), fatigue at the initial stage, and gradually develop the atrophy of limbs, laryngeal, and pharynx muscles, and subsequently expand to the atrophy of whole body muscle, which results in the motor dysfunction of limbs, the difficulty of speech and swallow, and eventually generate the respiratory failure. Most patients with ALS die from respiratory failure (Zarei et al., 2015). The etiology and pathogenesis of ALS are not known in 90% to 95% of the patients with ALS. Only about 5% to 10% of ALS cases are inherited (Zarei et al., 2015).

The current diagnosis of ALS is mainly based on the patient's signs and symptoms, and supplemented with some related auxiliary examinations to rule out other mimic diseases (Silani et al., 2011; Statland et al., 2015). No useful cure for ALS is found at present. Based on the information from current epidemiological investigations, ALS affects slightly more men than women, the age distribution of ALS peaks at 55 to 60 years for both men and women worldwide, and the onset age usually is around 60 years (Kiernan et al., 2011). The average survival periods from onset to death usually are 3–5 years. About 10% of patients with ALS survive longer than 10 years (Salameh et al., 2015).

In many countries and districts of the world, the epidemiological data of ALS has been unknown yet (Kiernan et al., 2011). In Europe, the prevalence of ALS is about 2.2 people per 100,000 per year for all ages (Kiernan et al., 2011). In the United States, the prevalence of ALS is ∼1.5 people per 100,000 per year for all ages (Mehta et al., 2016). The epidemiological survey of ALS in the Chinese mainland has been very deficient up to now. To obtain more estimation on the world epidemiology features of ALS, the epidemiological survey of ALS worldwide is needed to be improved further. To this end, we conducted a cross-sectional survey based on both population and hospitals in seven provinces of the Chinese mainland from 2015 to 2016, including prevalence, sexuality, onset age, geographical location, profession, chronic disease history, familial history, survival time, and both diagnosis and cure condition (hospitalization expenses). The detailed and large epidemiologic characteristics survey of ALS has been very deficient in the Chinese mainland. This epidemiologic investigation is the largest and most comprehensive survey about ALS on the Chinese mainland. Our study is an important addition to the population and hospital-based epidemiological survey of ALS in the Chinese mainland. Our survey supplied clinical data about the prevalence, the male and female ratio, the onset age, the rural and urban residents' onset, profession, the ratio of familial ALS, the major accompanying chronic diseases, the average survival duration, and the hospitalization expense of ALS in the population of the Chinese mainland, and more detailed epidemiologic information of ALS, and provided the epidemiologic evidence for the clinical prevention and treatment of ALS. Our data supplied the deficiency of ALS epidemiology in the Chinese mainland and provided more information on the world epidemiology of ALS.

## Methods

### Study design and procedures

In 2015–2016, we undertook a nationwide cross-sectional survey based on population and hospitals jointing Jiangxi Province Center for Disease Control and Prevention to more comprehensively understand the epidemiological characteristics of ALS in the Chinese mainland (Figure 1). This survey was approved by the ethics committee of the First Affiliated Hospital of Nanchang University (approval number: 2014-06-01). We obtained multilevel approvals from the local Center for Disease Control and Prevention, selected hospitals, and selected communities and villages. All participants were to participate voluntarily and gave oral consent.

### Screening of investigation regions

Based on the classification of the eastern, middle, and western regions of our country, we used a stratified multistage random cluster sampling design to obtain representative samples of people in general populations

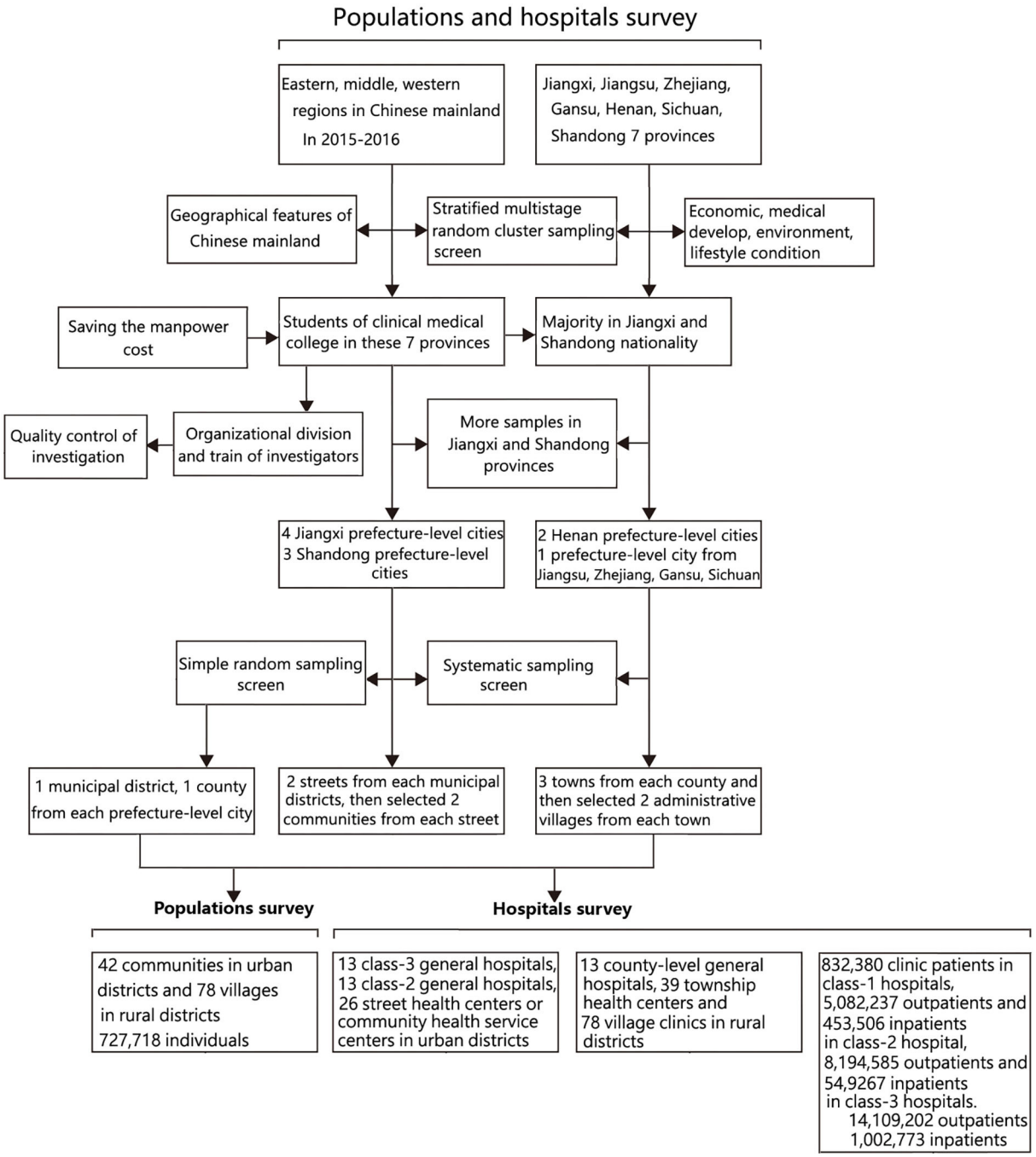


FIGURE 1
Selection of participants for population and hospital survey.

and hospitals at the different levels of districts. We selected seven provinces including Jiangxi, Jiangsu, Zhejiang, Gansu, Henan, Sichuan, and Shandong province. These districts differed in the economic, medical developmental, environmental, and lifestyle conditions, which possessed the representation statistically.

To save manpower cost, these students whose household registers were in these seven provinces and who studied in the Clinical Medical College of Nanchang University were recruited to perform this survey. Because the students of Jiangxi and Shandong nationality occupied the majority of them in the University of Nanchang at that time, we selected more

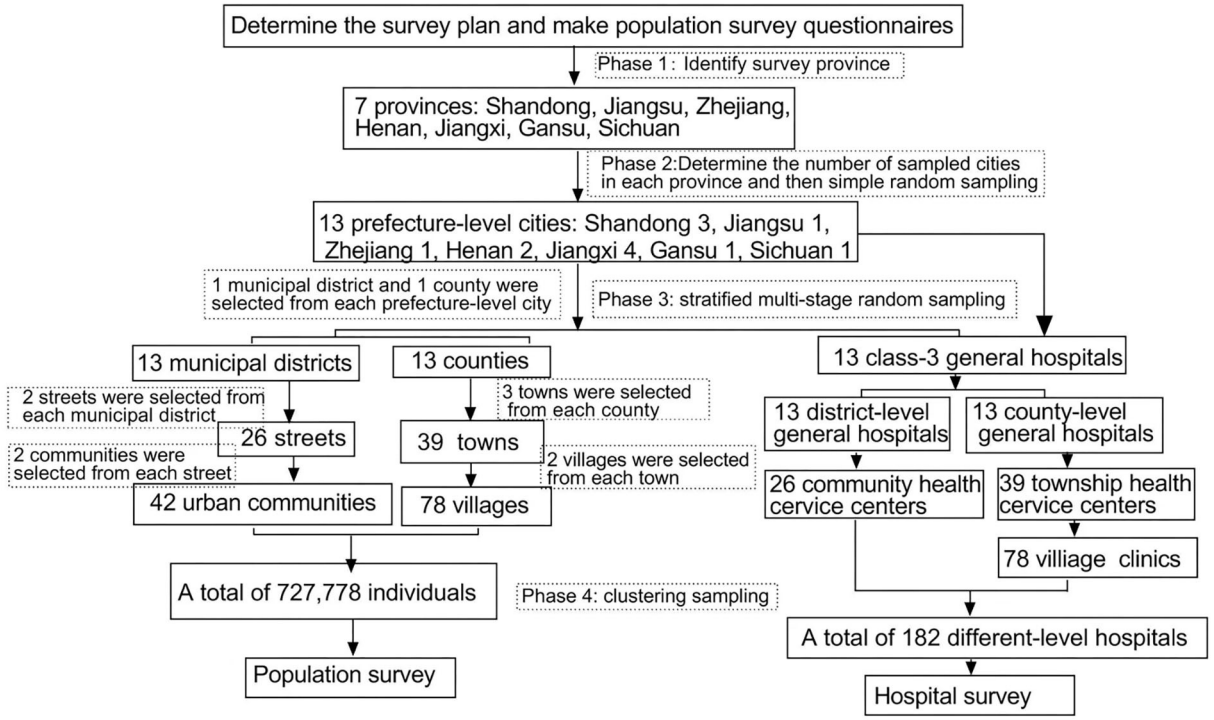


FIGURE 2
Flow chart of epidemiological survey.

samples from these two provinces than from other provinces. By probability proportionate to size sampling, we sampled four prefecture-level cities from Jiangxi province, three prefecture-level cities from Shandong province, two prefecture-level cities from Henan province, and one prefecture-level city for each province from the other four provinces (Jiangsu, Zhejiang, Gansu, and Sichuan). We surveyed 13 prefecture-level cities in total. By simple random sampling method, we selected one municipal district and one county from each prefecture-level city. We selected two streets from each municipal district and selected two communities from each street using the systematic sampling method. We selected three towns from each county and then selected two administrative villages from each town by the systematic sampling method. All regions selected based on the above-described statistically designed method were recruited as the investigation districts in this survey. The detail about the screening of investigation regions was in Figure 2.

## Organizational and division of investigators

Dr. Jie Zhang took the general directors of this survey, who were responsible for the establishment of schemes, personnel training, overall analysis of data, and report writing. About three to four neurologists of hospitals in prefecture-level cities of each province acted as the regional supervisors, and were responsible for organizing and implementing the schemes of prefecture-level cities, on-site supervision and the quality control, analysis of data, and report writing of prefecture-level cities. We trained 23 medical students including undergraduate and postgraduate students as investigators, whose household registers were in the selected prefecture-level cities. They were responsible for the implementation of an on-site investigation plan, the collection, arrangement, and analysis of original data, and report writing of municipal districts and counties.

## Population survey schemes

For each surveyed prefecture-level city, we extracted four communities and six villages. We finally selected 42 communities in urban districts and 78 villages in rural districts totally. We produced questionnaires, and took a household as a unit to conduct home visits, excluding those family members who had died or lived <3 months every year in the surveyed district (Figure 2).

## Hospital survey schemes

For the surveyed municipal districts (two streets) and sub-districts under its jurisdiction (four communities) of each prefecture-level city in this hospital survey, we, respectively, extracted a class-3 general hospital, a class-2 general hospital, and two street health centers or community health service centers. For the surveyed counties (three towns) and sub-districts under its jurisdiction (six administrative villages) of each prefecture-level city in this hospital survey, we, respectively, extracted a county-level general hospital, three township health centers, and six village clinics. We finally selected 13 class-3 general hospitals, 13 class-2 general hospitals, and 26 street health centers or community health service centers in urban districts, and 13 county-level general hospitals, 39 township health centers, and 78 village clinics in rural districts totally. All hospitals selected based on the above-described statistically designed method were recruited as investigation subjects in this hospital survey (Figure 2).

## Diagnosis of patients with ALS

The diagnosis of ALS followed the standardized diagnostic procedures of ALS (Brooks et al., 2000; Ludolph et al., 2015; Johnsen et al., 2019). In the population survey, investigators briefly asked questions about demographics and medical history at initial household assessments. If there were suspicious patients with ALS, they would be reported to the regional supervisors and taken to the local class-3 general hospital for a further diagnosis. To improve the accuracy of preliminary diagnoses, we repeated neurological examinations in all confirmed and suspected cases after 3 and 6 months using electromyography and the related lab examination by assessing disease progression, and re-examinations excluded those patients with ALS who refused another assessment, had died, or were lost to follow-up in the local class-3 general hospital. The neurologist director Prof. Renshi Xu made the final diagnosis based on all available information provided by investigators.

In the hospital survey, we searched patients with ALS in inpatients and outpatients through databases of hospitals. Once suspicious patients were found, they would be reported to regional supervisors, and if the patient had never been diagnosed in a tertiary hospital and could be contacted, he or she would be taken to the local class-3 general hospital for further diagnosis. To improve the accuracy of ALS diagnoses, we repeated neurological examinations in all definite and suspected cases following-up to 3 to 6 months using electromyography and related laboratory tests to further assess the ALS diagnosis in the local class-3 general hospital. Multiple investigations were conducted to exclude those patients who refused the further assessment, had died, or lost the follow-up. The neurologist director Prof. Renshi Xu made the final diagnosis based on all available information provided by investigators.

For all definite diagnostic ALS cases, we produced the investigation of questionnaires that involved general health situations, symptoms and signs, demographics, medical histories, profession, treatment processes, and costs.

## Quality control of investigation

Before the formal investigation, we selected Nanchang city to carry out a preliminary test to further improve the investigation plan and train all investigators (23 medical students) for 3 weeks in Nanchang city. Before finishing the household or hospital survey, investigators would conduct a comprehensive check of questionnaires. If there was any doubt, it was necessary to re-check and verify investigation contents, correct mistakes, and fill in missing items in time. The completion rate of the questionnaire investigation should be $\geq$99% and the error rate should be $<$1%. Regional supervisors should complete unqualified questionnaires and contact investigators to ascertain facts in time. During the investigation of fieldwork, staff members of the local Center for Disease Control and Prevention helped to communicate with communities or hospitals and supervisors monitored the on-site investigation interview of investigators.

## Statistical analysis

We used the SPSS statistical software of version 20.0 (SPSS, Chicago, IL, USA) for both data collection and management. All data of the cross-sectional survey based on population and hospitals including the epidemiological characteristics data of ALS were performed the statistical analysis by the SPSS software of version 20.0.

# Results

## Population survey

Our population survey covered 727,718 individuals, consisting of 160,983 (22.12%) Jiangxi province residents, 14,600 (2%) Jiangsu province residents, 10,600 (1.46%) Zhejiang province residents, 20,786 (2.86%) Gansu province residents, 52,081 (7.16%) Henan province residents, 18,950 (2.60%) Sichuan province residents, and 449,718 (61.80%) Shandong province residents (Table 1). The prevalence of ALS in seven provinces of the Chinese mainland was 1.24/100,000. Of these provinces, the prevalence of ALS in Zhejiang province was the highest (9.43/100,000) and the prevalence of ALS in Jiangsu province, Gansu province, Sichuan province, and Shandong province were 0/100,000 (Table 1).

TABLE 1 Prevalence of ALS in 7 provinces of China mainland in population survey.

| Districts | Number of ALS patients/ respondents | Number of respondents / total respondents (%) | Prevalence of ALS (1/100,000) |
|---|---|---|---|
| Jiangxi Province | 6/160,983 | 22.12 | 3.73 |
| Jiangsu Province | 0/14,600 | 2.00 | 0.00 |
| Zhejiang Province | 1/10,600 | 1.46 | 9.43 |
| Gansu Province | 0/20,786 | 2.86 | 0.00 |
| Henan Province | 2/52,081 | 7.16 | 3.84 |
| Sichuan Province | 0/18,950 | 2.60 | 0.00 |
| Shandong Province | 0/449,718 | 61.80 | 0.00 |
| Total | 9/727,718 | | 1.24 |

TABLE 2 Prevalence of ALS in urban and rural districts of China mainland in population survey.

| Districts | Number of ALS patients/ respondents | Respondents/ total respondents (%) | Prevalence of ALS (1/100,000) |
|---|---|---|---|
| Urban districts | 4/478,402 | 65.74 | 0.84 |
| Rural districts | 5/249,316 | 34.26 | 2.01 |
| Total | 9/727,718 | | 1.24 |

Our population survey consisted of 478,402 (65.74%) urban residents and 249,316 (34.26%) rural residents. The prevalence of ALS in rural residents (2.01/100,000) was higher than that in urban residents (0.84/100,000) (Table 2). Of the nine patients with ALS with a final diagnosis, seven (77.78%) individuals were male. The average onset time of sporadic ALS (sALS) was 54.57±10.65 years of age, the peak onset time was around 50 years old. In that, seven (77.78%) individuals were farmers and two (22.22%) had a chronic disease or ALS family history. The survival duration of sALS was 2.07±1.08 years, and familial ALS (fALS) ranged from 6 to 8 years. Among them, the ALS prevalences in male patients and farmers were in the higher percentage (Table 3).

## Hospital survey

All surveyed class-1 hospitals in the hospital survey involved 26 street health centers or community health service centers in urban districts, 39 township health centers, and 78 village clinics in rural districts. There were only outpatient departments in these hospitals. Two individuals with ALS were identified among

TABLE 3 General clinical and demographic information of ALS patients identified in population survey.

| ID | Gender | Age | Profession | Medical history | Family history | Survival duration |
|---|---|---|---|---|---|---|
| 1 | Male | 65 | Farmer | Unknown | None | 3 years |
| 2 | Male | 40 | Farmer | Unknown | None | 1 years |
| 3 | Male | 45 | Truck driver | Unknown | None | 0.5 years |
| 4 | Male | 62 | Farmer | Unknown | None | 1 years |
| 5 | Female | 74 | Farmer | Unknown | None | 4 years |
| 6 | Female | 46 | Unemployed | Diabetes | None | 3 years |
| 7 | Male | 31 | Farmer | Hepatitis | ALS | 8 years |
| 8 | Male | 40 | Farmer | Unknown | ALS | 6 years |
| 9 | Male | 50 | Farmer | Unknown | None | 2 years |

TABLE 4 Ratio of ALS outpatients and inpatients in different level hospitals of 7 provinces of China mainland in 2015–2016.

| | Number of ALS outpatients/ total outpatients (1/100,000) | Number of ALS inpatients/ total inpatients (1/100,000) |
|---|---|---|
| Class-1 hospitals | 2/832,380 (0.24/100,000) | |
| Class-2 hospitals | 12/5,082,237 (0.24/100,000) | 15/453,506 (3.31/100,000) |
| Class-3 hospitals | 112/8,194,585 (1.37/100,000) | 128/549,267 (23.30/100,000) |
| Total | 126/14,109,202 (0.89/100,000) | 143/1,002,773 (14.26/100,000) |

832,380 outpatients in these hospitals. The prevalence of ALS in class-1 hospital outpatients was 0.24/100,000 (Table 4).

All surveyed class-2 hospitals in the hospital survey involved 13 class-2 general hospitals in urban districts and 13 county-level general hospitals in rural districts. There were both outpatient and inpatient departments in these hospitals. Twelve individuals with ALS were identified among 5,082,237 outpatients in these hospitals. Fifteen individuals with ALS were identified among 453,506 inpatients in these hospitals. The prevalence of ALS in class-2 hospital outpatients was 0.24/100,000. The prevalence of ALS in class-2 hospital inpatients was 3.31/100,000 (Table 4, Figure 3A).

Among surveyed 13 class-3 hospitals in urban districts, 102 individuals with ALS were identified among 8,194,585 outpatients in these hospitals And 128 individuals with ALS were identified among 54,9267 inpatients in these hospitals. The prevalence of ALS in class-3 hospital outpatients was 1.37/100,000. The prevalence of ALS in class-3 hospital inpatients was 23.30/100,000 (Table 4, Figure 2A).

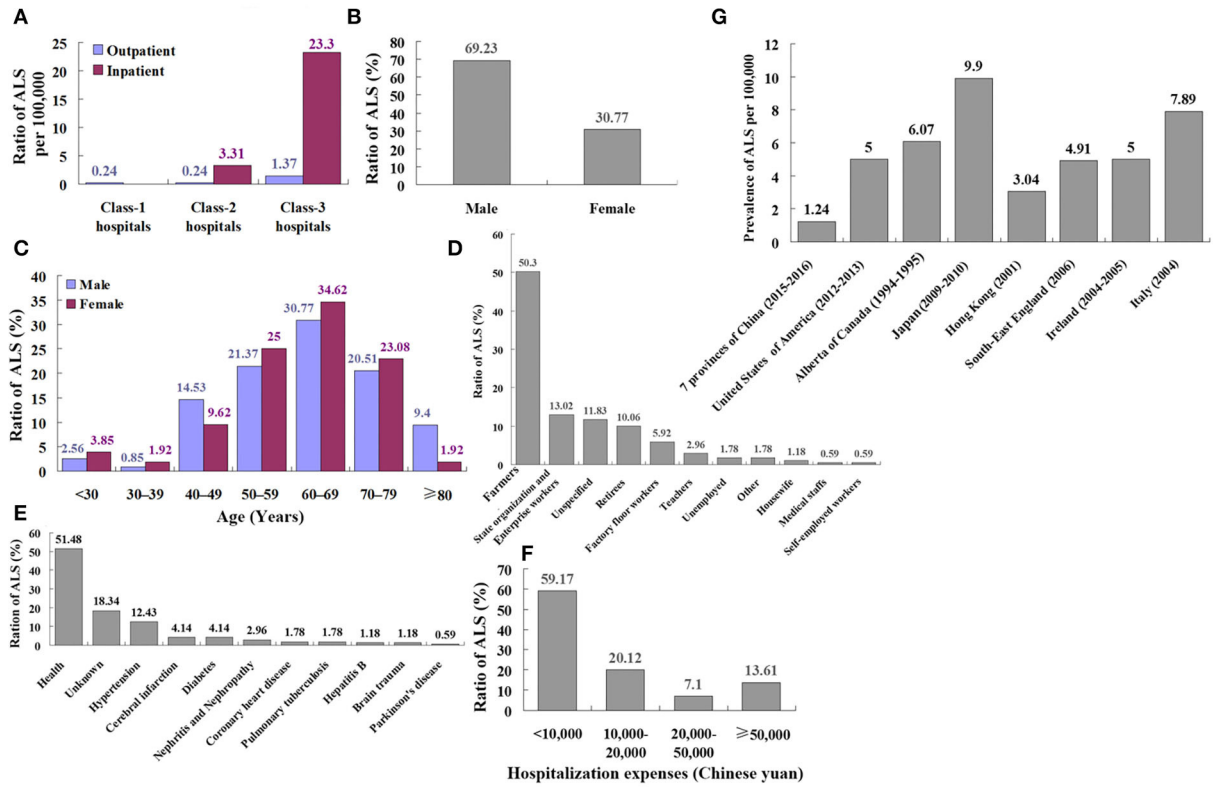


FIGURE 3
The comparison of survey data based on the hospital. **(A)** The comparison of ALS ratio between outpatients and inpatients in the different level hospitals of China involved 13 class-3 general hospitals, 13 class-2 general hospitals, and 26 street health centers or community health service centers in urban districts, and 13 county-level general hospitals, 39 township health centers and 78 village clinics in rural districts. The prevalence of ALS in inpatients was higher than that in outpatients. The total prevalence of ALS in class-3 hospital patients showed the highest. **(B)** The gender ratio of patients with ALS in the hospital survey, among 169 patients with ALS in the hospital survey, 117 (69.23%) patients with ALS were male and 52 (30.77%) were female. **(C)** The onset age ratio of patients with ALS in the hospital survey, and among those 169 patients, the percentage of 60 to 69 years was the highest, and the percentage of 30 to 39 years was the lowest. **(D)** The profession ratio of patients with ALS in the hospital survey, and among 169 patients with ALS in the hospital survey, farmers were the highest percentage (50.3%), and both medical staffs and self-employed workers were the lowest percentages (0.59%). **(E)** The medical history ratio of patients with ALS in hospital survey, and among 169 patients with ALS in the hospital survey, most patients with ALS had no chronic disease history (51.48%). **(F)** The hospitalization expenses ratio of patients with ALS in the hospital survey, and among 169 patients with ALS in the hospital survey, the hospitalization expenses of most patients with ALS (59.17%) were lower than 10,000 Chinese Yuan. **(G)** The comparison of ALS prevalence in different countries. Our results showed that the prevalence of ALS in seven provinces of the Chinese mainland was 1.24/100,000, which was less than the world other nations and districts including Chinese Hong Kong and similar to the European per year prevalence (2.2/100,000) and United States (1.5/100,000).

One hundred six individuals with ALS were identified among 14,109,202 outpatients in all hospitals. The prevalence of ALS in all hospital outpatients was 0.89/100,000. One hundred forty-three individuals with ALS were identified among 1,002,773 inpatients in all hospitals. The prevalence of ALS in all hospital inpatients was 14.26/100,000. In general, the prevalence of ALS in inpatients was higher than that in outpatients. The total prevalence of ALS in class-3 hospital patients showed the highest (1.37/100,000 outpatients and 23.30/100,000 inpatients) (Table 4, Figure 3A).

Among the 169 patients with ALS in the hospital survey, 117 (69.23%) were men and 52 (30.77%) were women (Figure 3B). The mean age of onset among male patients was 61.43 ± 12.66 years, and the mean age of onset among female patients was 59.98 ± 12.76 years. The percentage of 60 to 69 years was the highest and the percentage of 30 to 39 years was the lowest (Table 5, Figure 3C).

Among the 169 patients with ALS in the hospital survey, farmers were the highest percentage (50.3%) and medical staffs and self-employed workers were the lowest percentage (0.59%) (Table 2, Figure 3D). Most of the patients with ALS had no chronic disease history (51.48%). About 12.43% of patients had hypertension, 4.14% had cerebral infarction, 4.14% had diabetes, 2.96% had nephritis and nephropathy, 1.78% had coronary heart disease, 1.78% had pulmonary tuberculosis, 1.18% had hepatitis B, 1.18% had brain trauma, and 0.59% had Parkinson's disease (Figure 3E). Hospitalization expenses of most of the

TABLE 5 Epidemiological and clinical characteristics of ALS patients in hospital survey ($n = 169$).

| Characteristics | No. cases (n) (Total $n = 169$) | Ratio (%) |
|---|---|---|
| Gender | | |
| Male | 117 | 69.23 |
| Female | 52 | 30.77 |
| Age (years) | | |
| Male (Mean age 61.43±12.66) | | |
| <30 | 3 | 2.56 |
| 30–39 | 1 | 0.85 |
| 40–49 | 17 | 14.53 |
| 50–59 | 25 | 21.37 |
| 60–69 | 36 | 30.77 |
| 70–79 | 24 | 20.51 |
| ≥80 | 11 | 9.40 |
| Female (Mean age 59.98 ± 12.76) | | |
| <30 | 2 | 3.85 |
| 30–39 | 1 | 1.92 |
| 40–49 | 5 | 9.62 |
| 50–59 | 13 | 25.00 |
| 60–69 | 18 | 34.62 |
| 70–79 | 12 | 23.08 |
| ≥80 | 1 | 1.92 |
| Profession | | |
| Farmers | 85 | 50.30 |
| State organization and enterprise workers | 22 | 13.02 |
| Unspecified | 20 | 11.83 |
| Retirees | 17 | 10.06 |
| Factory floor workers | 10 | 5.92 |
| Teachers | 5 | 2.96 |
| Unemployed | 3 | 1.78 |
| Other | 3 | 1.78 |
| Housewife | 2 | 1.18 |
| Medical staffs | 1 | 0.59 |
| Self-employed workers | 1 | 0.59 |
| Medical history | | |
| Health | 87 | 51.48 |
| Unknown | 31 | 18.34 |
| Hypertension | 21 | 12.43 |
| Cerebral infarction | 7 | 4.14 |
| Diabetes | 7 | 4.14 |
| Nephritis and Nephropathy | 5 | 2.96 |
| Coronary heart disease | 3 | 1.78 |
| Pulmonary tuberculosis | 3 | 1.78 |
| Hepatitis B | 2 | 1.18 |
| Brain trauma | 2 | 1.18 |
| Parkinson's disease | 1 | 0.59 |
| Hospitalization expenses ($) | | |
| <10,000 | 100 | 59.17 |
| 10,000–20,000 | 34 | 20.12 |
| 20,000–50,000 | 12 | 7.10 |
| ≥50,000 | 23 | 13.61 |

TABLE 6 Comparison of ALS/MND prevalence in different countries or districts.

| Countries/Districts | Prevalence (per 100,000) | Duration |
|---|---|---|
| Seven provinces of China | 1.24 | 2015-2016 |
| United States of America | 5.00 | 2012-2013[1] |
| Alberta of Canada | 6.07 | 1994-1995[2] |
| Japan | 9.90 | 2009-2010[3] |
| Hong Kong | 3.04 | 2001[4] |
| South-East England | 4.91 | 2006[5] |
| Ireland | 5.00 | 2004-2005[6] |
| Italy | 7.89 | 2004[7] |

[1]Svenson et al., 1999; [2]Fong et al., 2005; [3]Abhinav et al., 2007; [4]Chiò et al., 2009; [5]Donaghy et al., 2009; [6]Doi et al., 2014; [7]Mehta et al., 2016.

patients with ALS (59.17%) were lower than 10,000 Chinese Yuan (Table 5, Figure 3F).

## Discussion

This large, cross-sectional survey study of ALS epidemiology in seven provinces of Chinese eastern, middle, and western regions involved a vast majority of Chinese geographic territory. In this study, we assessed the epidemiological data from population and hospital surveys. Our population survey involved 727,718 individuals including 61.80% (449,718) of Shandong peoples, 22.12% (160,983) of Jiangxi peoples, 7.16% (52,081) of Henan peoples, 2.86% (20,786) of Gansu peoples, 2.60% (18,950) of Sichuan peoples, 2% (14,600) of Jiangsu peoples, and 1.46% (10,600) of Zhejiang people (Table 1).

Our results showed that the prevalence of ALS in seven provinces of the Chinese mainland was 1.24/100,000, and the total prevalence of ALS was less than that of the world, other nations and districts including Chinese Hong Kong (Table 6, Figures 3G, 4, 5), was similar to the per year prevalence of Europe (2.2/100,000) and United States (1.5/100,000) (Kiernan et al., 2011; Mehta et al., 2016). But in the Chinese mainland, the 9.43/100,000 prevalence of ALS in Zhejiang province was the highest and was adjacent to the world's highest Japan (9.9/100,000) (Table 6, Figures 3G, 4, 5). The secondary was 3.84/100,000 in Henan and 3.73/100,000 in Jiangxi, respectively (Table 1), which was similar to that in the Chinese Hong Kong district (3.04/100,000) (Table 6, Figures 3G, 4, 5). Among them, 65.74% (478,402) were urban people and 34.26% (249,316) were rural people, and it was obvious that the ALS prevalence in rural residents (2.01/100,000) was higher than that in urban residents (0.84/100,000) (Table 2).

Of the nine definite ALS in the population survey, 77.78% were farmers, occupying more than 50% among them (Table 3). About 22.22% had an ALS family history, which was slightly more than the international reported ∼10% (Table 3), and 77.78% were sALS, which was slightly less than the international

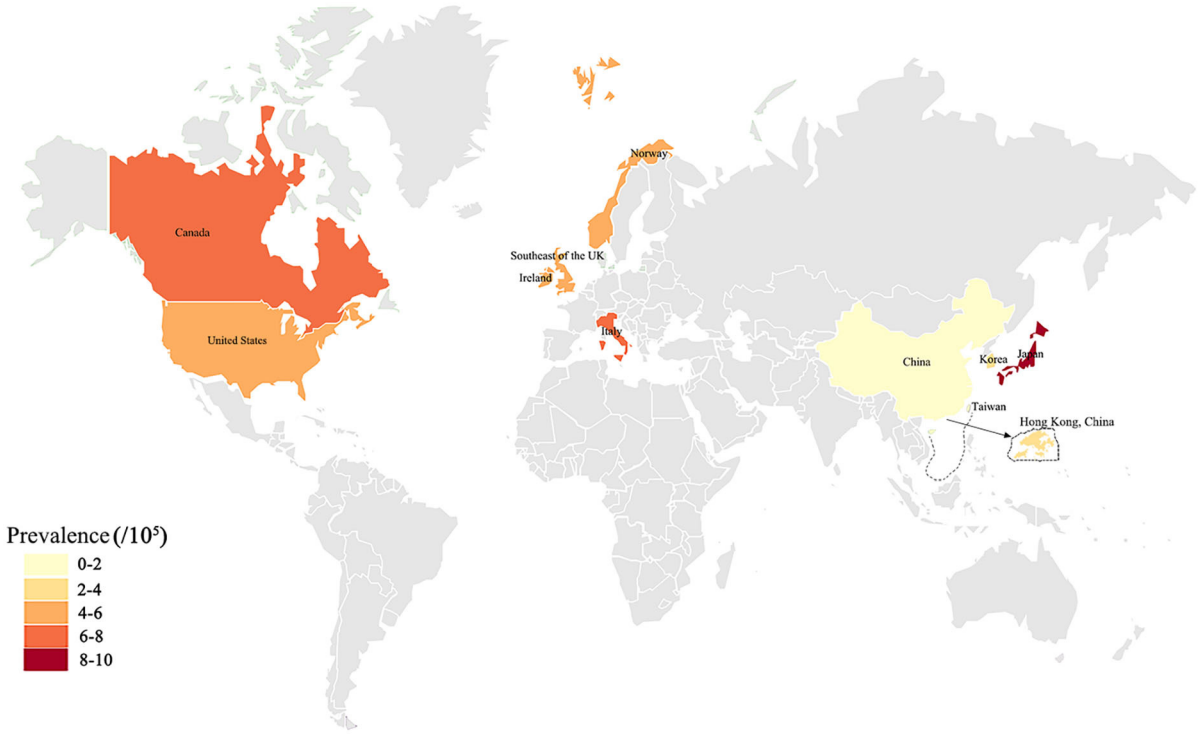


FIGURE 4
The previously reported ALS prevalence in the global different countries or districts was exhibited on the world map. The deep and shallow color indicated the high and low of ALS prevalence. Deeper color indicated higher ALS prevalence.

reported approximate 80% (Table 3) (Zarei et al., 2015). The survival duration of sALS was 0.5–4 years and the average survival was 2.07 ± 1.08 years, which was less than the previously reported around 3.5 years (Table 3) (Salameh et al., 2015). The survival duration of fALS ranged from 6 to 8 years in this survey. fALS in the previous survey existed a longer survival duration, someone lives more than 10 years, even exceeding 30 years or more long (Salameh et al., 2015). About 77.78% were male patients, 22.22% were female patients, male patients were significantly higher than female patients (Table 3), which was consistent with the information reported by previous investigation (Kiernan et al., 2011).

In the hospital survey, the prevalence of ALS was 0.24/100,000 in 832,380 outpatients in class-1 hospitals (Table 4). The prevalence of ALS was 0.24/100,000 in 5,082,237 outpatients and 3.31/100,000 in 453,506 inpatients in class-2 hospitals, respectively (Table 4, Figure 3A). The prevalence of ALS was 1.37/100,000 in 8,194,585 outpatients and 23.30/100,000 in 54,9267 inpatients in class-3 hospitals, respectively (Table 4, Figure 3A). The prevalence of ALS was 0.89/100,000 in 14,109,202 outpatients and 14.26/100,000 in 1,002,773 inpatients in all hospitals, respectively. There could be no doubt that the total prevalence of ALS in class-3 hospitals was the highest and the secondary was in class-2 hospitals. It was affirmative that the prevalence of ALS in both inpatients and outpatients in the class-3 hospital was higher than that in both class-2 and class-1 hospitals (Table 4, Figure 3A). The possible cause of the highest prevalence in class-3 hospitals is that ALS is a rare disease and the patients with ALS themselves are not completely confident of the diagnosis made by doctors in both class-1 and class-2 hospitals, as well as the doctors in both class-2 and class-1 find it difficult to diagnose ALS because of the limitation of doctor diagnostic ability and hospital medical condition.

Among the 169 definite patients with ALS in the hospital survey, 69.23% were male and 30.77% were female (Figure 3B), which was similar to the previous investigation reported information (Kiernan et al., 2011). The male mean age of onset was 61.43±12.66 years, the female mean age of onset was 59.98 ± 12.76 years, the peak onset age was at 60 to 69 years, and the lowest age of onset was 30 to 39 years (Table 5, Figure 3C). Among different professions, 50.3% were farmers, and medical staffs and self-employed workers were the lowest, only occupying 0.59% (Table 2, Figure 3D). About 51.48% of ALS had no chronic disease history (Figure 2E). The hospitalization expenses of 59.17% of patients with ALS were lower than 10,000 Chinese Yuan (Table 5, Figure 3F).

We conducted informative comparisons with previous studies in this study. Although the prevalence, onset age,

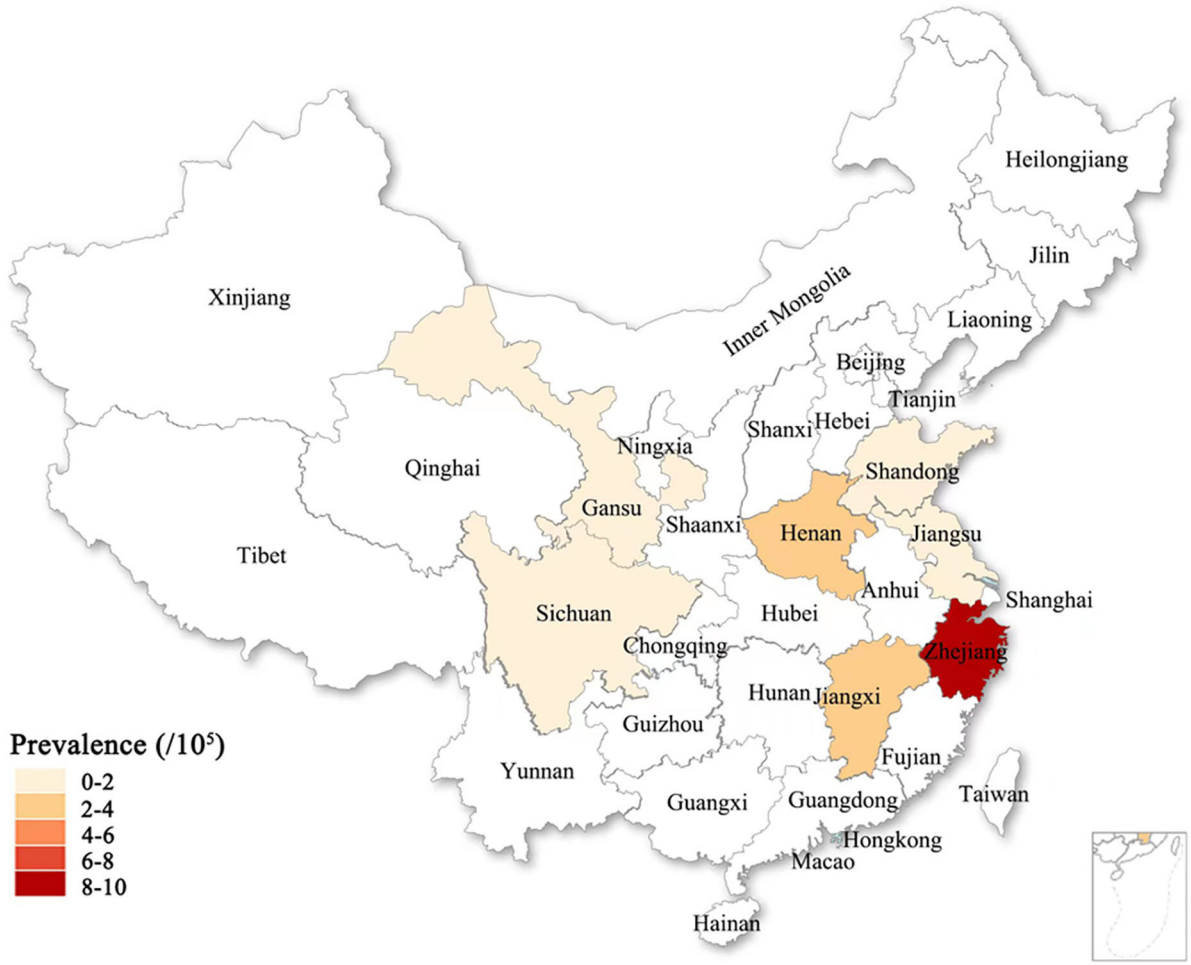


FIGURE 5
The ALS prevalence in seven provinces of the Chinese mainland in this survey was exhibited on the Chinese map. The deep and shallow color indicated the high and low of ALS prevalence. Deeper color indicated higher ALS prevalence.

geographical location, profession, chronic disease history, familial history, survival duration, and diagnostic and cured condition (Hospitalization expenses) of ALS in our study were slightly different than the corresponding rates in the previous study, the overall results were similar to the previous investigation data (Kiernan et al., 2011; Salameh et al., 2015). We speculate that the relatively low prevalence of ALS observed in our study implies greater omit diagnosis in the Chinese mainland because the criteria of ALS cannot be accurately realized by doctors in under class-2 hospitals. Although both population and hospital survey studies showed an increased risk of farmers and rural residents, our study did not show a statistically significant association between farmers, rural residents, and the development of ALS because of the limited sample. The multiple coexisting conditions seen in farmers and rural residents may confer greater susceptibility to ALS, such as associated environments, education and physical power, and so on, which is, in part, supported by the higher prevalence seen in our study than in previous studies (Ludolph et al., 2012; Zarei et al., 2015; Vasta et al., 2022). The profession in agriculture was risk work for the development of ALS in the Chinese mainland. As far as know, the information that the higher prevalence of ALS in rural residents and farmers was not reported before our study.

Our study demonstrated that the prevalence of ALS and hospitalization expenses were lower than global other countries and districts. For that, we doubted that the survey results did not represent that the prevalence of ALS in our country was really lower than in other countries and districts in the world. In fact, it might be that we weren't enough know the disease of ALS, especially for the non-neurological doctor, omitted lots of patients with ALS. Moreover, the major reason for lower hospitalization expenses might not be because patients with ALS do not have enough money for hospitalization cure but the

ALS treatment has exerted the national medical insurance of chronic disease in our country, and patients with ALS do not need to pay too much money for hospitalized treatment and the fact that mainly because lots of non-neurologists did not generally and comprehensively understand the normal processes of ALS diagnosis and treatments, which resulted in inaccurate diagnosis and treatment of ALS. This finding questions in our study reminder us to nationwide efforts to prevent to omit the diagnosis and the hospitalized treatment of ALS by spreading knowledge about the ALS diagnosis and treatment in popular peoples and non-neurological doctors, enhancing the training of ALS diagnosis and treatment in neurological doctors, improving the ratio of ALS diagnosis and the hospitalized treatment, and reducing the ratio of omitted diagnosis, providing better medical service for patients with ALS and improving the life quality of patients with ALS.

Our data further revealed that men were more likely to suffer from ALS than women. The survival duration of sALS was about 3 years and the survival duration of fALS was longer than that of sALS (Kiernan et al., 2011; Salameh et al., 2015). In addition, it seems that the peak onset age (60–69 years) in our study is later than the previous investigation data (Kiernan et al., 2011), for that, we thought that it could not completely exclude some patients with ALS or diagnosed by doctors at the initial or early stage of ALS, and it also could not completely exclude the retrospective survey of disease resulted in the mistaken judgment of accurate onset time.

Our study has some strengths. Our survey involved the representative regions of the population and hospitals in the Chinese mainland, which enabled the general evaluation of prevalence, sexuality, onset age, profession, survival time, and diagnostic and cured condition of ALS in the Chinese mainland. Of course, our study also exist some limitations, such as our study did not include all population and hospitals in the Chinese mainland, and also the survey of multiple centers. Therefore, our study might have some deficiencies and errors.

## Conclusion

In conclusion, the ALS hospitalization expenses in our survey were the lowest compared with the previously reported countries and districts. The farmer was a possible higher risk profession for ALS, and the majority of ALS were not accompanied by other chronic diseases. Besides, the general epidemiology features in our study were not found to be significantly different from the previous epidemiology survey. The prevalence, sexuality, onset age, and survival time of ALS were similar to the reported information in the previous epidemiological studies conducted in other countries and districts (Svenson et al., 1999; Fong et al., 2005; Abhinav et al., 2007; Chiò et al., 2009; Donaghy et al., 2009; Kiernan et al., 2011; Doi et al., 2014; Salameh et al., 2015; Mehta et al., 2016).

## 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

We declare that all human studies have been approved this survey by the Ethics Committee of First Affiliated Hospital of Nanchang University (Approval number: 2014-06-01). All participants were invited to participate voluntarily. The study protocol and procedures follow the ethical guidelines of the 1975 Declaration of Helsinki. A verbal informed consent was obtained from all subjects in this study in approved by from the local Center for Disease Control, selected hospitals, and selected communities and villages, which gave approval for verbal consent. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## Author contributions

JZ, XL, HL, SX, XW, and RX conceived the idea, designed the research, and wrote the manuscript. JZ, XL, HL, and SX performed the data collection, extraction, and analyses. XW and RX contributed to the data verification. All authors read, reviewed, and approved the final manuscript.

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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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## References

Abhinav, K., Stanton, B., Johnston, C., Hardstaff, J., Orrell, R. W., Howard, R., et al. (2007). Amyotrophic lateral sclerosis in South-East England: a population-based study. The South-East England register for amyotrophic lateral sclerosis (SEALS Registry). *Neuroepidemiology.* 29, 44–48. doi: 10.1159/000108917

Brooks, B. R., Miller, R. G., Swash, M., Munsat, T. L., and World Federation of Neurology Research Group on Motor Neuron Diseases. (2000). El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph. Lateral. Scler. Other Motor Neuron Disord.* 1, 293–299. doi: 10.1080/146608200300079536

Chiò, A., Mora, G., Calvo, A., Mazzini, L., Bottacchi, E., Mutani, R., et al. (2009). Epidemiology of ALS in Italy: a 10-year prospective population-based study. *Neurology.* 72, 725–731. doi: 10.1212/01.wnl.0000343008.26874.d1

Doi, Y., Atsuta, N., Sobue, G., Morita, M., and Nakano, I. (2014). Prevalence and incidence of amyotrophic lateral sclerosis in Japan. *J. Epidemiol.* 24, 494–499. doi: 10.2188/jea.JE20140059

Donaghy, C., O'Toole, O., Sheehan, C., Kee, F., Hardiman, O., and Patterson, V. (2009). An all-Ireland epidemiological study of MND, 2004-2005. *Eur. J. Neurol.* 16, 148–153. doi: 10.1111/j.1468-1331.2008.02361.x

Fong, C. C. Y., Cheng, T. S., Lam, K., Cheng, W. K., Mok, K. Y., Cheung, C. M., et al. (2005). An epidemiological study of motor neuron disease in Hong Kong. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 6, 164–168. doi: 10.1080/14660820510028412a

Johnsen, B., Pugdahl, K., Fuglsang-Frederiksen, A., Kollewe, K., Paracka, L., Dengler, R., et al. (2019). Diagnostic criteria for amyotrophic lateral sclerosis: a multicentre study of inter-rater variation and sensitivity. *Clin. Neurophysiol.* 130, 307–314. doi: 10.1016/j.clinph.2018.11.021

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., et al. (2011). Amyotrophic lateral sclerosis. *Lancet.* 377, 942–955. doi: 10.1016/ S0140-6736(10)61156-7

Ludolph, A., Drory, V., Hardiman, O., Nakano, I., Ravits, J., Robberecht, W., et al. (2015). A revision of the El Escorial criteria - 2015. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 16, 291–292. doi: 10.3109/21678421.2015.1049 183

Ludolph, A. C., Brettschneider, J., and Weishaupt, J. H. (2012). Amyotrophic lateral sclerosis. *Curr. Opin. Neurol.* 25, 530–535. doi: 10.1097/WCO.0b013e328356d 328

Mehta, P., Kaye, W., Bryan, L., Larson, T.,Copeland, T., Wu, J., et al. (2016). Prevalence of amyotrophic lateral sclerosis - United States, 2012-2013. *MMWR. Surveill. Summ.* 65, 1–12. doi: 10.15585/mmwr.ss6508a1

Salameh, J. S., Brown, R. H., and Berry, J. D. (2015). Amyotrophic lateral sclerosis: review. *Semin. Neurol.* 35, 469–476. doi: 10.1055/s-0035-1558984

Silani, V., Messina, S., Poletti, B., Morelli, C., Doretti, A., Ticozzi, N., et al. (2011). The diagnosis of amyotrophic lateral sclerosis in 2010. *Arch. Ital. Biol.* 149, 5–27. doi: 10.4449/aib.v149i1.1260

Statland, J. M., Barohn, R. J., McVey, A. L., Katz, J. S., and Dimachkie, M. M. (2015). Patterns of weakness, classification of motor neuron disease, and clinical diagnosis of sporadic amyotrophic lateral sclerosis. *Neurol. Clin.* 33, 735–748. doi: 10.1016/j.ncl.2015.07.006

Svenson, L. W., Cwik, V. A., and Martin, W. R. (1999). The prevalence of motor neurone disease in the Province of Alberta. *Can. J. Neurol. Sci.* 26, 119–122.

Vasta, R., Chia, R., Traynor, B. J., and Chi,ò, A. (2022). Unraveling the complex interplay between genes, environment, and climate in ALS. *EBioMedicine* 75, 103795. doi: 10.1016/j.ebiom.2021.103795

Zarei, S., Carr, K., Reiley, L., Diaz, K., Guerra, O., Altamirano, P. F., et al. (2015). A comprehensive review of amyotrophic lateral sclerosis. *Surg. Neurol. Int.* 6, 171. doi: 10.4103/2152-7806.169561

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# Extended effects of a wearable sensory prosthesis on gait, balance function and falls after 26 weeks of use in persons with peripheral neuropathy and high fall risk—The walk2Wellness trial

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**Background:** We recently reported that individuals with impaired plantar sensation and high fall risk due to sensory peripheral neuropathy (PN) improved gait and balance function following 10 weeks of use of Walkasins®, a wearable lower limb sensory prosthesis that provides directional specific mechanical tactile stimuli related to plantar pressure measurements during standing and walking (RxFunction Inc., Eden Prairie, MN, United States). Here, we report 26-week outcomes and compare pre- and in-study fall rates. We expected improvements in outcomes and reduced fall rates reported after 10 weeks of use to be sustained.

**Materials and methods:** Participants had clinically diagnosed PN with impaired plantar sensation, high fall risk (Functional Gait Assessment, FGA score $< 23$) and ability to sense tactile stimuli above the ankle at the location of the device. Additional outcomes included 10 m Gait Speed, Timed Up and Go (TUG), Four-Stage Balance Test, and self-reported outcomes, including Activities-Specific Balance Confidence scale and Vestibular Disorders Activities of Daily Living Scale. Participants tracked falls using a calendar.

**Results:** We assessed falls and self-reported outcomes from 44 individuals after 26 weeks of device use; 30 of them conducted in-person testing of clinical outcomes. Overall, improvements in clinical outcomes seen at 10 weeks of use remained sustained at 26 weeks with statistically significant

increases compared to baseline seen in FGA scores (from 15.0 to 19.2), self-selected gait speed (from 0.89 to 0.97 m/s), and 4-Stage Balance Test (from 25.6 to 28.4 s), indicating a decrease in fall risk. Non-significant improvements were observed in TUG and fast gait speed. Overall, 39 falls were reported; 31 of them did not require medical treatment and four caused severe injury. Participants who reported falls over 6 months prior to the study had a 43% decrease in fall rate during the study as compared to self-report 6-month pre-study (11.8 vs. 6.7 falls/1000 patient days, respectively, $p < 0.004$), similar to the 46% decrease reported after 10 weeks of use.

**Conclusion:** A wearable sensory prosthesis can improve outcomes of gait and balance function and substantially decreases incidence of falls during long-term use. The sustained long-term benefits in clinical outcomes reported here lessen the likelihood that improvements are placebo effects.

**Clinical trial registration:** ClinicalTrials.gov, identifier #NCT03538756.

KEYWORDS

**peripheral neuropathy, falls, balance, gait speed, neuromodulation, clinical trial, sensory prosthesis, wearable**

## Introduction

Persistent problems with gait and balance function may lead to falls, fractures, and other serious injuries in older adults (Bergen et al., 2016; Ganz and Latham, 2020; Moreland et al., 2020). In fact, clinical observations on the problem of falls and fall prevention go back to at least 1948 when Sheldon (1948) presented data in *The Lancet* on "Liability to Falls" in older adults, concluding that falling was "a clinical problem which has never received the attention it deserves." Today, we appreciate that causes of falls are complex and require a multifactorial clinical approach to arrive at a correct diagnosis and to provide specific and relevant therapeutic interventions (Cohen et al., 2015).

To address the problem of falls in older adults, various clinical practice guidelines for falls prevention and management have been developed (Montero-Odasso et al., 2021). The Centers for Disease Control and Prevention (CDC), for example, has established "STEADI" (STEADI, 2021), an evidence-based initiative to address fall prevention based on a curation of decades of research activities (Stevens and Phelan, 2013). At this time, advice provided through the STEADI initiative includes "Talk to your doctor"; "Do Strength and Balance Exercises"; "Have Your Eyes Checked"; and "Make Your Home Safer" (CDC, 2021). At a minimum, health care providers should screen their patients for fall risk, including older adults and individuals with neurodegenerative diseases that affect balance function. Simple interventions that should be considered to help reduce the risk for falls include medication management and vitamin D supplements to improve bone, muscle, and nerve health (Bergen et al., 2016). Further treatment of gait and balance issues may involve use of canes, walkers, and rehabilitation interventions with balance exercises (Richardson et al., 2001; Halvarsson et al., 2013; Melzer and Oddsson, 2013; Ganz and Latham, 2020), including Tai-Chi (Li and Manor, 2010; Manor et al., 2014; Quigley et al., 2014). Any functional benefits related to fall rates and gait function after long-term use of bilateral ankle foot orthoses appear limited (Wang et al., 2019).

In addition to other age-related risk factors, sensory peripheral neuropathy (PN), leading to impaired plantar sensation, is an independent risk factor for falls (Richardson and Hurvitz, 1995; Koski et al., 1998; Reeves et al., 2021), irrespective of whether the underlying cause is due to diabetes (Mustapa et al., 2016; Vinik et al., 2017), chemotherapy (Winters-Stone et al., 2017), or an unknown factor (Riskowski et al., 2012). The prevalence of PN in the United States population for those over age 40 is nearly 15% (Gregg et al., 2004) and 28.4% in persons with diabetes (Hicks et al., 2021). The incidence of injuries due to falls is 15 times higher in patients with diabetic PN than in healthy individuals (Cavanagh et al., 1992). Furthermore, polyneuropathy contributes independently to functional impairments, including difficulty walking and a tendency to fall (Hoffman et al., 2015), and those individuals more often incur fall-related injuries (Hanewinckel et al., 2017). A prospective study of older individuals with PN found that 65% fell during a one-year period and 30% reported an injury from a fall (DeMott et al., 2007).

Mechanoreceptors play an important role in proprioception (Fallon et al., 2005; Vaughan, 2021) and loss of sensory information from the lower limbs, including plantar mechanoreceptors alters balance performance (Meyer et al., 2004a,b). Furthermore, this sensory impairment affects the automaticity of walking, thereby increasing the need for cognitive attention to gait and mobility activities (Paul et al., 2005; Clark et al., 2014; Clark, 2015). Furthermore, several studies have reported problems with gait and balance function and the increased risk of falls in patients with PN (Menz et al., 2004; DeMott et al., 2007; Dixon et al., 2017; Lipsitz et al., 2018).

Additionally, low gait speed is a risk factor for falls (Studenski et al., 2003; Montero-Odasso et al., 2005), is an indicator of frailty (Kim et al., 2019), and is even a predictor of hospitalization and disability (Cesari et al., 2005; Dumurgier et al., 2009) as well as survival (Studenski et al., 2011). In fact, interventions designed to improve gait speed may actually increase survival (Hardy et al., 2007). Older adults with sensory impairment related to PN show a decline in gait speed that is four times higher than in healthy aging (Buracchio et al., 2010; Lipsitz et al., 2018).

Although strength and balance training in patients with PN may help reduce fall risk falls (Ites et al., 2011; Tofthagen et al., 2012; Streckmann et al., 2014; Morrison et al., 2018), strength training in patients with PN appears to have less impact on balance (Streckmann et al., 2014). Its effects are mainly compensatory and do not address impaired somatosensation, the root cause of balance problems related to PN. Furthermore, balance training activities must be specific (Oddsson et al., 2007) and must be conducted with sufficient intensity and frequency (Lipsitz et al., 2019) to be helpful, or benefits will be limited or absent (Kruse et al., 2010; Lipsitz et al., 2018, 2019). Clear guidelines regarding frequency of balance exercises are currently lacking although three sessions a week may be a minimum necessary to see an improvement (Kruse et al., 2010). Thus, although "Falls can be prevented" (CDC, 2021), they continue to be a large problem in older adults, indicating a continued need for novel solutions.

We recently reported from our multi-site clinical trial (walk2Wellness, NCT #03538756) that individuals with PN and a high risk of falls improved their Functional Gait Assessment (FGA) scores, (Wrisley et al., 2004), Gait Speed, and Timed Up and Go times following 10 weeks of home-based use of a wearable sensory prosthesis intended to substitute nerve function related to impaired plantar sensation (**Figure 1**, Walkasins by RxFunction Inc., Eden Prairie, MN, United States), (Oddsson et al., 2020). Previously, we found similar meaningful improvements in FGA scores and gait speed in participants with PN in a randomized crossover trial conducted in-clinic (Koehler-McNicholas et al., 2019), findings that were recently referred to as "presumably emulating" signals by fast-adapting cutaneous afferents (Vaughan, 2021). We also reported a decrease in the number of fall risk factors as well as fall rate from baseline to 10 weeks for individuals who reported falls in the 6 months preceding study participation (Oddsson et al., 2020).

Here we report extended long-term use data from the walk2Wellness trial for 44 participants after 26 weeks of Walkasins use. Furthermore, we analyzed falls during the 26 weeks of device use and compared to patient self-reports of falls from the 6 months preceding participation in the study. We expected improvements in clinical outcomes and lower fall rate seen after 10 weeks (Oddsson et al., 2020) to be sustained at 26 weeks.

## Materials and methods

Detailed descriptions of materials and methods have been reported previously (Oddsson et al., 2020), and are summarized below. The measures we use have been validated (Oddsson et al., 2020) and our primary outcome measure, the FGA, is recommended for assessment of walking balance in adults with neurologic conditions by current physical therapy Clinical Practice Guidelines (Moore et al., 2018). The walk2Wellness study is registered at ClinicalTrials.gov (#NCT03538756). **Figure 1** shows a picture of the Walkasins device components with a brief description of its functionality.

### Participants

The walk2Wellness clinical trial received approval for human subject research from Advarra IRB (formerly Quorum Review) for four sites and from the IRB Subcommittee, the Subcommittee on Research Safety, and the Research and Development Committee of the Minneapolis VA Health Care System (MVAHCS). Recruitment for the walk2Wellness trial began in October 2018 and ended at the last site in April 2021. Because of COVID-19 disruptions, the study ended earlier than planned at four sites; the fifth site completed the trial but with a reduced enrollment goal. Study site team members posted fliers at community businesses and clinics to advertise the trial; in addition, some of the sites received referrals from physicians who were informed about the study.

To participate in the trial, individuals met the following inclusion criteria: male or female; ages 21–90; a formal diagnosis of sensory PN prior to participating in the study as indicated by their medical record or a letter by a physician; self-reported problems with balance; ability for transfers or ambulation on level surfaces at fixed cadence as assessed by trained study personnel; FGA score $< 23$, the cut-off score for high fall risk (Wrisley and Kumar, 2010); ability to understand and provide informed consent; foot size to allow the Walkasins device to function properly, and ability to complete all functional outcome measures without the use of an assistive device in order to ensure sufficient motor function. Participants could use an assistive device at their discretion during their daily activities.


FIGURE 1
**(Left)** The two components of the Walkasins prosthetic device, the pressure sensitive flexible foot pad that is placed in the shoe and connects to the leg unit that contains a rechargeable battery, a microprocessor, supporting electronics, and four mechanical tactile stimulators. The embedded software algorithm evaluates pressure data and activates the mechanical tactile stimulators at relevant times during standing and walking to signal balance-related information to the afferent nervous system. **(Right)** A Walkasins user wearing the device in the process of turning it on. The Walkasins system is worn bilaterally (unilateral components depicted).

Individuals were excluded if they were unable to perceive the tactile stimuli from the Walkasins leg unit (**Figure 1**) or if they used an ankle-foot orthosis for ambulation that prevented donning the device. In addition, individuals having any of the following conditions were excluded from participation: acute thrombophlebitis; deep vein thrombosis; untreated lymphedema; a lesion of any kind, swelling, infection, inflamed area of skin, or eruptions on the lower leg near placement of the device; foot or ankle fractures; severe peripheral vascular disease; or any musculoskeletal or neurological condition that would prohibit use of the device, as determined by a clinician. Because of a potential to overload the pressure sensors in the foot pad, individuals weighing over 136 kg (300 lbs) were excluded from participation. The criteria to participate in the study were similar to receiving a prescription for the device in a clinic. Potential participants also agreed not to initiate any balance training (e.g., Tai-Chi, etc.) or balance-related therapy during the first 10 weeks of the trial (Oddsson et al., 2020). Furthermore, participants were blinded to their outcomes during the trial and not shared by study personnel.

## Study procedures and outcome measures

Before undergoing any study-related procedures, individuals signed the IRB-approved consent form and HIPAA authorization. after enrollment, site staff tested whether participants were able to perceive the tactile stimuli from the Walkasins leg unit. Anyone unable to feel the tactile stimuli was excluded from continued participation in the study. Individuals who were able to feel the tactile stimuli from the Walkasins leg unit bilaterally then completed a medical history form to assess common health issues and systemic diseases. They also provided information on falls they had experienced in the previous 6 and 12 months, including the number of falls and any injuries they sustained as a result. In addition, participants provided a list of their medications (medication name, indication, dose, and frequency), which was updated over the course of the study.

Participants then completed the Activities-Specific Balance Confidence (ABC) Questionnaire (Powell and Myers, 1995) to assess levels of balance self-confidence and the Vestibular Activities of Daily Living Scale (VADL), (Cohen et al., 2000) to evaluate the effects of vertigo and balance disorders on their ability to perform everyday activities independently. At baseline, weeks 10 and 26, site personnel also assessed participants using the Weinstein Enhanced Sensory Test (WEST) monofilament foot test (0.5, 2, 10, 50, and 200 g), as described previously (Oddsson et al., 2020). Staff also performed a vibration sensation test using a Rydel-Seiffer Tuning Fork to document loss of sensation (Kästenbauer et al., 2004; Oddsson et al., 2020). Scoring values $\leq$4 at the first metatarsal joint is categorized as abnormal (Kästenbauer et al., 2004).

During the baseline visit, participants donned the Walkasins devices and performed a standardized set of standing and walking balance activities focused on orientation and familiarization with the device, referred to as the Walkasins Learning Protocol (Koehler-McNicholas et al., 2019; Oddsson et al., 2020). Trained site personnel then assessed participants on the FGA, 10-Meter Walk Test (Perera et al., 2006), Timed Up and Go (TUG) (Mathias et al., 1986), and 4-Stage Balance Test (CDC, 2021). The clinical outcomes were standardized and performed by study personnel who were trained by one of

the investigators (DW). A sponsor representative conducted observation visits periodically during the study to ensure standardization across the sites (LJ).

After finishing the clinical outcome tests, participants completed the following self-reported outcome measures to provide information about their quality of life, pain level, and engagement in social activities: Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001), PROMIS Pain Interference Short Form 6b (Askew et al., 2016), PROMIS Pain Intensity Form 1a, Ability to Participate Short Form 8a (Hahn et al., 2016b), and Satisfaction with Participation in Social Roles Short Form 8a (Hahn et al., 2014, 2016a). At the conclusion of the baseline visit, site staff members trained the participants on caring for the device.

Participants left the baseline visit with the device and a calendar on which to note any fall events they experienced as well as their use of Walkasins. For consistency across sites, we used the World Health Organization's definition of a "fall": "A fall is an event which results in a person coming to rest inadvertently on the ground or floor or other lower level." When participants reported falls, study staff recorded the following: the fall date; severity (no injury, minor injury, or major injury); the level of treatment the individual sought or received, if any; the cause or causes of the fall (environmental factor, lost balance, lost consciousness, lost strength/felt weak, slipped, tripped, on new medication, other, unknown); and a description of the fall.

Unless prevented from doing so because of COVID-19 site restrictions, participants returned for in-person visits at weeks 2, 6, 10, and 26. These visits followed most of the same procedures as the baseline visit: balance questionnaires, clinical assessments, and self-reported outcome measures. As we have previously reported (Oddsson et al., 2020) we calculated ABC to FGA ratios at each assessment to measure the degree of internal self-perception of balance capability (ABC score) in relation to the externally observed walking balance performance (FGA score). We noted that a ratio of around 3.3 would be "expected" based on the maximum scores of the two scales. This construct aligns with a multifactorial causation model for falls emphasizing the importance of a "Realistic Appraisal of One's Own Abilities," (Hadjistavropoulos et al., 2011).

Between the in-person visits at weeks 10 and 26, study sites contacted participants via telephone at weeks 14, 18, and 22 to remind them of study requirements and to collect follow-up information regarding health changes, falls, adverse events, pain scores, device usage, and device functioning, as well as whether they had begun any physical therapy for their balance problems. If participants reported adverse events and/or falls during these contacts, site personnel recorded the details on the appropriate case report forms in REDCap Cloud, the electronic data capture system used in the study[1] (Encinitas, CA, United States).

1 https://www.redcapcloud.com/

## Statistical analysis and availability of data

An analysis of outcomes after 2, 6, and 10 weeks, with 10 weeks being the primary endpoint, was presented earlier (Oddsson et al., 2020). Here we focus on outcomes after extended long-term device use and compare outcomes to baseline for individuals participating in 26 weeks of device use. Graphical data from 2, 6 and 10 weeks are provided below for illustration. We calculated descriptive statistics (mean and standard deviation or median as appropriate) for all variables and tested for normality with the Shapiro-Wilk's test. We applied the two-proportion $Z$-test to compare proportion-based measures. We conducted a *post hoc* analysis to compare participants at baseline who reported falls in the previous 6 months (Pre-Fallers, $n = 25$) to those who did not (Pre-Non-Fallers, $n = 19$). Their baseline characteristics were compared using a $t$-test for independent samples or a Mann-Whitney $U$ test if data was not normally distributed based on a Shapiro-Wilk's test.

To compare baseline variables to those after 26 weeks of device use, we conducted a $t$-test for dependent samples or Wilcoxon signed-rank test in case variables were not normally distributed. We applied a Bonferroni's adjustment of significance levels for correlated measures, ranging from $p < 0.0167$ (0.05/3 for three comparisons) for a full correction (non-correlated measures, $r = 0$) and $p < 0.05$ for perfectly correlated measures ($r = 1$) (Uitenbroek, 1997). We calculated effect sizes using Cohen's $d_{rm}$ according to recommendations by Lakens (2013) and interpreted them according to Cohen (1988), i.e., 0.2 represents a small effect, 0.5 a medium effect, and 0.8 a large effect size. Ninety-five percent confidence intervals of effect sizes were estimated according to Algina et al. (2005).

We performed statistical analysis with the Analysis-ToolPak module in Microsoft Excel 2016 and the Real Statistics Resource Pack software, release 6.8 (Zaiontz, 2020). Baseline characteristics of participants in the study from the four clinical sites are presented in **Table 1**. We have pooled the data for the continued analysis presented here. The raw data supporting the conclusions of this article will be made available to qualified researchers without undue reservation.

# Results

## Enrollment and allocation

Our previous report with 45 participants at the 10-week primary endpoint (Oddsson et al., 2020) was published early due to the unknown circumstances at the time related to COVID-19, which had caused recruitment holds and suspension of in-person testing activities at the research sites. Over time, the trial continued with the enrollment of additional participants

TABLE 1 Characteristics of the 44 individuals from the four different clinical sites enrolled in the study who reached 26 weeks of participation.

| | n | Age (yrs) | Height (m) | Weight (kg) | #ChrD | FGA score | Gait speed self-selected (m/s) | Gait speed fast (m/s) | TUG (s) | 4-Stage balance test (s) | ABC score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **VAMC** | 18 | 74.4 (6.0) | 1.76 (0.07) | 96.9 (14.1) | 9.6 (2.3) | 15.4 (4.1) | 0.82 (0.13) | 1.27 (0.36) | 13.5 (2.9) | 24.8 (7.4) | 68.2 (15.8) |
| **Baylor** | 11 | 74.6 (9.4) | 1.75 (0.08) | 87.0 (15.2) | 8.2 (2.9) | 15.4 (4.5) | 0.88 (0.25) | 1.33 (0.39) | 13.3 (6.3) | 28.1 (8.0) | 55.6 (18.8) |
| **M Health Fairview** | 5 | 71.2 (10.7) | 1.75 (0.14) | 84.4 (12.0) | 6.8 (5.3) | 15.6 (2.1) | 1.07 (0.21) | 1.32 (0.20) | 10.5 (1.4) | 26.4 (3.8) | 64.1 (7.0) |
| **Harvard** | 10 | 71.5 (8.0) | 1.76 (0.13) | 86.7 (16.9) | 6.6 (3.5) | 15.0 (3.0) | 0.95 (0.31) | 1.25 (0.45) | 12.8 (3.3) | 25.3 (7.6) | 60.8 (15.3) |

Values represent Mean (Standard Deviation). #ChrD, Number of Chronic Diseases.

who are part of the current report as illustrated in the flow chart in Figure 2. Figure 2 shows the flow chart for the study as it was continued from the 10-week primary endpoint assessment that we have previously reported (Oddsson et al., 2020). Text boxes with dashed lines in Figure 2 indicate enrollment numbers for the initial 10-week report.

Of the 85 individuals who were assessed for eligibility, a total of 69 participants were enrolled in the trial (17 more than in previous report, cf. Figure 2). Sixty participants took part in the 2-week follow up visit, 55 in the 6-week follow-up visit, and 51 in the 10-week follow-up visit (six more than in the previous report). Eleven of the 18 participants who discontinued interventions through the 10-week follow-up visit stopped participation due to circumstances related to COVID-19 (cf. Figure 2). (Note: due to the timing of study initiation at Johns Hopkins and the onset of the pandemic, the three participants enrolled at the site were unable to complete most in-person assessments. When the situation did not improve, the sponsor decided to terminate Johns Hopkins as a research site. None of the participants had completed follow-up datasets through week 10, so their results were not included in this analysis.)

Forty-four of the 51 participants who reached the 10-week follow-up assessment were analyzed after their 26-week assessment. Due to circumstances related to COVID-19, including lockdowns at the research sites, 14 of the 44 participants were unable to participate in the 26-week in-person outcomes testing (FGA, 10MWT, TUG, and 4-Stage Balance Test). All 44 participants, however, were assessed on self-reported outcomes over the phone, and all 44 participants provided reports of falls throughout the 26-week period. Consequently, we report baseline assessment of clinical outcomes for all 44 participants, comparing those who reported falling in the prior 6 months (Pre-Fallers) to those who did not (Pre-Non-Fallers). We also present data over time for the 30 participants who completed the 26-week in-person assessment (weeks 0, 2, 6, 10, and 26, Figure 2), comparing the baseline assessments of those 30 participants to their 26-week assessment.

Seven of the 44 participants discontinued the intervention after the 10-week assessment (cf. Figure 2); one passed away; two had been enrolled in solely the 10-week protocol; three expressed device dissatisfaction, and one experienced an unrelated adverse event.

## Baseline characteristics and outcomes

Baseline characteristics for the 44 individuals who were evaluated at 26 weeks are shown in Table 1 and were substantially similar to those reported previously (Oddsson et al., 2020). Participants across all sites were pooled for the continued analysis.

The baseline characteristics of the 44 participants are shown in Table 2. Characteristics are also reported separately for participants who reported falling in the 6 months preceding the study (Pre-F, $n = 25$) and participants who did not report a fall (Pre-NF, $n = 19$). Overall, characteristics reported previously for participants entering the trial were maintained (Oddsson et al., 2020) except for some substantiated differences between the Pre-F and Pre-NF participants that were now statistically significant (Table 2). In addition to previous observations, baseline self-selected gait speed and ABC scores were significantly different between the Pre-F and Pre-NF groups, 0.83 vs. 0.97 m/s and 57.8 vs. 69.6%, respectively (Table 2). A higher observed mean value in PHQ-9 score for the Pre-F group was nearly statistically significant (5.7 vs. 3.3, respectively, $p = 0.052$). Mean values for all PROMIS measures were near 50, and any differences were well within 10 (Table 2) corresponding to one standard deviation for these measures, indicating any observed differences were minor (Askew et al., 2016; Hahn et al., 2016a,b).

## Clinical outcomes

Improvements in FGA scores were seen across all individuals, irrespective of their baseline FGA scores and appeared similar after 2-, 6-, 10-, and 26-weeks of device use (Figure 3). This finding is indicated by the regression lines in Figure 3 being shifted above the line of equality. Figure 3 also illustrates similar improvements in FGA scores for the Pre-F group and the Pre-NF group (filled vs. open symbols). Interestingly, the slope of the regression line was

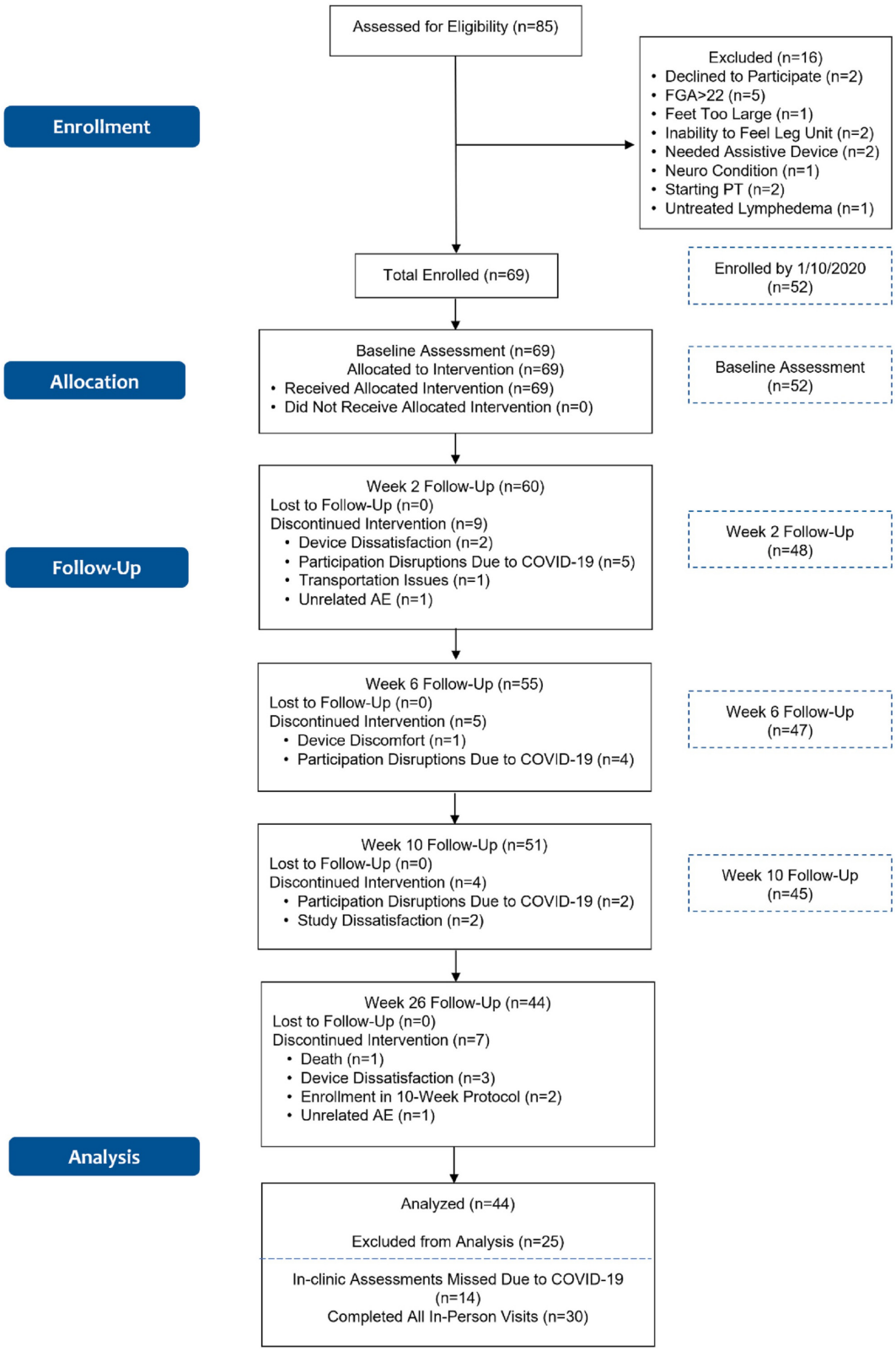


FIGURE 2
Flowchart of the walk2Wellness trial. Data for the primary endpoint at 10 weeks, shown to the right for reference, were published earlier (Oddsson et al., 2020). Due to COVID-19 site lockdowns, in-person outcomes could be assessed from 30 of the 44 participants. Self-reported outcomes and fall events were assessed from all 44 participants who reached the 26-week follow-up visit.

TABLE 2 Baseline characteristics for participants reaching the 26-week assessment ($n$ = 44).

| Baseline assessment | All $n$ = 44 | Pre-F $n$ = 25 | Pre-NF $n$ = 19 | $P$-level |
|---|---|---|---|---|
| Gender Female (n) | 8 of 44 (18%) | 8 of 25 (32%) | 0 of 19 (0%) | <**0.01** |
| Use of Assistive Device (n) | 25 of 44 (57%) | 18 of 25 (72%) | 7 of 19 (37%) | **0.02** |
| Gait speed self-selected < 0.7 m/s (n) | 8 of 44 (18%) | 7 of 25 (28%) | 1 of 19 (5%) | 0.052 |
| Timed Up and Go > 12 s (n) | 21 of 44 (48%) | 16 of 25 (64%) | 5 of 19 (26%) | **0.013** |
| 4-Stage Balance Test < 30 s (n) | 28 of 44 (64%) | 18 of 25 (72%) | 10 of 19 (53%) | 0.19 |
| ABC Score < 67% (n) | 24 of 44 (54%) | 17 of 25 (68%) | 7 of 19 (37%) | **0.04** |
| Fallen in Last 6 months (n) | 25 of 44 (57%) | 25 of 25 (100%) | 0 of 19 (0%) | n/a |
| Fallen in Last 12 months (n) | 31 of 44 (70%) | 25 of 25 (100%) | 6 of 19 (32%) | <**0.0001** |
| Number of Falls 6 months | 53 | 53 | 0 | n/a |
| Number of Falls 12 months | 106 | 97 of 106 (92%) | 9 of 106 (8%) | <**0.0001** |
| | **Mean (SD) $n$ = 44** | **Mean (SD) $n$ = 25** | **Mean (SD) $n$ = 19** | ***P*-level** |
| Age (yrs) | 73.5 (7.8) | 73.1 (7.9) | 73.9 (7.8) | 0.72 |
| Height (m) | 1.76 (0.09) | 1.76 (0.12) | 1.76 (0.05) | 0.96 |
| Weight (kg) | 90.7 (15.3) | 89.7 (17.3) | 92.0 (12.5) | 0.63 |
| BMI (kg/m$^2$) | 29.4 (4.6) | 29.0 (5.2) | 29.8 (3.8) | 0.59 |
| FGA score | 15.3 (3.7) | 13.9 (3.4) | 17.3 (3.3) | <**0.002** |
| Gait Speed, Self-selected (m/s) | 0.89 (0.23) | 0.83 (0.23) | 0.97 (0.21) | <**0.05** |
| Gait Speed, Fast (m/s) | 1.29 (0.36) | 1.13 (0.29) | 1.50 (0.35) | <**0.0005** |
| TUG (s) | 12.9 (4.0) | 14.1 (4.4) | 11.4 (2.8) | <**0.026** |
| 4-Stage Balance Test (s) | 25.9 (7.2) | 24.5 (6.3) | 27.8 (8.1) | 0.14 |
| Fall-Risk Factors* (n of 7) | 3.4 (1.5) | 4.3 (0.9) | 2.2 (1.1) | <**0.0001** |
| # Chronic Conditions | 8.3 (3.3) | 8.8 (3.3) | 7.5 (3.3) | 0.21 |
| ABC Score (%) | 62.9 (16.2) | 57.8 (14.0) | 69.6 (16.8) | <**0.02** |
| VADL Mean Score | 3.7 (1.1) | 4.1 (1.0) | 3.1 (0.8) | <**0.001** |
| VAS Pain Score (0–10) | 2.9 (2.1) | 3.0 (2.1) | 2.8 (2.2) | 0.89 |
| PHQ-9 | 4.7 (4.0) | 5.7 (4.5) | 3.3 (2.9) | 0.052 |
| Pain InterferencePROMIS® 6b | 50.9 (8.1) | 52.7 (7.7) | 48.5 (8.1) | 0.09 |
| Satisfaction Social RolesPROMIS® 8a | 49.8 (7.0) | 49.8 (6.7) | 49.9 (7.5) | 0.96 |
| Ability to ParticipatePROMIS® 8a | 49.9 (7.4) | 48.4 (6.7) | 51.8 (8.0) | 0.13 |

Participants who reported having fallen in the past 6 months (Pre-F) and those who did not (Pre-NF) are reported separately. Values represent Mean (Standard Deviation). Column $p$-level shows significance level for comparison between the Pre-F and Pre-NF groups. In bold if $p < 0.05$.
*Fall-risk factors assessed in the current study included, recent history of falls (Tinetti and Kumar, 2010), PN diagnosis (Richardson and Hurvitz, 1995), FGA score <23 (Wrisley and Kumar, 2010), TUG >12 s (CDC 2017), 4-Stage Balance Test <30 s (CDC, 2017), Gait Speed <0.7 m/s (Studenski et al., 2003, Montero-Odasso et al., 2005), and ABC score <67% (Lajoie and Gallagher, 2004).

<1 at all assessments indicating larger improvements in the lower range of baseline FGA scores mostly representing Pre-F group participants (cf. Table 3 and further below). After 26 weeks of device use, FGA scores increased across all individuals from 15.0 to 19.2 ($p < 0.00001$, Table 3) indicating a large effect size (Cohen's $d_{rm}$ = 1.38, Table 3). Furthermore, self-selected gait speed increased from 0.89 m/s to 0.97 m/s ($p$ = 0.02), a medium effect size (Cohen's $d_{rm}$ = 0.49); and the 4-Stage Balance Test improved from 25.6 s to 28.4 s ($p < 0.01$), representing a small effect size (Cohen's $d_{rm}$ = 0.32). An increase seen in fast gait speed (1.30 to 1.37 m/s) did not reach statistical significance ($p$ = 0.07). Changes in Rydel-Seiffer tuning fork testing scores suggested a small decrease in sensitivity at the site of the lateral malleolus (mean 3.8 to 3.2, $p$ = 0.032, Cohen's $d_{rm}$ = 0.32), while a noted decrease at the MTP joint did not reach statistical significance ($p$ = 0.07).

The Pre-F cohort ($n$ = 18) improved their mean FGA score from 13.7 at baseline to 18.1 at 26 weeks ($p < 0.0001$, Table 3) representing a large effect size (Cohen's $d_{rm}$ = 1.58). Their observed increases in self-selected gait speed from 0.82 to 0.90 m/s and from 1.13 to 1.23 m/s for fast gait speed did not reach statistical significance ($p$ = 0.12 and $p$ = 0.07, respectively). The Pre-F group showed a decrease in vibration sensation at the

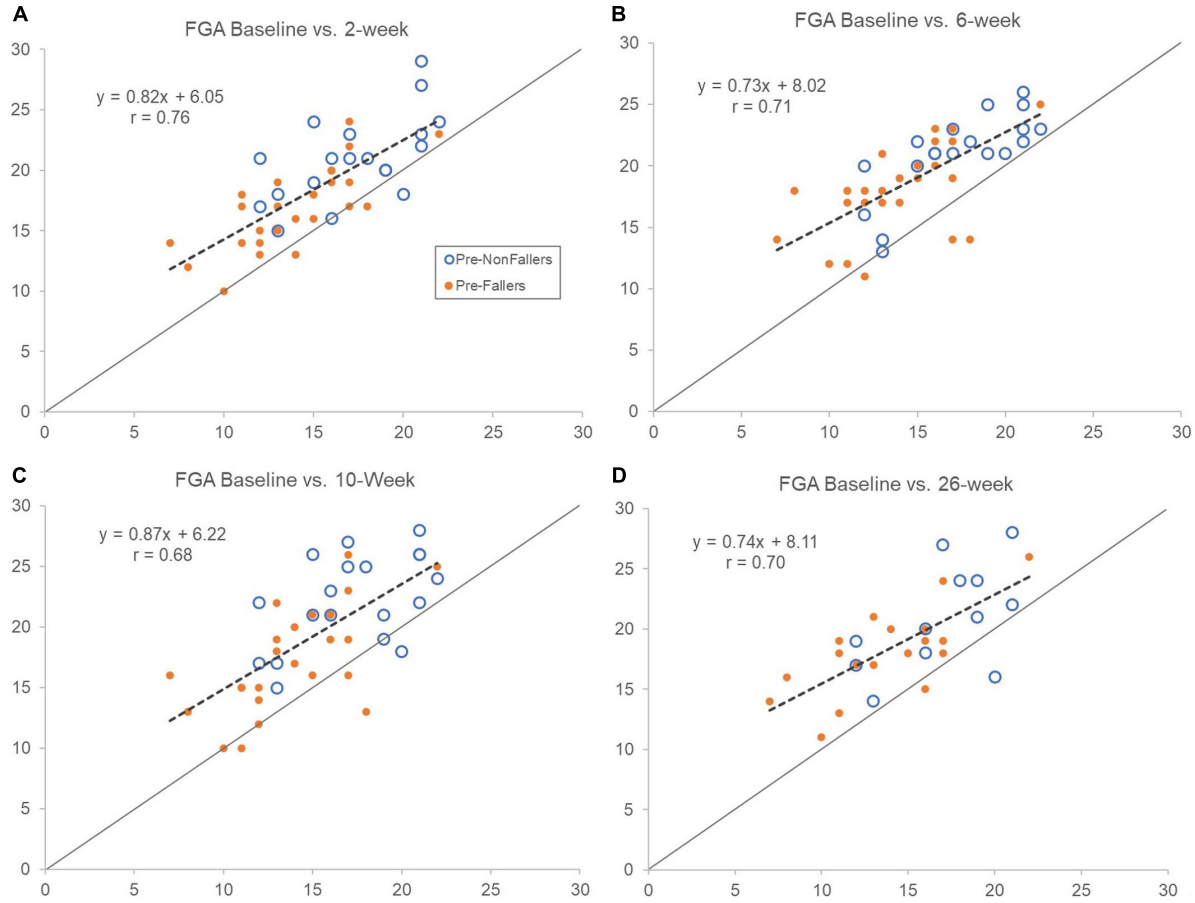


FIGURE 3
Comparing FGA scores at baseline (horizontal axes) with assessments after 2 **(A)**, 6 **(B)**, 10 **(C)**, and 26 **(D)** weeks (vertical axes) of device use. Open symbols represent pre-study non-fallers and filled ones are pre-study fallers. Dashed lines show regression line for the whole group. Forty-four participants completed in clinic outcomes testing up to 10 weeks (primary endpoint), and 30 participants completed the 26-week assessment in person. Scores above line of equality indicate improvements and below a decrement in FGA score compared to baseline scores. Notice that regression line slopes are less than 1 indicating slightly larger improvements in FGA score for those with lower baseline scores. Overall, improvements observed after 2 weeks of use appeared sustained throughout the 26 weeks of use.

first MTP joint (mean 2.9 to 1.8, $p < 0.005$) and at the lateral malleolus that did not reach statistical significance (mean 3.75 to 3.25, $p = 0.07$).

The Pre-NF group ($n = 12$) increased their mean FGA score from 17.0 at Baseline to 20.8 at 26 weeks ($p < 0.004$, Cohen's $d_{rm} = 1.23$, Table 3). A small increase in self-selected gait speed from 1.00 m/s to 1.08 m/s did not reach statistical significance ($p = 0.06$). Other clinical outcomes, including Rydel-Seiffer tuning fork sensitivity testing scores, remained unchanged at 26 weeks compared to baseline (Table 3).

The mean values of all in-person outcomes across the 26 weeks of device use for all participants are depicted in Figure 4 and are shown separately for the Pre-F and the Pre-NF groups. In general, the behavior over time of the two subgroups was qualitatively similar, although the Pre-NF group consistently performed better than the Pre-F cohort.

## Self-reported outcomes, device use and Activities-Specific Balance Confidence to Functional Gait Assessment ratios

Self-reported outcomes for the 44 participants who completed assessments through 26 weeks are shown in Table 4. Across all participants, a slight but statistically significant increase was seen in PROMIS Ability to Participate scores after 26 weeks of device use (49.9 to 52.6, $p < 0.05$). All baseline values were maintained at 26 weeks for the Pre-F group while we noted an increase in the PROMIS Satisfaction with Social Roles scores (49.9 to 55.5, $p < 0.006$) for the Pre-NF group.

Participants documented their weekly device use in a calendar and were asked to report it back during phone calls at weeks 14, 18, 22, and 26. Their average reported weekly

TABLE 3 Clinical outcomes and Rydel-Seiffer vibration sensation screening for the 30 individuals who were able to complete all in-person assessments at baseline and after 26 weeks as well as the subgroups of Pre-Fallers and Pre-Non-Fallers.

| **All** | **Baseline Mean (SD)** $n = 30$ | **26-week Mean (SD)** $n = 30$ | ***P*-level** | **Cohen's $d_{rm}$ (95% CI's)** |
|---|---|---|---|---|
| FGA score | 15.0 (3.9) | **19.2 (4.1)** *(0.69)* 0.036* | **<0.00001** | **1.38 (0.82–1.94)** |
| Gait speed self-selected (m/s) | 0.89 (0.26) | **0.97 (0.28)** *(0.80)* 0.040* | **0.02** | **0.49 (−0.03–1.0)** |
| Gait speed fast (m/s) | 1.30 (0.41) | 1.37 (0.42) | 0.07 | n/a |
| TUG (s) | 13.2 (4.6) | 12.3 (3.4) | 0.20 | n/a |
| 4-Stage Balance Test (s) | 25.6 (7.2) | **28.4 (5.7)** *(0.65)* 0.034* | **<0.01** | **0.47 (−0.05–0.98)** |
| R/L 1st MTP joint | 2.8 (2.5) | 2.2 (2.4) | 0.07 | n/a |
| R/L lateral malleolus | 3.8 (2.2) | **3.2 (2.1)** *(0.60)* 0.032* | **0.032** | **0.32 (−0.19–0.83)** |
| R/L patella | 4.0 (2.2) | 4.0 (1.8) | 0.91 | n/a |
| **Pre-Fallers** | **Baseline Mean (SD)** $n = 18$ | **26-week Mean (SD)** $n = 18$ | ***P*-level** | **Cohen's $d_{rm}$ (95% CI's)** |
| FGA score | 13.7 (3.7) | **18.1 (3.6)** *(0.72)* 0.037* | **<0.0001** | **1.58 (0.83–2.33)** |
| Gait speed self-selected (m/s) | 0.82 (0.25) | 0.90 (0.29) | 0.12 | n/a |
| Gait speed fast (m/s) | 1.13 (0.33) | 1.23 (0.37) | 0.07 | n/a |
| TUG (s) | 14.5 (5.0) | 13.0 (3.9) | 0.14 | n/a |
| 4-Stage Balance Test (s) | 23.5 (6.5) | **27.7 (5.4)** *(0.69)* 0.037* | **<0.02** | **0.82 (0.14–1.50)** |
| R/L 1st MTP joint | 2.9 (2.4) | **1.8 (2.0)** *(0.55)* 0.03* | **0.005** | **0.58 (−0.09–1.24)** |
| R/L lateral malleolus | 3.75 (2.1) | 3.25 (1.9) | 0.07 | n/a |
| R/L patella | 4.2 (2.0) | 4.0 (1.9) | 0.64 | n/a |
| **Pre-Non-Fallers** | **Baseline Mean (SD)** $n = 12$ | **26-week Mean (SD)** $n = 12$ | ***P*-level** | **Cohen's $d_{rm}$ (95% CI's)** |
| FGA score | 17.0 (3.3) | **20.8 (4.3)** *(0.56)* 0.031* | **0.004** | **1.23 (0.36–2.10)** |
| Gait speed self-selected (m/s) | 1.00 (0.25) | 1.08 (0.25) | 0.06 | n/a |
| Gait speed fast (m/s) | 1.56 (0.40) | 1.59 (0.42) | 0.63 | n/a |
| TUG (s) | 11.1 (3.1) | 11.3 (2.5) | 0.78 | n/a |
| 4-Stage Balance Test (s) | 28.7 (7.4) | 29.5 (6.1) | 0.65 | n/a |
| R/L 1st MTP joint | 2.5 (2.8) | 2.7 (2.8) | 0.76 | n/a |
| R/L lateral malleolus | 3.7 (2.5) | 3.1 (2.4) | 0.21 | n/a |
| R/L patella | 3.7 (2.5) | 4.0 (1.7) | 0.68 | n/a |

Statistical significance is indicated in bold. Bonferroni's adjustment of significance levels for correlated measures was applied (Uitenbroek, 1997).
Values in (italics) indicate Pearson's correlation coefficient followed by the adjusted significance level required for an overall significance of 0.05 as marked with *.
Cohen's $d_{rm}$ indicates effect size for change between baseline and 26 weeks where 0.2 is represents a small effect, 0.5 a medium effect, and 0.8 a large effect. Values in parenthesis show 95% confidence interval. "Estimate the effect size for single-group pretest-posttest designs" (Morris and DeShon, 2002).

device use was 5.1 ± 0.4 days. Participants reporting that they used the device weekly either "Every Day" or "At least 5 Days" was 71.8 ± 10.5%. An average of 94.8 ± 3.3% of reporting participants stated they used the device "1–2 Days" or more per week.

We also sought to monitor participants' self-perceived balance confidence (Lajoie and Gallagher, 2004) in relation to their assessed gait function performance over time in the trial by measuring changes in the ABC to FGA ratio, which is shown in **Figure 5**. **Figure 5** illustrates two observations. First, the ABC/FGA ratio for the overly confident individuals gradually decreased from high values at baseline to week 6 when the ratio essentially aligned with the low self-confidence participants, who maintained a consistent

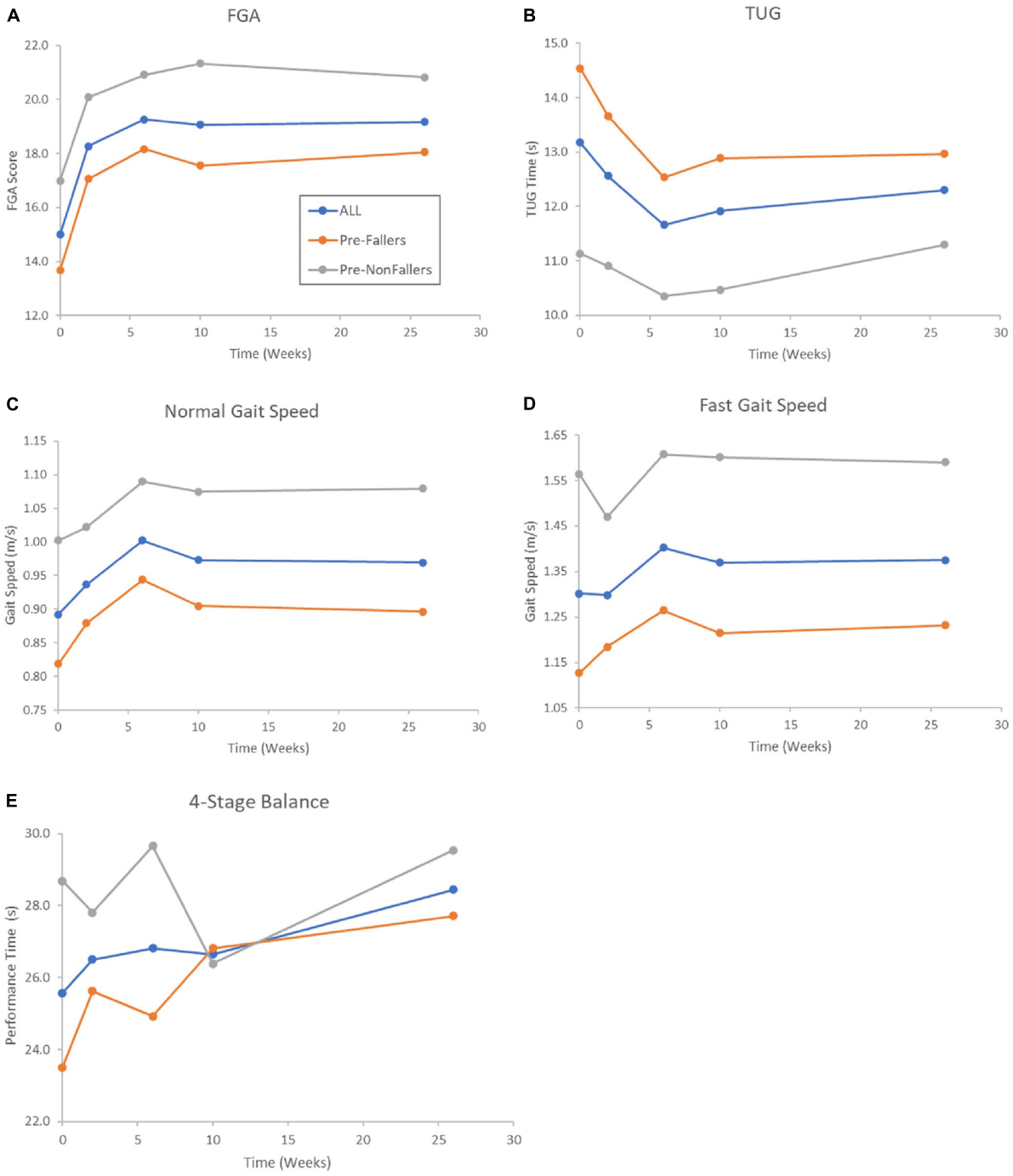


FIGURE 4
Averages of clinical outcomes across the in-person assessments at baseline (0), 2, 6, 10, and 26 weeks for the 30 participants (blue lines and symbols) who were able to be tested in clinic after 26 weeks of device use for the in-clinic outcomes FGA **(A)**, TUG **(B)**, gait speed **(C,D)**, and the Four-Stage Balance test **(E)**. Gray symbols and lines represent the pre-study non-fallers ($n$ = 12 of 30) and orange lines and symbols represent pre-study fallers ($n$ = 18 of 30). Notice that most improvements appeared to peak after 6–10 weeks of device use, followed by a leveling off until 26 weeks.

ABC/FGA ratio of about 3.5 throughout the 26-week period of the trial (Figure 5, left $y$-axis). Second, both groups of participants increased their FGA scores in a similar fashion although the overly confident participants showed higher levels of improvement in their FGA scores (Figure 5, right $y$-axis).

TABLE 4 Results from self-reported outcomes for the 44 individuals who completed all assessments.

| | Baseline Mean (SD) $n = 44$ | 26-week Mean (SD) $n = 44$ | *P*-level |
|---|---|---|---|
| ABC-Score (%) | 62.9 (16.2) | 65.1 (14.8) | 0.39 |
| VADL Mean Score | 3.70 (1.06) | 3.46 (1.10) | 0.08 |
| VAS Score (0–10) | 2.9 (2.1) | 2.8 (2.2) | 0.66 |
| PHQ-9 | 4.7 (4.0) | 3.9 (4.2) | 0.14 |
| Pain Interference PROMIS® 6b | 50.9 (8.1) | 51.1 (8.3) | 0.88 |
| Satisfaction Social Roles PROMIS® 8a | 49.8 (7.0) | 51.9 (8.3) | 0.075 |
| Ability to Participate PROMIS® 8a | 49.9 (7.4) | 52.6 (8.6) | **<0.05** |
| **Pre-Fallers ($n = 25$)** | **Baseline Mean (SD)** | **26-week Mean (SD)** | |
| ABC-Score (%) | 57.8 (13.9) | 59.1 (14.4) | 0.73 |
| VADL Mean Score | 4.14 (1.00) | 3.95 (0.79) | 0.32 |
| VAS Score (0–10) | 3.0 (2.1) | 3.1 (1.9) | 0.76 |
| PHQ-9 | 5.7 (4.7) | 5.1 (4.5) | 0.22 |
| Pain Interference PROMIS® 6b | 52.7 (8.0) | 52.9 (7.3) | 0.86 |
| Satisfaction Social Roles PROMIS® 8a | 49.8 (6.7) | 49.2 (6.8) | 0.69 |
| Ability to Participate PROMIS® 8a | 48.4 (7.1) | 50.0 (7.1) | 0.12 |
| **Pre-Non-Fallers ($n = 19$)** | **Baseline Mean (SD)** | **26-week Mean (SD)** | |
| ABC-Score (%) | 69.6 (17.1) | 73.0 (12.0) | 0.34 |
| VADL Mean Score | 3.11 (0.86) | 2.81 (1.00) | 0.14 |
| VAS Score (0–10) | 2.8 (2.2) | 2.4 (2.4) | 0.35 |
| PHQ-9 | 3.3 (2.9) | 2.4 (3.5) | 0.35 |
| Pain Interference PROMIS® 6b | 48.5 (8.1) | 48.6 (9.2) | 0.96 |
| Satisfaction Social Roles PROMIS® 8a | 49.9 (7.7) | 55.5 (8.9) | **0.006** |
| Ability to Participate PROMIS® 8a | 51.8 (7.5) | 55.0 (10.0) | 0.18 |

Data are shown separately for the group as a whole and for Pre-Fallers (having reported fallen in the prior 6 months) and Pre-Non-Fallers (no falls reported in the prior 6 months). Statistical significance is indicated in bold.

## Falls data

Falls were reported throughout the 26-week period for all 44 participants and separately for the Pre-F ($n = 25$) and Pre-NF ($n = 19$) groups, respectively (Table 5). The 44 participants reported a total of 53 falls over 6 months prior to participating in the trial while 39 falls were documented during the 26 weeks of the trial, corresponding to a pre-study mean fall rate of 6.7 falls/1000 patient days (median = 5.6 falls/1000 patient days) and a post study mean fall rate of 4.8 falls/1000 patient days (median = 0 falls/1000 patient days). Wilcoxon Signed Rank test was used to compare pre- and post-study fall rates since the data was not normally distributed (Shapiro–Wilk W-statistic = 0.82, $p < 0.0001$). Across all participants, the median of the post-study fall rate was lower than the pre-study fall rate (Table 5, $p = 0.044$), reflecting a 28% decrease in fall rate. Of the 44 participants, 25 had fallen in the past 6 months (Pre-F); and after 26 weeks, 20 of the 44 participants had reported falling. Overall, 31 of the 39 falls required no treatment while eight falls (∼20%) required treatment with four of those (∼10%) causing severe injury (two fractures) (Table 5).

The 25 participants in the Pre-F cohort reported 31 falls after 26 weeks compared to 53 falls pre-study corresponding to a pre-study fall rate of 11.8 falls/1000 patient days (median = 11.1), which decreased to 6.7 falls/1000 patient days at 26 weeks (median = 5.0), a 43% decrease in fall rate ($p = 0.0043$). Of the 25 Pre-F participants, 12 did not fall during the study, a 48% statistically significant decrease in number of fallers ($p < 0.0001$). Of the 31 falls experienced by the Pre-F cohort, six (∼20%) required treatment (Table 5). Three of the four falls that led to severe injury occurred in the Pre-F group.

Seven of the 19 Pre-NF participants reported falling during the trial ($p < 0.0001$) leading to an increase in fall rate from zero to 2.3 falls/1000 patient days at 26 weeks that was statistically significant ($p = 0.023$). A total of eight falls were reported by the seven Pre-NF participants who fell during the study (Table 5). Two of the eight falls in the group required treatment, and one fall led to a severe injury.

Figure 6 illustrates the cumulative sum of falls based on 6-month pre-study fall reports from the participants and the falls recorded by the Pre-F cohort during the trial. The 53 pre-study falls were randomly distributed across the 6-month pre-study period for illustration purposes since the exact time of their occurrence prior to the trial were not known (see Figure 6). Note that 53 falls over a period of 6 months corresponds to 53/180∼0.29 falls/day, which becomes the slope

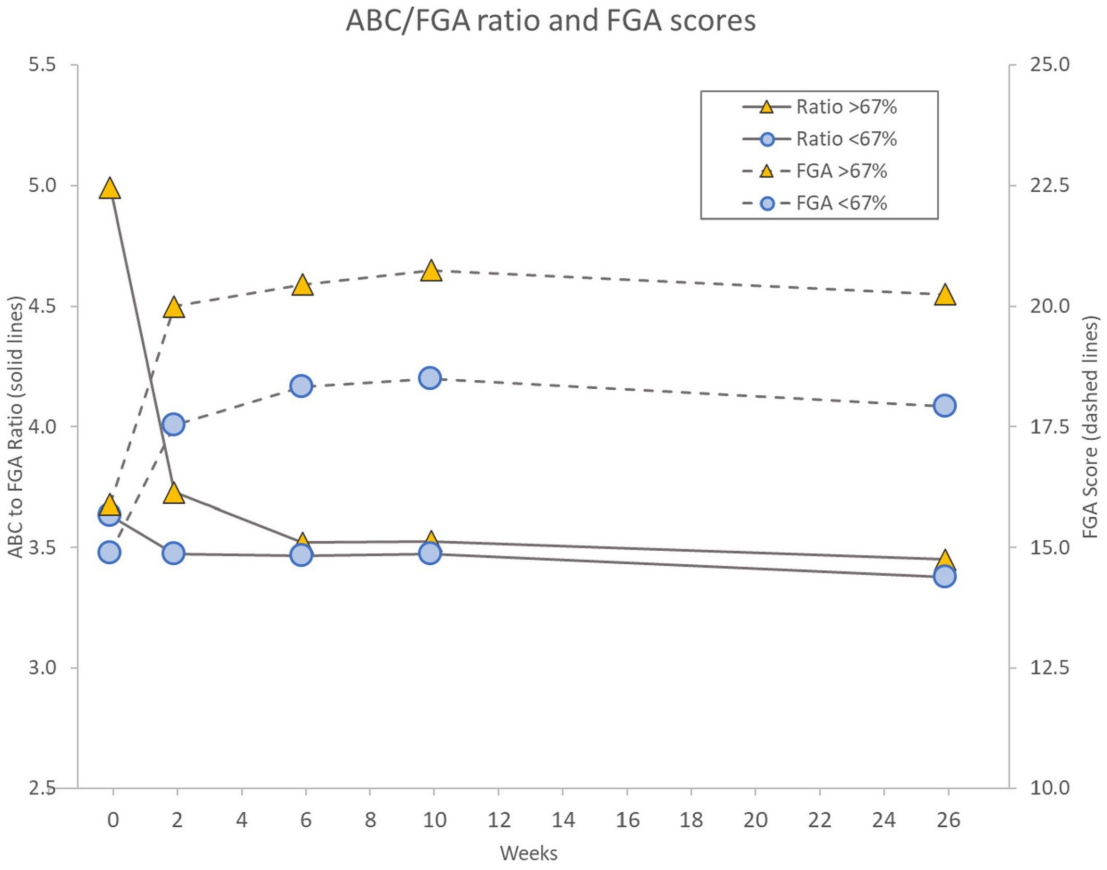


FIGURE 5
Illustration of changes in ABC/FGA ratio (left axis, solid lines), an indication of self-perceived balance confidence in relation to externally observed gait function performance, for participants with baseline ABC scores >67% (high balance-confidence, orange triangles) and those with ABC scores ≤67% (low balance-confidence, blue circles). The associated changes over time in FGA scores for the same two groups are also shown (right axis, dashed lines). Notice that both groups had similar FGA scores at baseline, which led to a dramatic difference in the ABC/FGA ratio at baseline. Over time, the ratio for the high balance-confidence participants gradually decreased and aligned with the low balance-confidence group at ~3.5, mainly due to an increased FGA score and a maintained ABC score. The low balance-confidence group maintained an ABC/FGA ratio ~3.5 throughout the 26 weeks associated with a proportional increase in ABC and FGA scores. Interestingly, the high balance-confidence group showed a higher increase in FGA score than the low balance-confidence group.

of the blue regression line in Figure 6, irrespective of the random distribution of the pre-study falls. The in-study falls are presented on the day they occurred according to participant reports (see Figure 6). The in-study decrease of falls quantified in Table 5 can be visually observed over time in Figure 6 as a lower slope for the best fit regression line (blue-dotted line pre-study vs. orange-dotted line in-study). It should be noted that the lower slope appears to begin after about 20 days of device use.

# Discussion

## Key findings

Walkasins is a device that provides mechanical tactile stimuli related to foot pressure for individuals with PN and gait and balance problems. Overall, our findings from analyzing outcomes after 26 weeks of wearing Walkasins show that improvements in outcomes seen after 10 weeks of use are sustained longer term (Oddsson et al., 2020). The FGA score was improved from 15.0 at baseline to 19.2 after 26 weeks of device use across all participants, a large effect size (Cohen's $d_{rm}$ = 1.38) and an increase that is beyond the MCID for community-dwelling older adults (Beninato et al., 2014). Interestingly, these improvements were seen for individuals across the full range of baseline FGA scores, from the lowest of 7 to the highest of 22 (Figure 3) with a tendency to be higher for individuals in the lower FGA range. Furthermore, similar improvements were seen for the Pre-F and Pre-NF cohorts. Moreover, the 46% decrease in fall rate, compared to pre-study falls we reported after 10 weeks of use, was sustained at 43% after 26 weeks, an important observation further discussed below. Weekly device use continued to be high and similar to after

TABLE 5 Parameters related to falls and fall risk assessed at baseline and at 26 weeks.

| All ($n$ = 44) | Baseline (SD) | 26-weeks (SD) | $P$-level |
|---|---|---|---|
| #Falls (pre-6 mo and in study, $n$ = 44) | 53 | 39 | n/a |
| Fall Rate Mean (#/1000 patient days, pre-6 mo and in study, $n$ = 44) | 6.7 (7.7) | 4.8 (7.7) | n/a |
| Fall Rate Median | 5.6 | 0 | **0.044*** |
| #Fallers (pre-6 mo and at 6 mo in study, $n$ = 44) | 25 | 20 | 0.28 |
| Falls no treatment sought | n/a | 31 | n/a |
| Falls treatment sought + severe injury | n/a | 4 + 4 | n/a |
| **Pre-Fallers ($n$ = 25)** | | | |
| #Falls (pre-6 mo and in study, $n$ = 25) | 53 | 31 | n/a |
| Fall Rate Mean (#/1000 patient days, pre-6 mo and in study, $n$ = 25) | 11.8 (6.7) | 6.7 (9.5) | n/a |
| Fall Rate Median | 11.1 | 5.0 | **0.0043*** |
| #Fallers (pre-6 mo and at 6 mo in study, $n$ = 25) | 25 | 13 | **<0.0001** |
| Falls no treatment sought | n/a | 25 | n/a |
| Falls treatment sought + severe injury | n/a | 3 + 3 | n/a |
| **Pre-Non-Fallers ($n$ = 19)** | | | |
| #Falls (pre-6 mo and in study, $n$ = 19) | 0 | 8 | n/a |
| Fall Rate Mean (#/1000 patient days, pre-6 mo and in study, $n$ = 19) | 0 (0) | 2.3 (3.3) | n/a |
| Fall Rate Median | 0 | 0 | **0.023*** |
| #Fallers (pre-6 mo and at 6 mo in study, $n$ = 19) | 0 | 7 | **<0.0001** |
| Falls no treatment sought | n/a | 6 | n/a |
| Falls treatment sought + severe injury | n/a | 1 + 1 | n/a |

All 44 individuals were asked to report their falls throughout the 26-week period. Data for Pre-Fallers and Pre-Non-Fallers are presented separately. Fall rates are reported in number of falls per 1000 patient days. Fall rates were not normally distributed (Shapiro–Wilk W-stat = 0.82). Statistical significance is indicated in bold.
*Wilcoxon Signed Rank test.

10 weeks [5.1 vs. 5.3 days/week, respectively (Oddsson et al., 2020)].

All clinical outcomes improved compared to baseline, but changes in TUG and 4-Stage Balance did not reach statistical significance, likely because the study was underpowered for these measures (**Table 3**). Previous work has found that older individuals with diabetic PN walk slower than their healthy cohort (Menz et al., 2004), and older adults with peripheral sensory impairment show a 0.046 m/s/year. decline in gait speed (Lipsitz et al., 2018). This decrease is more than 3.5 times higher than declines reported in healthy aging (Buracchio et al., 2010). With this in mind, it is encouraging to report a statistically significant 0.08 m/s increase in gait speed gait speed across all participants in this trial, which is beyond a small meaningful change (0.05 m/s) and close to the range for a substantial meaningful change (0.10 m/s) (Perera et al., 2006).

Participants had a decrease in sensitivity to vibration, an overall small decrease at the lateral malleolus and a larger change at the MTP joint in the Pre-Faller group. Such changes did not occur at the 10-week assessment (Oddsson et al., 2020). This decrease in tuning fork vibration perception may indicate a progression of some participants' PN and increased sensory loss. In spite of such changes, none of the participants stated that they were unable to perceive the stimuli provided by the device, suggesting they were still benefiting from its use as confirmed by improved clinical outcomes.

## Balance exercise interventions and their limitations

We can compare our findings to interventions using different forms of physical exercise in patients with PN since we see similar improvements in outcomes from simply wearing the device in the current trial. Several review studies conclude that balance training is "the most effective exercise intervention" (Ites et al., 2011; Tofthagen et al., 2012; Streckmann et al., 2014; Morrison et al., 2018), while strength training in patients with PN appears to have less impact on balance (Streckmann et al., 2014), likely due to lack of specificity related to balance (Oddsson et al., 2007). Although such interventions may improve balance outcomes, effects are essentially compensatory since they do not address the root cause of the balance problem in these patients, namely their impaire plantar sensation due to PN. Furthermore, any exercise activity would need to be maintained on a regular basis long-term or benefits would gradually be lost (Melzer and Oddsson, 2013; van Waart et al., 2015), sometimes referred to as the "use it or lose it" principle (Hart, 2021). This phenomenon is problematic because individuals sometimes become sedentary due to health problems unrelated to their balance disorders. Likewise, long-term compliance with exercise interventions is a challenge, due to motivation or lack of safety resources, and has been reported to be low with over half of

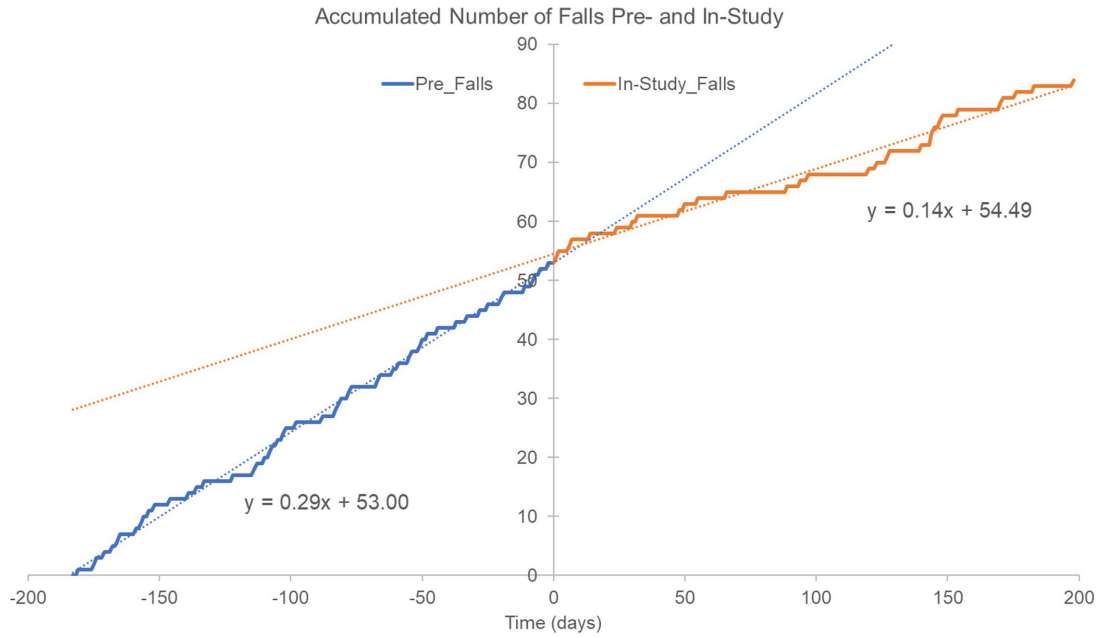


FIGURE 6
Accumulated number of falls reported 6 months pre-study (blue trace and blue dotted linear regression line) and falls documented in-study (orange trace and orange dotted regression line). The 53 falls reported for the prior 6 months would correspond to 0.29 falls/day (53/180∼0.29), which represents the slope of the regression line (blue trace). For illustration purposes, the 53 pre-study falls were randomly distributed across the 6 months since their exact time occurrence was unknown. In-study falls are shown as they occurred and were reported by participants throughout the 26 weeks. Notice how the rate of in-study falls appear to begin deviating from pre-study fall rate (slope of dotted blue line) after approximately 20 days of device use.

participants not being compliant (Sluijs et al., 1993; Alexandre et al., 2002), likely causing diminished benefits of the exercise intervention.

The improvements in clinical outcomes we report here, were gained from a single, brief training visit on the use of the sensory prosthesis and then simply by wearing it during regular daily activities. By comparison, obtaining the benefits of an exercise intervention requires the presence and engagement by the individual, at multiple therapy visits. In fact, during the first 10 weeks of use, we prohibited participants from joining in any physical therapy or balance-related exercise activities to help isolate the effect of the device (Oddsson et al., 2020). Knowing, however, that such interventions may help improve gait and balance function (Tofthagen et al., 2012), we felt it would potentially be unsafe and even unethical to prevent participation in those activities beyond the primary endpoint at 10 weeks. Therefore, to control for any potential effects of balance exercise interventions, we asked participants during follow-up phone calls at 14, 18, and 22 weeks whether they had begun any physical therapy for their balance problems. Not surprisingly, only one of the participants answered "yes" to this question, emphasizing the above-mentioned challenge with exercise engagement and compliance, and further suggesting that long-term use of the device was the main cause of improvement in clinical outcomes.

## Exercise dosing vs. daily exposure from sensory prosthesis use

These improvements in clinical outcomes are not surprising, because the mechanisms for improved gait and balance function from exercise interventions are different than the mechanisms for using a sensory prosthesis to replace an important component of somatosensory balance input, in this case from plantar mechanoreceptors (Meyer et al., 2004; Fallon et al., 2005; Strzalkowski et al., 2018). Traditional balance training activities stimulate existing sensorimotor integration by challenging function with a series of successively more difficult motor tasks. This kind of treatment gradually improves performance based on important and well-known principles of training (Oddsson et al., 2007). Such improvement may be attributable to training of specific splinter skills, and some improvement may be due to compensation for balance issues from impaired plantar sensation or address other balance related impairments. Users of a sensory prosthesis, however, receive new functional sensory stimuli on a nearly continuous basis that are highly specific to standing and walking and replace their impaired sense; and these new stimuli provide sensory information that the central nervous system expects to receive from the lower extremities during such activities (Guertin, 2012, Clark, 2015).

To analyze this contrast further, a research trial of traditional balance training interventions typically provides one-hour balance stimulation sessions about 2–3 times per week for 3–6 months e.g., (Shumway-Cook et al., 1997; Wolf et al., 2001, 2003; Li et al., 2005; Li and Manor, 2010; Melzer and Oddsson, 2013; Manor et al., 2014). Assuming 100% compliance, this example would add up to an overall exercise dose of no more than 72 h of focused therapeutic balance stimulation over 6 months. In a typical therapy practice, the dose would more likely be in the range of 8–40 h, for several reasons (HS Cohen, EdD, OTR, and personal communication). Patients sometimes miss visits due to schedule conflicts. Patients may shorten the total length of treatment either because they feel they have reached their maximum level of improvement, because they dislike the hassle of taking the time for the therapy visit, or because they cannot get transportation. In addition, some insurance programs may refuse to pay for more than a limited number of therapy visits. In the case of exposure to balance stimuli from a sensory prosthesis, however, participants used the device on average 36.1 hours/week with more than 80% using the device over 21 hours/week (Oddsson et al., 2020). Over 26 weeks this use would amount to a range from around 500 to almost 1000 h of specific gait and balance stimulation, essentially an order of magnitude higher than a regular balance training intervention. Furthermore, the device is intended to be used on a continuous basis, which furthers compliance.

Participants may not be physically active and receiving sensory stimuli the entire abovementioned time. However, they will regularly experience hundreds of specific balance stimuli/hour that are intimately integrated into their regular standing and walking activities and that are likely to cause related changes in balance behavior. Over time, this feedback may lead to a reweighting of sensory information that is relevant for balance function (Sienko et al., 2018), as has previously been suggested in older adults performing in-home balance training using vibrotactile sensory stimulation related to trunk tilt (Noohi et al., 2017, Bao et al., 2018) further supported by recently reported changes in brain connectivity (Bao et al., 2022).

Interestingly, early observations from a pilot study of five participants in the cohort studied here, who completed 26 weeks of device use, show neuroplastic changes in brain network connectivity related to postural control and balance that were associated with improved changes in FGA scores (Hsu et al., 2021). This finding indicates a direct effect of the sensory prosthesis initiating plastic changes related to sensorimotor interaction and postural control. Related observations on sensorimotor neuroplastic effects associated with peripheral afferent activity have previously been made following a novel amputation strategy, agonist-antagonist myoneural interface, intended to maintain neuromuscular communication of the lost limb (Srinivasan et al., 2020). This surgical technique intends to promote proprioceptive feedback and cause central sensorimotor plastic changes that may facilitate control of a prosthetic limb (Srinivasan et al., 2020). In a similar fashion, the current non-invasive sensory prosthesis is externally providing new tactile afference, essentially through the same dermatomes that signaled plantar pressure information from mechanoreceptors that have become functionally deafferented due to PN (Fallon et al., 2005; Koehler-McNicholas et al., 2019; Vaughan, 2021).

## Activities-Specific Balance Confidence to Functional Gait Assessment ratios, self-reported outcomes and device use

On the ABC, 67% is the cut-off for high-fall risk (Lajoie and Gallagher, 2004). We previously reported that participants with baseline ABC scores below 67% improved their ABC scores after 10 weeks of device use, whereas those with ABC scores above 67% did not, although both subgroups improved their FGA scores (Oddsson et al., 2020). Since these two categories of participants had similar baseline FGA scores, the discrepancy in ABC scores led to seemingly high baseline ABC to FGA ratios in participants with high ABC scores (Oddsson et al., 2020; **Figure 5**). We posited that the high ratio individuals were either too confident or simply unaware of their abilities or impairments, a construct that aligns with a proposed multifactorial causation model for falls emphasizing the importance of a "Realistic Appraisal of One's Own Abilities," (Hadjistavropoulos et al., 2011). This ratio indicates the degree of internal self-perception of balance capability (ABC score) in relation to the externally observed performance (FGA score). A ratio of around 3.3 would be "expected" based on the maximum scores of the two scales (Powell and Myers, 1995; Wrisley and Kumar, 2010). We further noted that after 6 weeks of device use, the overly confident participants appeared to "normalize" their self-perception indicating a more sensible perception of their abilities and align with those holding more expected ABC scores. This change was mainly due to an improvement in their FGA score and a maintained or slightly decreased ABC score.

We show here that this observation is preserved after 26 weeks of device use (**Figure 5**). This change in self confidence is important because it is a fall risk factor. As the data suggest, this ratio is a modifiable risk factor. Therefore, the ratio can and should be measured during studies of balance interventions.

Furthermore, the current data (**Figure 5**) shows that the high-confidence individuals appear to improve their FGA scores more than the low confidence individuals. Since the baseline FGA scores were similar for these two cohorts, it could be argued that the higher balance-confidence individuals may have "naturally" challenged themselves more in their daily activities, triggering a larger increase in FGA score. Correspondingly, the low balance-confidence individuals may have "explored" their improved functional capabilities less than

the high confidence group leading to a less beneficial long-term effect. We postulate that individuals with low balance confidence would potentially benefit further from a guided interaction through physical and occupational therapy or other means, whereas the high confidence individuals may be better at "self-coaching" their renewed capabilities. Consequently, to provide an individually optimized rehabilitation strategy for falls prevention and self-efficacy, clinicians should monitor outcomes that measure both balance outcomes and balance confidence, similar to the ratio we have used to allow this construct to be investigated further (Hadjistavropoulos et al., 2011; Soh et al., 2021).

Additional observations related to self-report measures were in line with our previous reporting (Oddsson et al., 2020), overall showing marginal changes and likely being less clinically meaningful since they were near the average for the United States population (Hahn et al., 2014, 2016a,b; Askew et al., 2016). The continued high level of device use, on average more than five days a week and over 70% of participants using it every day or at least 5 days/week, suggests that users find the device helpful.

## Falls data

One of the most important findings from this trial is the 43% decrease in fall rate for participants with a fall history following 26 weeks of device use. We reported an encouraging 46% decrease already after 10 weeks of use (Oddsson et al., 2020), while appreciating that 10 weeks would not be long enough for a relevant comparison to 6 months of pre-study data. Consequently, the similar statistically significant decrease after 26 weeks provides a stronger argument for a meaningful decrease in falls. In fact, even including the pre-study non-fallers in the analysis showed a statistically significant decrease in fall rate over 26 weeks of device use (**Table 5**). Note, however, that fall rate was not a primary outcome in this trial. In fact, we gathered pre-study falls data primarily as a means of describing our study population, and we monitored falls during the trial as a subset of adverse events. Thus, we enrolled participants at high fall risk based on their FGA scores, irrespective of whether or not they had fallen in the past. This procedure allowed us to conduct a *post hoc* analysis and investigate any trend in the data indicating an actual decrease in falls. A comparison with historical self-report data on prior falls is far from ideal as compared to a parallel arm trial with a control group, but other investigators have found that asking individuals to recall past falls commonly leads to an underreporting of actual falls; even recalling the past 3 months led to 25% underreporting of falls (Hannan et al., 2010), and 26% did not recall falls over the prior 6 months (Cummings et al., 1988). Under such circumstances, the 43% decrease in falls reported here probably underestimates the real effect.

The data on injuries reported from falls are probably clinically significant. Previous studies have reported that 65% of older individuals with PN fell over a year with 30% reporting an injury from a fall (DeMott et al., 2007). In a study of individuals with chemotherapy-induced PN, over 40% experienced falls, and more than 40% of falls resulted in an injury, two-thirds of which were fractures (Komatsu et al., 2019). In this study, 57% of participants reported falling in the 6 months prior to the study while 45% fell during 6 months of the trial. Eight of the 39 falls during the trial (20.5%) led to injuries where treatment was sought, half of those considered serious. Consequently, both fall rate and injury rate are lower than previous reports of similar populations (DeMott et al., 2007; Komatsu et al., 2019).

## Study limitations

This study had several limitations, as we have previously outlined (Oddsson et al., 2020). It was an unblinded, single arm trial. Our decision to conduct a single arm trial was based on several factors, including previous results from an in-clinic randomized control cross-over trial showing improved FGA scores when using the device turned on compared to off (Koehler-McNicholas et al., 2019). In addition, data from the first-in-human, long-term use study showed remarkable improvements in clinical outcomes in a patient with PN using the device for a year following over 5 months of balance physical therapy with limited improvement (Wrisley et al., 2021). This individual continues to use the device daily, now for more than 4 years. Also, blinding participants in a study using this kind of intervention is challenging and may not really be viable unless some form of deception is used since a requirement to use the device is perceiving the tactile stimuli (Oddsson et al., 2020). Consequently, the most feasible placebo treatment for a control group would likely be wearing a device that is non-functional.

An important strength of the trial includes our real-life inclusion/exclusion criteria that were essentially the same as the requirements for receiving a prescription for the device outside of a research trial. These requirements are aligned with the intentions of a pragmatic clinical trial to advance applicability of study findings (Patsopoulos, 2011; Gill et al., 2021). Only 7% of participants (6 of 85) were excluded due to medical circumstances (**Figure 2**). We wanted the participants to realistically reflect patients who are seen in the clinic, and we did not try to screen for only those individuals who may be the best responders. In fact, our participants reported an average of 8.3 chronic conditions, had multiple fall-risk factors, and polypharmacy (mean of 8 subscription medications).

Furthermore, the long-term benefits we report here combined with the participants not receiving any useful

feedback about their outcomes or systematic encouragement during their assessments and knowing that only one individual decided to participate in a balance therapy intervention, decreases the chance these effects are placebo (Finniss et al., 2010; Enck et al., 2013; Coste and Montel, 2017). A review of placebo effects in 47 randomized control trials found large effects in subjective outcomes and small effects in clinical outcomes, which is the opposite of what we report here, further suggesting effects are due to device use. Additional strengths include our use of recommended standardized objective clinical outcome measures (Moore et al., 2018) and the involvement of multiple clinical sites from different regions using different evaluators.

## Conclusion

Patients with peripheral neuropathy who have gait and balance problems with a high risk of falls including a history of falls improve their walking balance and decrease their fall rates from long-term use of a wearable non-invasive sensory prosthesis.

## Data availability statement

The original contributions presented in this 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 Advarra IRB (formerly Quorum Review IRB), serving as the Institutional Review Board (IRB) of record for three of the participating sites under the study protocol. The three sites include Baylor College of Medicine, Houston, TX; Hebrew SeniorLife, a Harvard Medical School Affiliate, Boston, MA; and M Health Fairview, Minneapolis, MN. The IRB Subcommittee, the Subcommittee on Research Safety, and the Research and Development Committee of the Minneapolis VA Health Care System (MVAHCS) also approved the trial. The study is registered on ClinicalTrials.gov (#NCT03538756). All necessary patient/participant consent has been obtained and the appropriate institutional forms have been archived. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

LO was involved in conception, study design, data analysis, database development, and wrote the first draft of the manuscript. TB, II, and PM performed data acquisition. HC was involved in design and data analysis. LJ was involved in training and monitoring, data analysis, and drafted early sections. DK was involved in design and data acquisition. LL and BM were involved in study design. YR developed the REDCap Cloud database, training, monitoring, data analysis and drafted the early sections. DW was involved in conception, design, and outcomes standardization training. SK-M was part of conception, design, data acquisition, and analysis. All authors contributed to data interpretation, critical review and manuscript revisions, read, and approved version to be submitted.

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## Conflict of interest

LO is an inventor of the technology, Co-Founder of RxFunction Inc., a shareholder in the company, serves on its Board of Directors, and is an employee of the company. LJ and YR are employees and shareholders of RxFunction Inc.

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.

## Publisher's note



## References

Alexandre, N. M., Nordin, M., Hiebert, R., and Campello, M. (2002). Predictors of compliance with short-term treatment among patients with back pain. *Rev. Panam. Salud. Publica.* 12, 86–94. doi: 10.1590/S1020-49892002000800003

Algina, J., Keselman, H. J., and Penfield, R. D. (2005). Effect sizes and their intervals: The two-level repeated measures case. *Educ. Psychol. Meas.* 65, 241–258. doi: 10.1177/0013164404268675

Askew, R. L., Cook, K. F., Revicki, D. A., Cella, D., and Amtmann, D. (2016). Evidence from diverse clinical populations supported clinical validity of PROMIS pain interference and pain behavior. *J. Clin. Epidemiol.* 73, 103–111. doi: 10.1016/j.jclinepi.2015.08.035

Bao, T., Carender, W. J., Kinnaird, C., Barone, V. J., Peethambaran, G., Whitney, S. L., et al. (2018). Effects of long-term balance training with vibrotactile sensory augmentation among community-dwelling healthy older adults: A randomized preliminary study. *J. Neuroeng. Rehabil.* 15:5. doi: 10.1186/s12984-017-0339-6

Bao, T., Noohi, F., Kinnaird, C., Carender, W. J., Barone, V. J., Peethambaran, G., et al. (2022). Retention effects of long-term balance training with vibrotactile sensory augmentation in healthy older adults. *Sensors (Basel)* 22:3014. doi: 10.3390/s22083014

Beninato, M., Fernandes, A., and Plummer, L. S. (2014). Minimal clinically important difference of the functional gait assessment in older adults. *Phys. Ther.* 94, 1594–1603. doi: 10.2522/ptj.20130596

Bergen, G., Stevens, M. R., and Burns, E. R. (2016). Falls and fall injuries among adults aged =65 years–United States, 2014. *MMWR Morb. Mortal Wkly. Rep.* 65, 993–998. doi: 10.15585/mmwr.mm6537a2

Buracchio, T., Dodge, H. H., Howieson, D., Wasserman, D., and Kaye, J. (2010). The trajectory of gait speed preceding mild cognitive impairment. *Arch. Neurol.* 67, 980–986. doi: 10.1001/archneurol.2010.159

Cavanagh, P. R., Derr, J. A., Ulbrecht, J. S., Maser, R. E., and Orchard, T. J. (1992). Problems with gait and posture in neuropathic patients with insulin-dependent diabetes mellitus. *Diabet. Med.* 9, 469–474. doi: 10.1111/j.1464-5491.1992.tb01819.x

CDC (2017). *STEADI–older adult fall prevention*. Available online at: https://www.cdc.gov/steadi/materials.html (accessed May 5, 2018)

CDC (2021). *Older adult fall prevention*. Available online at: https://www.cdc.gov/falls/facts.html (accessed November 1, 2021)

Cesari, M., Kritchevsky, S. B., Penninx, B. W., Nicklas, B. J., Simonsick, E. M., Newman, A. B., et al. (2005). Prognostic value of usual gait speed in well-functioning older people–results from the health, aging and body composition study. *J. Am. Geriatr. Soc.* 53, 1675–1680. doi: 10.1111/j.1532-5415.2005.53501.x

Clark, D. J. (2015). Automaticity of walking: Functional significance, mechanisms, measurement and rehabilitation strategies. *Front. Hum. Neurosci.* 9:246. doi: 10.3389/fnhum.2015.00246

Clark, D. J., Christou, E. A., Ring, S. A., Williamson, J. B., and Doty, L. (2014). Enhanced somatosensory feedback reduces prefrontal cortical activity during walking in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 69, 1422–1428. doi: 10.1093/gerona/glu125

Cohen, H. S., Kimball, K. T., and Adams, A. S. (2000). Application of the vestibular disorders activities of daily living scale. *Laryngoscope* 110, 1204–1209. doi: 10.1097/00005537-200007000-00026

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. New York, NY: Routledge Academic.

Cohen, M. A., Miller, J., Shi, X., Sandhu, J., and Lipsitz, L. A. (2015). Prevention program lowered the risk of falls and decreased claims for long-term services among elder participants. *Health Aff. (Millwood)* 34, 971–977. doi: 10.1377/hlthaff.2014.1172

Coste, J., and Montel, S. (2017). Placebo-related effects: A meta-narrative review of conceptualization, mechanisms and their relevance in rheumatology. *Rheumatology (Oxford)* 56, 334–343. doi: 10.1093/rheumatology/kew274

Cummings, S. R., Nevitt, M. C., and Kidd, S. (1988). Forgetting falls. The limited accuracy of recall of falls in the elderly. *J. Am. Geriatr. Soc.* 36, 613–616. doi: 10.1111/j.1532-5415.1988.tb06155.x

DeMott, T. K., Richardson, J. K., Thies, S. B., and Ashton-Miller, J. A. (2007). Falls and gait characteristics among older persons with peripheral neuropathy. *Am J Phys Med Rehabil* 86, 125–132. doi: 10.1097/PHM.0b013e31802ee1d1

Dixon, C. J., Knight, T., Binns, E., Ihaka, B., and O'Brien, D. (2017). Clinical measures of balance in people with type two diabetes: A systematic literature review. *Gait Posture* 58, 325–332. doi: 10.1016/j.gaitpost.2017.08.022

Dumurgier, J., Elbaz, A., Ducimetière, P., Tavernier, B., Alpérovitch, A., and Tzourio, C. (2009). Slow walking speed and cardiovascular death in well functioning older adults: Prospective cohort study. *BMJ* 339:b4460. doi: 10.1136/bmj.b4460

Enck, P., Bingel, U., Schedlowski, M., and Rief, W. (2013). The placebo response in medicine: Minimize, maximize or personalize? *Nat. Rev. Drug Discov.* 12, 191–204. doi: 10.1038/nrd3923

Fallon, J. B., Bent, L. R., McNulty, P. A., and Macefield, V. G. (2005). Evidence for strong synaptic coupling between single tactile afferents from the sole of the foot and motoneurons supplying leg muscles. *J. Neurophysiol.* 94, 3795–3804. doi: 10.1152/jn.00359.2005

Finniss, D. G., Kaptchuk, T. J., Miller, F., and Benedetti, F. (2010). Biological, clinical, and ethical advances of placebo effects. *Lancet* 375, 686–695. doi: 10.1016/S0140-6736(09)61706-2

Ganz, D. A., and Latham, N. K. (2020). Prevention of falls in community-dwelling older adults. *N. Engl. J. Med.* 382, 734–743. doi: 10.1056/NEJMcp1903252

Gill, T. M., Bhasin, S., Reuben, D. B., Latham, N. K., Araujo, K., Ganz, D. A., et al. (2021). Effect of a multifactorial fall injury prevention intervention on patient well-being: The STRIDE Study. *J. Am. Geriatr. Soc.* 69, 173–179. doi: 10.1111/jgs.16854

Gregg, E. W., Sorlie, P., Paulose-Ram, R., Gu, Q., Eberhardt, M. S., Wolz, M., et al. (2004). Prevalence of lower-extremity disease in the US adult population >=40 years of age with and without diabetes: 1999-2000 national health and nutrition examination survey. *Diabet. Care* 27, 1591–1597. doi: 10.2337/diacare.27.7.1591

Guertin, P. A. (2012). Central pattern generator for locomotion: Anatomical, physiological, and pathophysiological considerations. *Front. Neurol.* 3:183. doi: 10.3389/fneur.2012.00183

Hadjistavropoulos, T., Delbaere, K., and Fitzgerald, T. D. (2011). Reconceptualizing the role of fear of falling and balance confidence in fall risk. *J. Aging Health* 23, 3–23. doi: 10.1177/0898264310378039

Hahn, E. A., DeWalt, D. A., Bode, R. K., Garcia, S. F., DeVellis, R. F., Correia, H., et al. (2014). New english and spanish social health measures will facilitate evaluating health determinants. *Health Psychol.* 33, 490–499. doi: 10.1037/hea0000055

Hahn, E. A., Kallen, M. A., Jensen, R. E., Potosky, A. L., Moinpour, C. M., Ramirez, M., et al. (2016b). Measuring social function in diverse cancer populations: Evaluation of measurement equivalence of the patient reported outcomes measurement information system. *Psychol. Test Assess. Model* 58, 403–421.

Hahn, E. A., Beaumont, J. L., Pilkonis, P. A., Garcia, S. F., Magasi, S., DeWalt, D. A., et al. (2016a). The PROMIS satisfaction with social participation measures demonstrated responsiveness in diverse clinical populations. *J. Clin. Epidemiol.* 73, 135–141. doi: 10.1016/j.jclinepi.2015.08.034

Halvarsson, A., Franzén, E., Farén, E., Olsson, E., Oddsson, L., and Ståhle, A. (2013). Long-term effects of new progressive group balance training for elderly people with increased risk of falling–a randomized controlled trial. *Clin. Rehabil.* 27, 450–458. doi: 10.1177/0269215512462908

Hanewinckel, R., Drenthen, J., Verlinden, V. J. A., Darweesh, S. K. L., van der Geest, J. N., Hofman, A., et al. (2017). Polyneuropathy relates to impairment in daily activities, worse gait, and fall-related injuries. *Neurology* 89, 76–83. doi: 10.1212/WNL.0000000000004067

Hannan, M. T., Gagnon, M. M., Aneja, J., Jones, R. N., Cupples, L. A., Lipsitz, L. A., et al. (2010). Optimizing the tracking of falls in studies of older participants: Comparison of quarterly telephone recall with monthly falls calendars in the MOBILIZE Boston Study. *Am. J. Epidemiol.* 171, 1031–1036. doi: 10.1093/aje/kwq024

Hardy, S. E., Perera, S., Roumani, Y. F., Chandler, J. M., and Studenski, S. A. (2007). Improvement in usual gait speed predicts better survival in older adults. *J. Am. Geriatr. Soc.* 55, 1727–1734. doi: 10.1111/j.1532-5415.2007.01413.x

Hart, D. A. (2021). "Learning from human responses to deconditioning environments: Improved understanding of the "Use It or Lose It" principle. *Front. Sports Act Living* 3:685845. doi: 10.3389/fspor.2021.685845

Hicks, C. W., Wang, D., Windham, B. G., Matsushita, K., and Selvin, E. (2021). Prevalence of peripheral neuropathy defined by monofilament insensitivity in middle-aged and older adults in two US cohorts. *Sci. Rep.* 11:19159. doi: 10.1038/s41598-021-98565-w

Hoffman, E. M., Staff, N. P., Robb, J. M., St Sauver, J. L., Dyck, P. J., and Klein, C. J. (2015). Impairments and comorbidities of polyneuropathy revealed by population-based analyses. *Neurology* 84, 1644–1651. doi: 10.1212/WNL.0000000000001492

Hsu, C. L., Iloputaife, I., Oddsson, L., Manor, B., and Lipsitz, L. (2021). Six-Month lower-leg sensory stimulation augments neural network connectivity associated with improved gait. *Innovation in aging* 5(Suppl. 1), 960–961. doi: 10.1093/geroni/igab046.3439

Ites, K. I., Anderson, E. J., Cahill, M. L., Kearney, J. A., Post, E. C., and Gilchrist, L. S. (2011). Balance interventions for diabetic peripheral neuropathy: A systematic review. *J. Geriatr. Phys. Ther.* 34, 109–116. doi: 10.1519/JPT.0b013e318212659a

Kästenbauer, T., Sauseng, S., Brath, H., Abrahamian, H., and Irsigler, K. (2004). The value of the Rydel-Seiffer tuning fork as a predictor of diabetic polyneuropathy compared with a neurothesiometer. *Diabet. Med.* 21, 563–567. doi: 10.1111/j.1464-5491.2004.01205.x

Kim, D. H., Glynn, R. J., Avorn, J., Lipsitz, L. A., Rockwood, K., Pawar, A., et al. (2019). Validation of a claims-based frailty index against physical performance and adverse health outcomes in the health and retirement study. *J. Gerontol. A Biol. Sci. Med. Sci.* 74, 1271–1276. doi: 10.1093/gerona/gly197

Koehler-McNicholas, S. R., Danzl, L., Cataldo, A. Y., and Oddsson, L. I. E. (2019). Neuromodulation to improve gait and balance function using a sensory neuroprosthesis in people who report insensate feet–A randomized control cross-over study. *PLoS One* 14:e0216212. doi: 10.1371/journal.pone.0216212

Komatsu, H., Yagasaki, K., Komatsu, Y., Yamauchi, H., Yamauchi, T., Shimokawa, T., et al. (2019). Falls and functional impairments in breast cancer patients with chemotherapy-induced peripheral neuropathy. *Asia Pac. J. Oncol. Nurs.* 6, 253–260. doi: 10.4103/apjon.apjon_7_19

Koski, K., Luukinen, H., Laippala, P., and Kivelä, S. L. (1998). Risk factors for major injurious falls among the home-dwelling elderly by functional abilities. A prospective population-based study. *Gerontology* 44, 232–238. doi: 10.1159/000022017

Kroenke, K., Spitzer, R. L., and Williams, J. B. (2001). The PHQ-9: Validity of a brief depression severity measure. *J. Gen. Intern. Med.* 16, 606–613. doi: 10.1046/j.1525-1497.2001.016009606.x

Kruse, R. L., Lemaster, J. W., and Madsen, R. W. (2010). Fall and balance outcomes after an intervention to promote leg strength, balance, and walking in people with diabetic peripheral neuropathy: "Feet first" randomized controlled trial. *Phys. Ther.* 90, 1568–1579. doi: 10.2522/ptj.20090362

Lajoie, Y., and Gallagher, S. P. (2004). Predicting falls within the elderly community: Comparison of postural sway, reaction time, the Berg balance scale and the Activities-specific Balance Confidence (ABC) scale for comparing fallers and non-fallers. *Arch. Gerontol. Geriatr.* 38, 11–26. doi: 10.1016/S0167-4943(03)00082-7

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Front. Psychol.* 4:863. doi: 10.3389/fpsyg.2013.00863

Li, F., Harmer, P., Fisher, K. J., McAuley, E., Chaumeton, N., Eckstrom, E., et al. (2005). Tai Chi and fall reductions in older adults: A randomized controlled trial. *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 187–194. doi: 10.1093/gerona/60.2.187

Li, L., and Manor, B. (2010). Long term Tai Chi exercise improves physical performance among people with peripheral neuropathy. *Am. J. Chin. Med.* 38, 449–459. doi: 10.1142/S0192415X1000797X

Lipsitz, L. A., Macklin, E. A., Travison, T. G., Manor, B., Gagnon, P., Tsai, T. I, et al. (2019). A cluster randomized trial of Tai Chi vs health education in subsidized housing: The MI-WiSH study. *J. Am. Geriatr. Soc.* 67, 1812–1819. doi: 10.1111/jgs.15986

Lipsitz, L. A., Manor, B., Habtemariam, D., Iloputaife, I., Zhou, J., and Travison, T. G. (2018). The pace and prognosis of peripheral sensory loss in advanced age: Association with gait speed and falls. *BMC Geriatr.* 18:274. doi: 10.1186/s12877-018-0970-5

Manor, B., Lough, M., Gagnon, M. M., Cupples, A., Wayne, P. M., and Lipsitz, L. A. (2014). Functional benefits of tai chi training in senior housing facilities. *J. Am. Geriatr. Soc.* 62, 1484–1489. doi: 10.1111/jgs.12946

Mathias, S., Nayak, U. S., and Isaacs, B. (1986). Balance in elderly patients: The "get-up and go" test. *Arch. Phys. Med. Rehabil.* 67, 387–389.

Melzer, I., and Oddsson, LIe (2013). Improving balance control and self-reported lower extremity function in community-dwelling older adults: A randomized control trial. *Clin. Rehabil.* 27, 195–206. doi: 10.1177/0269215512450295

Menz, H. B., Lord, S. R., St George, R., and Fitzpatrick, R. C. (2004). Walking stability and sensorimotor function in older people with diabetic peripheral neuropathy. *Arch. Phys. Med. Rehabil.* 85, 245–252. doi: 10.1016/j.apmr.2003.06.015

Meyer, P. F., Oddsson, L. I., and De Luca, C. J. (2004a). Reduced plantar sensitivity alters postural responses to lateral perturbations of balance. *Exp. Brain Res.* 157, 526–536. doi: 10.1007/s00221-004-1868-3

Meyer, P. F., Oddsson, L. I., and De Luca, C. J. (2004b). The role of plantar cutaneous sensation in unperturbed stance. *Exp. Brain Res.* 156, 505–512. doi: 10.1007/s00221-003-1804-y

Montero-Odasso, M. M., Kamkar, N., Pieruccini-Faria, F., Osman, A., Sarquis-Adamson, Y., Close, J., et al. (2021). Evaluation of clinical practice guidelines on fall prevention and management for older adults: A systematic review. *JAMA Netw. Open* 4:e2138911.

Montero-Odasso, M., Schapira, M., Soriano, E. R., Varela, M., Kaplan, R., Camera, L. A., et al. (2005). Gait velocity as a single predictor of adverse events in healthy seniors aged 75 years and older. *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 1304–1309. doi: 10.1093/gerona/60.10.1304

Moore, J. L., Potter, K., Blankshain, K., Kaplan, S. L., O'Dwyer, L. C., and Sullivan, J. E. (2018). A core set of outcome measures for adults with neurologic conditions undergoing rehabilitation: A Clinical Practice Guideline. *J. Neurol. Phys. Ther.* 42, 174–220. doi: 10.1097/NPT.0000000000000229

Moreland, B., Kakara, R., and Henry, A. (2020). Trends in nonfatal falls and fall-related injuries among adults aged =65 years–United States, 2012-2018. *MMWR Morb. Mortal Wkly. Rep.* 69, 875–881. doi: 10.15585/mmwr.mm6927a5

Morris, S. B., and DeShon, R. P. (2002). Combining effect size estimates in meta-analysis with repeated measures and independent-groups designs. *Psychol. Methods* 7, 105–125. doi: 10.1037/1082-989X.7.1.105

Morrison, S., Simmons, R., Colberg, S. R., Parson, H. K., and Vinik, A. I. (2018). Supervised balance training and wii fit-based exercises lower falls risk in older adults with type 2 diabetes. *J. Am. Med. Dir. Assoc.* 19, .e187–.e185. doi: 10.1016/j.jamda.2017.11.004

Mustapa, A., Justine, M., Mohd Mustafah, N., Jamil, N., and Manaf, H. (2016). Postural control and gait performance in the diabetic peripheral neuropathy: A systematic review. *Biomed. Res. Int.* 2016:9305025. doi: 10.1155/2016/9305025

Noohi, F., Kinnaird, C., DeDios, Y. I, Kofman, S., Wood, S., Bloomberg, J., et al. (2017). Functional brain activation in response to a clinical vestibular test correlates with balance. *Front. Syst. Neurosci.* 11:11. doi: 10.3389/fnsys.2017.00011

Oddsson, L. I. E., Bisson, T., Cohen, H. S., Jacobs, L., Khoshnoodi, M., Kung, D., et al. (2020). The effects of a wearable sensory prosthesis on gait and balance function after 10 weeks of use in persons with peripheral neuropathy and high fall risk–The walk2Wellness trial. *Front. Aging Neurosci.* 12:592751. doi: 10.3389/fnagi.2020.592751

Oddsson, L. I. E., Boissy, P., and Melzer, I. (2007). How to improve gait and balance function in elderly individuals—compliance with principles of training. *Eur. Rev. Aging Phys. Act* 4, 15–23. doi: 10.1007/s11556-007-0019-9

Patsopoulos, N. A. (2011). A pragmatic view on pragmatic trials. *Dialogues Clin. Neurosci.* 13, 217–224. doi: 10.31887/DCNS.2011.13.2/npatsopoulos

Paul, S. S., Ada, L., and Canning, C. G. (2005). Automaticity of walking–implications for physiotherapy practice. *Phys. Ther. Rev.* 10, 15–23. doi: 10.1179/108331905X43463

Perera, S., Mody, S. H., Woodman, R. C., and Studenski, S. A. (2006). Meaningful change and responsiveness in common physical performance measures in older adults. *J. Am. Geriatr. Soc.* 54, 743–749. doi: 10.1111/j.1532-5415.2006.00701.x

Powell, L. E., and Myers, A. M. (1995). The activities-specific balance confidence (ABC) scale. *J. Gerontol. A Biol. Sci. Med. Sci.* 50A, M28–M34. doi: 10.1093/gerona/50A.1.M28

Quigley, P. A., Bulat, T., Schulz, B., Friedman, Y., Hart-Hughes, S., Richardson, J. K., et al. (2014). Exercise interventions, gait, and balance in older subjects with distal symmetric polyneuropathy: A three-group randomized clinical trial. *Am. J. Phys. Med. Rehabil.* 93, 1–12. doi: 10.1097/PHM.0000000000000052

Reeves, N. D., Orlando, G., and Brown, S. J. (2021). Sensory-motor mechanisms increasing falls risk in diabetic peripheral neuropathy. *Medicina (Kaunas)* 57:457. doi: 10.3390/medicina57050457

Richardson, J. K., and Hurvitz, E. A. (1995). Peripheral neuropathy: A true risk factor for falls. *J. Gerontol. A Biol. Sci. Med. Sci.* 50, M211–M215. doi: 10.1093/gerona/50A.4.M211

Richardson, J. K., Sandman, D., and Vela, S. (2001). A focused exercise regimen improves clinical measures of balance in patients with peripheral neuropathy. *Arch. Phys. Med. Rehabil.* 82, 205–209. doi: 10.1053/apmr.2001.19742

Riskowski, J. L., Quach, L., Manor, B., Menz, H. B., Lipsitz, L. A., and Hannan, M. T. (2012). Idiopathic peripheral neuropathy increases fall risk in a population-based cohort study of older adults. *J. Foot Ankle Res.* 5:19. doi: 10.1186/1757-1146-5-S1-P19

Sheldon, J. H. (1948). Some aspects of old age. *Lancet* 1, 621–624. doi: 10.1016/S0140-6736(48)90811-3

Shumway-Cook, A., Gruber, W., Baldwin, M., and Liao, S. (1997). The effect of multidimensional exercises on balance, mobility, and fall risk in community-dwelling older adults. *Phys. Ther.* 77, 46–57. doi: 10.1093/ptj/77.1.46

Sienko, K. H., Seidler, R. D., Carender, W. J., Goodworth, A. D., Whitney, S. L., and Peterka, R. J. (2018). Potential mechanisms of sensory augmentation systems on human balance control. *Front. Neurol.* 9:944. doi: 10.3389/fneur.2018.00944

Sluijs, E. M., Kok, G. J., and van der Zee, J. (1993). Correlates of exercise compliance in physical therapy. *Phys. Ther.* 73, 771–782. doi: 10.1093/ptj/73.11.771

Soh, S. L., Tan, C. W., Thomas, J. I., Tan, G., Xu, T., Ng, Y. L., et al. (2021). Falls efficacy: Extending the understanding of self-efficacy in older adults towards managing falls. *J. Frailty Sarcopenia Falls* 6, 131–138. doi: 10.22540/JFSF-06-131

Srinivasan, S. S., Tuckute, G., Zou, J., Gutierrez-Arango, S., Song, H., Barry, R. L., et al. (2020). Agonist-antagonist myoneural interface amputation preserves proprioceptive sensorimotor neurophysiology in lower limbs. *Sci. Transl. Med.* 12:eabc592. doi: 10.1126/scitranslmed.abc5926

STEADI, C. (2021). *STEADI—Older Adult Fall Prevention*. Available online at: https://www.cdc.gov/steadi/ (accessedDecember 10, 2021)

Stevens, J. A., and Phelan, E. A. (2013). Development of STEADI: A fall prevention resource for health care providers. *Health Promot. Pract.* 14, 706–714. doi: 10.1177/1524839912463576

Streckmann, F., Zopf, E. M., Lehmann, H. C., May, K., Rizza, J., Zimmer, P., et al. (2014). Exercise intervention studies in patients with peripheral neuropathy: A systematic review. *Sports Med* 44, 1289–1304. doi: 10.1007/s40279-014-0207-5

Strzalkowski, N. D. J., Peters, R. M., Inglis, J. T., and Bent, L. R. (2018). Cutaneous afferent innervation of the human foot sole: What can we learn from single-unit recordings? *J. Neurophysiol.* 120, 1233–1246. doi: 10.1152/jn.00848.2017

Studenski, S., Perera, S., Patel, K., Rosano, C., Faulkner, K., Inzitari, M., et al. (2011). Gait speed and survival in older adults. *JAMA* 305, 50–58. doi: 10.1001/jama.2010.1923

Studenski, S., Perera, S., Wallace, D., Chandler, J. M., Duncan, P. W., Rooney, E., et al. (2003). Physical performance measures in the clinical setting. *J. Am. Geriatr. Soc.* 51, 314–322. doi: 10.1046/j.1532-5415.2003.51104.x

Tinetti, M. E., and Kumar, C. (2010). The patient who falls: "It's always a trade-off". *JAMA* 303, 258–266. doi: 10.1001/jama.2009.2024

Tofthagen, C., Visovsky, C., and Berry, D. L. (2012). Strength and balance training for adults with peripheral neuropathy and high risk of fall: Current evidence and implications for future research. *Oncol. Nurs. Forum* 39, E416–E424. doi: 10.1188/12.ONF.E416-E424

Uitenbroek, D. G. (1997). *SISA-Binomial*. Available online at: https://www.quantitativeskills.com/sisa/calculations/bonfer.htm (accessed May, 2020)

van Waart, H., Stuiver, M. M., van Harten, W. H., Geleijn, E., Kieffer, J. M., Buffart, L. M., et al. (2015). Effect of low-intensity physical activity and moderate- to high-intensity physical exercise during adjuvant chemotherapy on physical fitness, fatigue, and chemotherapy completion rates: Results of the paces randomized clinical trial. *J. Clin. Oncol.* 33, 1918–1927. doi: 10.1200/JCO.2014.59.1081

Vaughan, G. M. (2021). The roles of mechanoreceptors in muscle and skin in human proprioception. *Curr. Opin. Physiol.* 21, 48–56. doi: 10.1016/j.cophys.2021.03.003

Vinik, A. I., Camacho, P., Reddy, S., Valencia, W. M., Trence, D., Matsumoto, A. M., et al. (2017). Aging, Diabetes, And Falls. *Endocr. Pract.* 23, 1117–1139. doi: 10.4158/EP171794.RA

Wang, C., Goel, R., Zhang, Q., Lepow, B., and Najafi, B. (2019). Daily use of bilateral custom-made ankle-foot orthoses for fall prevention in older adults: A randomized controlled trial. *J. Am. Geriatr. Soc.* 67, 1656–1661. doi: 10.1111/jgs.15929

Winters-Stone, K. M., Horak, F., Jacobs, P. G., Trubowitz, P., Dieckmann, N. F., Stoyles, S., et al. (2017). Falls, functioning, and disability among women with persistent symptoms of chemotherapy-induced peripheral neuropathy. *J. Clin. Oncol.* 35, 2604–2612. doi: 10.1200/JCO.2016.71.3552

Wolf, B., Feys, H., De Weerdt, J., van, der Meer, Noom, M., and Aufdemkampe, G. (2001). Effect of a physical therapeutic intervention for balance problems in the elderly: A single-blind, randomized, controlled multicentre trial. *Clin. Rehabil.* 15, 624–636. doi: 10.1191/0269215501cr456oa

Wolf, S. L., Sattin, R. W., Kutner, M., O'Grady, M., Greenspan, A. I., and Gregor, R. J. (2003). Intense tai chi exercise training and fall occurrences in older, transitionally frail adults: A randomized, controlled trial. *J. Am. Geriatr. Soc.* 51, 1693–1701. doi: 10.1046/j.1532-5415.2003.51552.x

Wrisley, D. M., and Kumar, N. A. (2010). Functional gait assessment: Concurrent, discriminative, and predictive validity in community-dwelling older adults. *Phys. Ther.* 90, 761–773. doi: 10.2522/ptj.20090069

Wrisley, D. M., Marchetti, G. F., Kuharsky, D. K., and Whitney, S. L. (2004). Reliability, internal consistency, and validity of data obtained with the functional gait assessment. *Phys. Ther.* 84, 906–918. doi: 10.1093/ptj/84.10.906

Wrisley, D. M., McLean, G., Hill, J. B., and Oddsson, L. I. E. (2021). Long-term use of a sensory prosthesis improves function in a patient with peripheral neuropathy: A case report. *Front. Neurol.* 12:655963. doi: 10.3389/fneur.2021.655963

Zaiontz, C. (2020). *Real statistics resource pack (Release 6.8)*. Available online at: http://www.real-statistics.com/ (accessed August 2021).

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# Mild cognitive impairment is associated with poor gait performance in patients with Parkinson's disease

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Cognitive impairment may be commonly accompanied by gait disturbance in patients with Parkinson's disease (PD). However, it is still controversial whether gait disturbance is associated with mild cognitive impairment (MCI) and which cognitive function has a more important effect on specific gait parameter. Our objective was to investigate the association of gait parameters with MCI and the correlation between performance on comprehensive neuropsychological tests and gait parameters in PD patients. We enrolled 257 patients with *de novo* PD (111 PD-normal cognition and 146 PD-MCI). All patients underwent comprehensive neuropsychological tests and gait evaluation using the GAITRite system. We used logistic regression analysis and partial correlation to identify the association between gait parameters and MCI and correlations between neuropsychological performance and gait parameters. Gait velocity (odds ratio [OR] = 0.98, 95% confidence interval [CI] = 0.97–0.99) and stride length (OR = 0.98; 95% CI = 0.97–0.99) were associated with MCI in patients with PD. Specifically, gait velocity, stride length, and double support ratio were only associated with attention and frontal-executive function performance in patients with PD. Our findings provide insight into the relationship between gait disturbance and MCI in patients with PD. Furthermore, the evaluation of gait disturbance is necessary for PD patients with cognitive impairment.

KEYWORDS

**Parkinson's disease, cognitive impairment, gait disturbance, attention, frontal executive function**

Abbreviations: AR, akinetic rigid PD; COWAT, controlled oral word association test; CV, coefficient of variation; K-BNT, Korean version of the Boston naming test; MCI, mild cognitive impairment; PD-NC, Parkinson's disease-normal cognition; RCFT, Ray Osterrieth complex figure test; SVLT, Seoul Verbal Learning Test; TD, tremor-dominant PD; UPDRS, Unified Parkinson's Disease Rating Scale; WMH, white matter hyperintensity.

# Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and is characterized by progressive motor deficits, including bradykinesia, resting tremor, rigidity, and gait disturbance.

Although gait disturbance is a dopa-responsive symptom at early stage of PD, the response attenuates as the disease progresses (Morris et al., 1994), which in turn leads to debilitating features including freezing of gait and/or falls in patients with advanced PD (Lord et al., 2014). Recently, it has been recognized that non-motor symptoms including cognitive decline commonly occur in PD. The prevalence of mild cognitive impairment (MCI) in patients with PD have found to range from approximately 40–60% at the time of diagnosis (Baiano et al., 2020).

Patients with PD-mild cognitive impairment (PD-MCI) are at a higher risk of poor prognosis including gait disturbance (Fereshtehnejad et al., 2015). Furthermore, cognitive control is needed to compensate for impaired gait function in patients with PD. Given that these findings, the association between cognitive impairment and gait disturbance has become emerged. Specifically, previous studies have found that specific gait parameters are associated with cognition in patients with PD (Rochester et al., 2004, 2005; Wild et al., 2013; Yogev-Seligmann et al., 2013). However, because other studies have shown null results (Rochester et al., 2008; Amboni et al., 2012; Lord et al., 2014), it is still controversial whether gait disturbance may be associated with the presence of MCI and which cognitive function has a more important effect on specific gait parameters in patients with PD. Previous studies had relatively small sample sizes and focused only on global cognitive or executive function; therefore, they did not cover other cognitive domains.

Motor phenotype in patients with PD can be classified into akinetic rigid PD (AR) and tremor-dominant PD (TD). Patients with AR suffer from bradykinesia, rigidity and/or axial symptoms, while the TD is characterized by a tremor. Recently, there is a growing body of evidence that early stage of patients with AR are more likely to have poor prognosis including faster cognitive decline, higher risk of developing dementia, and severe gait disturbance than those with TD (Fereshtehnejad et al., 2015; Wojtala et al., 2019; Yang et al., 2021). However, despite of the differences in severity of cognitive impairment and gait disturbance between the motor phenotype, previous studies did not consider whether gait parameters were associated with specific cognitive functions in relation to motor phenotype.

Therefore, the first objective of our study was to check the differences the relationship between gait parameters and MCI in relation to motor phenotype. The second objective of our study was to investigate the association of gait parameters and the presence of fall or freezing of gait with mild cognitive impairment (MCI) in patients with PD. The second objective was to explore the correlation between performance in comprehensive neuropsychological tests and gait parameters in patients with PD. Given that patients with PD have impaired gait function and then cognitive compensation is necessary to keep gait performance, we hypothesized that MCI was associated with poor gait performance in patients with PD.

# Materials and methods

## Study participants

We recruited 257 patients with *de novo* PD (167 with PIGD and 90 with TDP) at the Movement Disorder Clinic of Korea University, Guro Hospital in Seoul, Korea, from January 2018 to December 2020. All patients underwent comprehensive movement disorder evaluations, including the Unified Parkinson's Disease Rating Scale (UPDRS), Non-Motor Symptoms Scale (Koh et al., 2012), brain magnetic resonance imaging, standardized neuropsychological tests (Seoul Neuropsychological Screening Battery 2nd edition) (Kang et al., 2019, 2021b), and comprehensive gait evaluation using the GAITRite system (CIR System Inc., USA) before the initiation of PD medication. The time interval between the comprehensive neuropsychological test and gait evaluation was less than 1 month. We excluded patients who exhibited any of the following conditions: (1) Parkinsonism due to offending drugs; (2) history of PD treatment in other hospitals; (3) dementia on a comprehensive neuropsychological test; (4) severe white matter hyperintensity (WMH) defined as deep WMH $\geq$ 25 mm and periventricular WMH $\geq$ 10 mm on fluid-attenuated inversion recovery image; (5) territorial infarction, lobar hemorrhage, brain tumor, and hydrocephalus or other structural lesions; and (6) history of psychiatric illness, including major depressive disorder, bipolar disorder, and schizophrenia.

Patients with PD were dichotomized as TD or AR using a modified ratio based on UPDRS III (Schiess et al., 2000; Kang et al., 2016). Specifically, the tremor/akinetic-rigidity ratio was calculated from the patient's mean tremor and akinetic-rigidity scores. Tremor was assessed using a nine-item scale that included a history of left or right tremor (two items), rest tremors of the face/lips/chin and each limb (five items), and postural tremor of the right and left upper extremities (two items). Akinetic-rigidity was assessed using a 12-item scale that included passive range of motion rigidity of the neck and each extremity (five items), rapid opening/closing of the hands (one item), finger tapping (one item), rising from a chair (one item), posture and postural instability (two items), gait (one item), and body bradykinesia (one item). Each item was rated 0–4, with 0 representing the absence of symptoms/normal activity and 4 representing the significant presence of the symptom. Mean tremor and akinetic-rigidity scores were calculated, and then the ratio (tremor/akinetic-rigidity score) was determined.

Patients with a ratio >1.0 were defined as TD, while those with a ratio ≤1.0 were defined as AR.

All participants with PD were patients with PD-normal cognition (PD-NC) and those with PD-MCI. All patients with PD-NC met the following criteria: (1) diagnosis of PD based on the UK Brain Bank criteria (Hughes et al., 1992); (2) no objective cognitive impairment or objective cognitive impairment in only one neuropsychological test; (3) no medical history likely to affect cognitive function based on Christensen's health screening criteria (Christensen et al., 1991); and (4) no significant impairment in activities of daily living. All patients with PD-MCI met the MDS level II criteria for PD-MCI: objective cognitive impairment below −1.0 SD in at least two neuropsychological tests, in which two tests were impaired in one cognitive domain or one test was impaired in two different cognitive domains (Litvan et al., 2012).

This study was approved by the Institutional Review Board of Korea University, Guro Hospital. Written informed consent was obtained from all patients.

## Assessment of gait disturbance related symptoms and gait parameters

Patients reported symptoms of falling and freezing of gait during interviews. Falling was assessed using the question, "did you have a history of falls during the past year?" (Pickering et al., 2007). The freezing of gait was assessed using the FOG questionnaire, and an FOG questionnaire item score ≥ 1 was defined as having freezing of gait (Giladi et al., 2000).

Patients underwent a comprehensive gait evaluation regarding spatial and temporal parameters of gait dynamics using the GAITRite system with a 4.6-m-long walkway (Kang et al., 2021a). Average spatiotemporal parameters, such as velocity, stride length, cadence, step length coefficient of variation (CV), and double support ratio, were measured during 10-times forward walking.

## Assessment of cognitive function

All patients underwent neuropsychological tests using the Seoul Neuropsychological Screening Battery 2nd edition (Kang et al., 2019). We opted to use 10 cognitive measurements of representative and important neuropsychological tests to evaluate cognitive function in five cognitive domains as follows: (1) attention, the Digit Span Test backward and Stroop Test (word reading); (2) language, the Korean version of the Boston Naming Test (K-BNT) and animal component of the Controlled Oral Word Association Test (COWAT); (3) visuospatial function, the Rey–Osterrieth Complex Figure Test (RCFT) copying Test and Clock Drawing Test; (4) memory, the Seoul Verbal Learning Test (SVLT) delayed recall (verbal memory) and RCFT delayed recall (visual memory); and (5) frontal executive function, the phonemic component of the COWAT and the Stroop Test (color reading). Continuous numerical values were converted to z-scores using the standardized norms for age and education presented in the SNSB-II, and z-scores were used in the analysis.

## Statistical analyses

We used the independent *t*-test and chi-square test to compare the demographic and clinical characteristics between the PD-NC and PD-MCI groups. To assess the interaction effect of cognitive status (PD-NC or PD-MCI) and motor phenotype (AR or TD) on gait disturbance, we performed a two-way ANOVA with interaction test on gait parameters (velocity, stride length, cadence, step length CV, and double support ratio). To determine the association of gait-related symptoms (easy falling, freezing of gait, and gait ignition failure) and gait parameters (velocity, stride length, cadence, step length CV, and double support ratio) with MCI, we used logistic regression, including the presence of gait-related symptoms (easy falling, freezing of gait, and gait ignition failure) or gait parameters (velocity, stride length, cadence, step length CV, and double support ratio) as separate independent variables after controlling for age, sex, disease duration and motor phenotype. Finally, we used partial correlations after controlling for age, sex, disease duration, and motor phenotype to explore the correlation between gait parameters and specific neuropsychological performance.

All reported *p*-values were two-sided, and the significance level was set at 0.05. All analyses were performed using R version 3.6.1 (Institute for Statistics and Mathematics, Vienna, Austria).[1]

# Results

## Clinical characteristics of the study participants

Among the 257 patients with PD, 111 were PD-NC and 146 were PD-MCI. Patients with PD-MCI were older than those with those with PD-NC ($p$ = 0.036). The proportion of females ($p$ = 0.056), disease duration ($p$ = 0.611), ratio of easy falling ($p$ = 0.215) and gait ignition failure ($p$ = 0.288), cadence ($p$ = 0.161), step length variability ($p$ = 0.812), and double support ratio ($p$ = 0.051) did not differ between the PD-NC and PD-MCI groups (**Table 1**). Patients with PD-MCI were more likely to experience freezing of gait ($p$ = 0.028) and had slower velocity ($p$ = 0.014), and shorter stride length ($p$ = 0.021) than those with PD-NC (**Table 1**).

1 www.R-project.org

TABLE 1 Demographic variables and gait profiles of study participants.

| | PD-NC ($n$ = 111) | PD-MCI ($n$ = 146) | *P-value* |
|---|---|---|---|
| **Motor phenotype** | | | |
| AR/TD | 40 (36.0%)/71 (64.0%) | 50 (34.2%)/96 (65.8%) | 0.766 |
| **Demographics** | | | |
| Age (years) | 69.8 ± 7.3 | 71.9 ± 8.0 | 0.036 |
| Sex, females | 71 (64.0%) | 76 (52.1%) | 0.056 |
| Education (years) | 8.4 ± 4.7 | 8.7 ± 4.9 | 0.677 |
| Disease duration | 25.8 ± 27.1 | 27.6 ± 27. | 0.611 |
| Hypertension | 51 (45.9%) | 72 (49.3%) | 0.592 |
| Diabetes | 21 (18.9%) | 37 (25.3%) | 0.222 |
| Easy falling | 6 (5.4%) | 14 (9.6%) | 0.215 |
| Freezing of gait | 7 (6.3%) | 22 (15.1%) | 0.028 |
| Gait ignition failure | 6 (5.4%) | 13 (8.9%) | 0.288 |
| **Gait parameters** | | | |
| Velocity (m/s) | 84.5 ± 20.5 | 77.7 ± 23.1 | 0.014 |
| Stride length (m) | 94.8 ± 20.0 | 88.3 ± 23.8 | 0.021 |
| Cadence | 107.8 ± 14.6 | 105.6 ± 10.8 | 0.161 |
| Step length CV | 2.6 ± 2.6 | 2.5 ± 2.2 | 0.812 |
| Double support (%) | 30.4 ± 5.4 | 32.0 ± 7.2 | 0.051 |

Values are presented as the mean ± standard deviation. PD-NC, Parkinson's disease-normal cognition; PD-MCI, Parkinson's disease-mild cognitive impairment; AR, akinetic rigid-type PD; TD, tremor-dominant-type PD; CV, coefficient of variation.

The interaction effects of cognitive status (PD-NC or PD-MCI) and motor phenotype (AR or TD) on gait parameters were not significant (Table 2).

## Association of gait related symptoms or gait parameters with Parkinson's disease-mild cognitive impairment

Freezing of gait (odds ratio [OR] = 2.84; 95% confidence interval [CI] = 1.08−7.43), slower gait velocity (OR = 0.98; 95% CI = 0.97−0.99), and shorter stride length (OR = 0.98; 95% CI = 0.97−0.99) were associated with PD-MCI (Table 3).

TABLE 3 Odds ratio for PD-MCI in patients with PD.

| | PD-MCI | |
|---|---|---|
| | OR* (95% CI) | *p* |
| **Symptoms** | | |
| Easy falling | 1.68 (0.60–4.72) | 0.322 |
| Freezing of gait | 2.84 (1.08–7.43) | 0.034 |
| Gait ignition failure | 1.71 (0.59–4.97) | 0.328 |
| **Gait parameters** | | |
| Velocity | 0.98 (0.97–0.99) | 0.022 |
| Stride length | 0.98 (0.97–0.99) | 0.021 |
| Cadence | 0.99 (0.97–1.01) | 0.273 |
| Step length CV | 0.98 (0.88–1.09) | 0.756 |
| Double support ratio | 1.04 (1.00–1.09) | 0.070 |

MCI, mild cognitive impairment; PD, Parkinson's disease; OR, odds ratio; CI, confidence interval; CV, coefficient of variation. *Adjusted OR for MCI was obtained using logistic regression analyses with each gait-related symptom or gait parameter as a single independent predictor, after controlling for age, sex disease duration, and motor phenotype.

## Relationship between gait parameters and performance in neuropsychological tests

Table 4 shows the correlation between gait parameters and performance on neuropsychological tests. Gait velocity was positively correlated with performance in the Digit Span Test backward ($r$ = 0.241, $p$ = 0.001), semantic ($r$ = 0.199, $p$ = 0.005), and phonemic component of the COWAT ($r$ = 0.232, $p$ = 0.001), and the color reading portion of the Stroop Test ($r$ = 0.215, $p$ = 0.002) in patients with PD (Figure 1). Stride length was also positively correlated with performance in the Digit Span Test backward ($r$ = 0.250, $p$ < 0.001), RCFT copying Test ($r$ = 0.156, $p$ = 0.029), semantic ($r$ = 0.222, $p$ = 0.002), phonemic component of the COWAT ($r$ = 0.250, $p$ < 0.001), and color reading portion of the Stroop Test ($r$ = 0.263, $p$ < 0.001) in patients with PD (Figure 1). The double support ratio was negatively correlated with the performance in the Digit Span Test backward ($r$ = −0.195, $p$ = 0.006), semantic component of the COWAT ($r$ = −0.159, $p$ = 0.026), phonemic component of the COWAT ($r$ = −0.183, $p$ = 0.010), and color reading portion of the Stroop Test ($r$ = −0.201, $p$ = 0.005) in patients with PD.

TABLE 2 Differences in gait parameters in relation to cognitive status and motor phenotype.

| Variables | Interaction effect (cognitive status × motor phenotype) *F*-value/*p*-value* | Main effect 1 (cognitive status) *F*-value/*p*-value | Main effect 2 (motor phenotype) *F*-value/*p*-value |
|---|---|---|---|
| Velocity | 2.08/0.151 | 6.65/0.011 | 23.53/ < 0.001 |
| Stride length | 2.66/0.104 | 6.05/0.015 | 30.42/ < 0.001 |
| Cadence | 0.09/0.765 | 1.96/0.162 | 0.01/0.935 |
| Step length CV | 0.05/0.821 | 0.06/0.811 | 5.45/0.020 |
| Double support | 1.88/0.172 | 3.49/0.063 | 14.79/ < 0.001 |

*$p$ for interaction effect was obtained using the two-way ANOVA with interaction test (diagnosis × motor phenotype) on gait parameters. CV, coefficient of variation.

TABLE 4 Correlation between gait parameters and neuropsychological tests.

| | Velocity | Stride length | Cadence | Step length CV | Double support ratio |
|---|---|---|---|---|---|
| DST backward | 0.241** | 0.250*** | 0.062 | −0.105 | −0.195* |
| BNT | 0.042 | 0.101 | −0.099 | −0.036 | −0.051 |
| RCFT copy | 0.094 | 0.156* | −0.082 | −0.100 | −0.031 |
| SVLT dr | 0.039 | 0.046 | −0.017 | −0.066 | −0.018 |
| RCFT dr | 0.065 | 0.090 | −0.049 | −0.056 | −0.034 |
| COWAT animal | 0.199* | 0.222** | 0.001 | −0.064 | −0.159* |
| COWAT phonemic | 0.232** | 0.250*** | 0.015 | −0.095 | −0.183* |
| Stroop CR | 0.215** | 0.263*** | −0.012 | −0.134 | −0.201* |

CV, coefficient of variation; DST, Digit Span Test; BNT, Boston Naming Test; RCFT, Rey–Osterrieth Complex Figure Test; SVLT, Seoul Verbal Learning Test; COWAT, Controlled Oral Word Association Test; CR, color reading. *$p$-value < 0.05, **$p$-value < 0.005, ***$p$-value < 0.001.

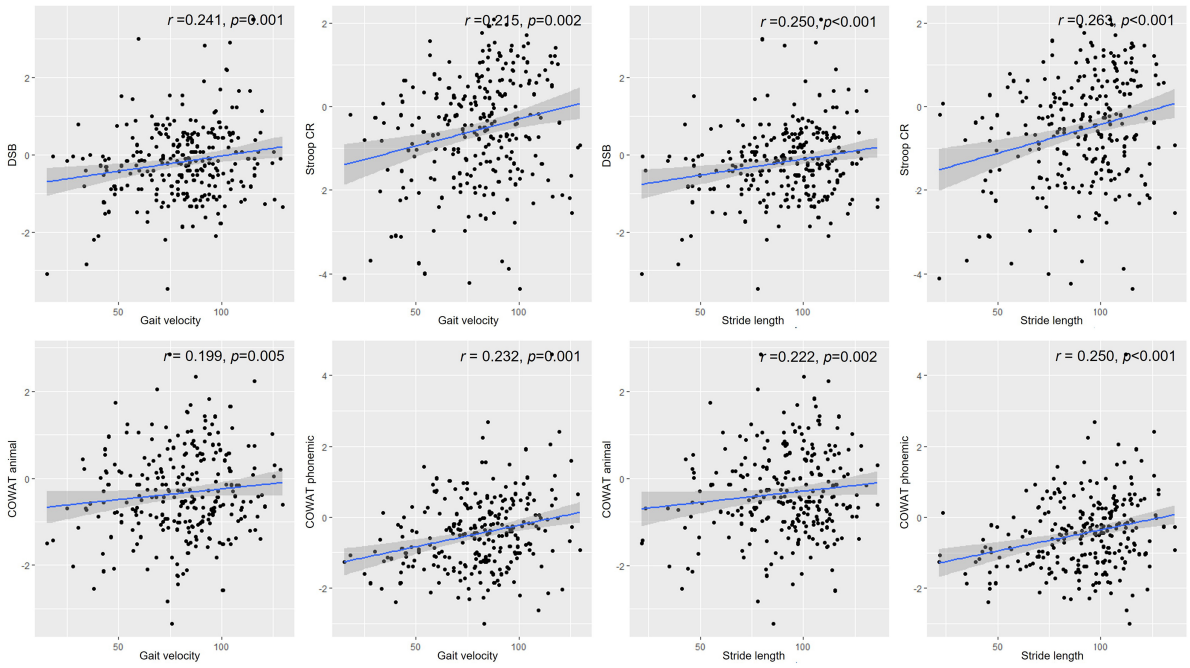


FIGURE 1
Correlations between gait parameters and neuropsychological performance. Values depicted in the scatter plot represent gait velocity or stride length on the X-axis and score in the Digit Span Test backward, Stroop color reading, animal component of COWAT, or phonemic component of COWAT on the Y-axis. DSB, Digit Span Test backward; Stroop CR, Stroop color reading; COWAT animal, animal component of COWAT; COWAT phonemic, phonemic component of COWAT.

## Discussion

In this large cohort study, we investigated the association between gait parameters and cognitive function in patients with *de novo* PD. The main findings of this study were as follows. First, gait velocity or stride length were associated with PD-MCI. Second, poor cognitive performances in attention and frontal-executive function were associated with slower gait velocity, shorter stride length, and higher double support ratio in patients with PD. Taken together, our findings provide insight into the relationship between gait disturbance and the presence of MCI in patients with PD.

Patients with AR have a more widespread PD pathology burden (Nutt, 2016) and worse gait performance than those with TD, and therefore cognitive compensation is more needed in AR motor phenotype, we expected that the relationship between gait disturbance and MCI was more stronger in patients with AR than TD. However, in contrary to our expectations, we found that the relationship of gait disturbance with MCI was not different between patients with AR and TD.

Our first major finding was that slower gait velocity or shorter stride length was associated with MCI in patients with PD. These results are consistent with that of previous findings, which demonstrated that patients with PD-MCI showed slower velocity and shorter stride length than those

with PD-NC (Morris et al., 1996; Amboni et al., 2012). Shared neural substrate degeneration and neurotransmitter deficits may explain our findings. Specifically, fronto-striatal neurodegeneration causes gait disturbance and subcortical-type cognitive impairment (Pillon et al., 1993) in patients with PD. Previous studies have also reported that hypometabolism in the prefrontal cortex was observed in the early stages of PD (Chu et al., 2019), which correlates with gait disturbance and cognitive impairment (Stuart et al., 2020). Given that there is growing evidence that the cholinergic system may be a pivotal contributor not only to cognitive performance but also to gait function in PD (Bohnen et al., 2011; Müller and Bohnen, 2013), cholinergic denervation in the basal forebrain and pedunculopontine may be another reason for the association between cognition and gait. Recent studies have supported that cholinergic deficits in the basal forebrain and mesencephalic locomotor area are responsible for cognitive impairment and gait disturbance including falls and freezing of gait (Bohnen et al., 2009, 2014).

Our second major finding was that poor cognitive performances in attention and frontal-executive function were associated with slower gait velocity, shorter stride length, and higher double support ratio only in patients with PD. Our results are consistent with that of previous studies showing that gait-related cognitive domains include attention and frontal-executive function, which are mainly correlated with velocity and stride length (Lord et al., 2010). This relationship may be explained by the facts that cognitive compensation to maintain the gait function is closely related to attention and frontal-executive function. Gait, an automatic motor skill to control movement in healthy individuals, is impaired in patients with PD, leading to an increased demand for attention and frontal-executive function (Yogev et al., 2005). In fact, patients with PD showed higher prefrontal activity during walking to compensate for gait control than healthy individuals (Stuart et al., 2019; Stuart and Mancini, 2020). Given that the prefrontal cortex is a well-known neural substrate for attention and frontal-executive function (Kang et al., 2019, 2021b), cognitive demand on attention and frontal-executive function may increase during walking in patients with PD.

Our study had several limitations that should be addressed. First, because this study had a cross-sectional design, a causal relationship between gait disturbance and cognitive impairment could not be concluded. Second, we did not address the effects of dual tasks on gait. Dual tasks are considered a sensitive condition to detect underlying gait disturbance and cognitive impairment in patients with PD because dual tasks aggravate gait performance, and the magnitude of worsening may be associated with underlying cognitive function. Finally, given that 59 (23.0%) out of 257 patients with PD had less than 1-year follow-up period, we could not completely rule out the possibility that a few patients diagnosed with PD at first would progress to multiple system atrophy or progressive supranuclear gaze palsy. However, we excluded the participants who had red flag sign including supranuclear gaze palsy, cerebellar sign, Babinski sign with hyperreflexia, stridor and atrophy of putamen, superior cerebellar peduncle, middle cerebellar peduncle, midbrain, pons or cerebellum on MRI at baseline evaluation to increase the diagnostic accuracy of *de novo* PD. However, our study is noteworthy because we comprehensively reported an association between cognitive impairment and gait disturbance in a large number of patients with *de novo* PD. Our findings suggest that the evaluation of gait disturbance is necessary for PD patients with cognitive impairment, while further longitudinal studies are needed to understand the complex mechanism of our findings.

## 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 study protocol was reviewed and approved by the Institutional Review Board of Korea University Guro Hospital. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

SK analyzed and interpreted the data and drafted the manuscript for intellectual content. JK and JL played major roles in data acquisition. S-BK acquired the data, designed and conceptualized the study, and revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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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.

## Publisher's note



## References

Amboni, M., Barone, P., Iuppariello, L., Lista, I., Tranfaglia, R., Fasano, A., et al. (2012). Gait patterns in Parkinsonian patients with or without mild cognitive impairment. *Mov. Disord.* 27, 1536–1543. doi: 10.1002/mds.25165

Baiano, C., Barone, P., Trojano, L., and Santangelo, G. (2020). Prevalence and clinical aspects of mild cognitive impairment in Parkinson's disease: A meta-analysis. *Mov. Disord.* 35, 45–54. doi: 10.1002/mds.27902

Bohnen, N. I., Albin, R. L., Müller, M. L., and Chou, K. (2011). Advances in therapeutic options for gait and balance in Parkinson's disease. *US Neurol.* 7, 100–108. doi: 10.17925/usn.2011.07.02.100

Bohnen, N. I., Frey, K. A., Studenski, S., Kotagal, V., Koeppe, R. A., Constantine, G. M., et al. (2014). Extra-nigral pathological conditions are common in Parkinson's disease with freezing of gait: An in vivo positron emission tomography study. *Mov. Disord.* 29, 1118–1124. doi: 10.1002/mds.25929

Bohnen, N. I., Müller, M. L., Koeppe, R. A., Studenski, S. A., Kilbourn, M. A., Frey, K. A., et al. (2009). History of falls in Parkinson disease is associated with reduced cholinergic activity. *Neurology* 73, 1670–1676. doi: 10.1212/WNL.0b013e3181c1ded6

Christensen, K. J., Multhaup, K. S., Nordstrom, S., and Voss, K. (1991). A cognitive battery for dementia: Development and measurement characteristics. *Psychol. Assess.* 3, 168–174. doi: 10.1037/1040-3590.3.2.168

Chu, J. S., Liu, T. H., Wang, K. L., Han, C. L., Liu, Y. P., Michitomo, S., et al. (2019). The metabolic activity of caudate and prefrontal cortex negatively correlates with the severity of idiopathic Parkinson's disease. *Aging Dis.* 10, 847–853. doi: 10.14336/ad.2018.0814

Fereshtehnejad, S. M., Romenets, S. R., Anang, J. B., Latreille, V., Gagnon, J. F., and Postuma, R. B. (2015). New clinical subtypes of parkinson disease and their longitudinal progression: A prospective cohort comparison with other phenotypes. *JAMA Neurol.* 72, 863–873. doi: 10.1001/jamaneurol.2015.0703

Giladi, N., Shabtai, H., Simon, E. S., Biran, S., Tal, J., and Korczyn, A. D. (2000). Construction of freezing of gait questionnaire for patients with parkinsonism. *Parkinson. Related Disord.* 6, 165–170. doi: 10.1016/s1353-8020(99)00062-0

Hughes, A. J., Daniel, S. E., Kilford, L., and Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *J. Neurol. Neurosurg. Psychiatry* 55, 181–184. doi: 10.1136/jnnp.55.3.181

Kang, S. H., Park, Y. H., Kim, J. P., Kim, J. S., Kim, C. H., Jang, H., et al. (2021b). Cortical neuroanatomical changes related to specific neuropsychological deficits in subcortical vascular cognitive impairment. *NeuroImage Clin.* 30:102685. doi: 10.1016/j.nicl.2021.102685

Kang, S. H., Kim, J., Kim, I., Moon, Y. A., Park, S., and Koh, S. B. (2021a). Dance intervention using the feldenkrais method improves motor, and non-motor symptoms and gait in Parkinson's disease: A 12-month study. *J. Mov. Disord.* [Epub ahead of print]. doi: 10.14802/jmd.21086

Kang, S. H., Lee, H. M., Seo, W. K., Kim, J. H., and Koh, S. B. (2016). The combined effect of REM sleep behavior disorder and hyposmia on cognition and motor phenotype in Parkinson's disease. *J Neurol Sci.* 368, 374–378. doi: 10.1016/j.jns.2016.07.057

Kang, S. H., Park, Y. H., Lee, D., Kim, J. P., Chin, J., Ahn, Y., et al. (2019). The cortical neuroanatomy related to specific neuropsychological deficits in Alzheimer's continuum. *Dement. Neurocogn. Disord.* 18, 77–95. doi: 10.12779/dnd.2019.18.3.77

Koh, S. B., Kim, J. W., Ma, H. I., Ahn, T. B., Cho, J. W., Lee, P. H., et al. (2012). Validation of the korean-version of the nonmotor symptoms scale for Parkinson's disease. *J. Clin. Neurol.* 8, 276–283. doi: 10.3988/jcn.2012.8.4.276

Litvan, I., Goldman, J. G., Tröster, A. I., Schmand, B. A., Weintraub, D., Petersen, R. C., et al. (2012). Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement disorder society task force guidelines. *Mov. Disord.* 27, 349–356. doi: 10.1002/mds.24893

Lord, S., Galna, B., Coleman, S., Yarnall, A., Burn, D., and Rochester, L. (2014). Cognition and gait show a selective pattern of association dominated by phenotype in incident Parkinson's disease. *Front. Aging Neurosci.* 6:249. doi: 10.3389/fnagi.2014.00249

Lord, S., Rochester, L., Hetherington, V., Allcock, L. M., and Burn, D. (2010). Executive dysfunction and attention contribute to gait interference in 'off' state Parkinson's Disease. *Gait Post.* 31, 169–174. doi: 10.1016/j.gaitpost.2009.09.019

Morris, M. E., Iansek, R., Matyas, T. A., and Summers, J. J. (1994). The pathogenesis of gait hypokinesia in Parkinson's disease. *Brain* 117, 1169–1181. doi: 10.1093/brain/117.5.1169

Morris, M. E., Iansek, R., Matyas, T. A., and Summers, J. J. (1996). Stride length regulation in Parkinson's disease. Normalization strategies and underlying mechanisms. *Brain* 119, 551–568. doi: 10.1093/brain/119.2.551

Müller, M. L., and Bohnen, N. I. (2013). Cholinergic dysfunction in Parkinson's disease. *Curr. Neurol. Neurosci. Rep.* 13:377. doi: 10.1007/s11910-013-0377-9

Nutt, J. G. (2016). Motor subtype in Parkinson's disease: Different disorders or different stages of disease? *Mov. Disord.* 31, 957–961. doi: 10.1002/mds.26657

Pickering, R. M., Grimbergen, Y. A., Rigney, U., Ashburn, A., Mazibrada, G., Wood, B., et al. (2007). A meta-analysis of six prospective studies of falling in Parkinson's disease. *Mov. Disord.* 22, 1892–1900. doi: 10.1002/mds.21598

Pillon, B., Deweer, B., Agid, Y., and Dubois, B. (1993). Explicit memory in Alzheimer's, Huntington's, and Parkinson's diseases. *Arch. Neurol.* 50, 374–379. doi: 10.1001/archneur.1993.00540040036010

Rochester, L., Hetherington, V., Jones, D., Nieuwboer, A., Willems, A. M., Kwakkel, G., et al. (2004). Attending to the task: Interference effects of functional tasks on walking in Parkinson's disease and the roles of cognition, depression, fatigue, and balance. *Arch. Phys. Med. Rehabilit.* 85, 1578–1585. doi: 10.1016/j.apmr.2004.01.025

Rochester, L., Hetherington, V., Jones, D., Nieuwboer, A., Willems, A. M., Kwakkel, G., et al. (2005). The effect of external rhythmic cues (auditory and visual) on walking during a functional task in homes of people with Parkinson's disease. *Arch. Phys. Med. Rehabilit.* 86, 999–1006. doi: 10.1016/j.apmr.2004.10.040

Rochester, L., Nieuwboer, A., Baker, K., Hetherington, V., Willems, A. M., Kwakkel, G., et al. (2008). Walking speed during single and dual tasks in Parkinson's disease: Which characteristics are important? *Mov. Disord.* 23, 2312–2318. doi: 10.1002/mds.22219

Schiess, M. C., Zheng, H., Soukup, V. M., Bonnen, J. G., and Nauta, H. J. (2000). Parkinson's disease subtypes: Clinical classification and ventricular cerebrospinal fluid analysis. *Parkinson. Related Disord.* 6, 69–76. doi: 10.1016/s1353-8020(99)00051-6

Stuart, S., Belluscio, V., Quinn, J. F., and Mancini, M. (2019). Pre-frontal cortical activity during walking and turning is reliable and differentiates across young, older adults and people with Parkinson's disease. *Front. Neurol.* 10:536. doi: 10.3389/fneur.2019.00536

Stuart, S., and Mancini, M. (2020). Prefrontal cortical activation with open and closed-loop tactile cueing when walking and turning in parkinson disease: A pilot study. *J. Neurol. Phys. Ther.* 44, 121–131. doi: 10.1097/npt.0000000000000286

Stuart, S., Morris, R., Giritharan, A., Quinn, J., Nutt, J. G., and Mancini, M. (2020). Prefrontal cortex activity and gait in Parkinson's disease with cholinergic and dopaminergic therapy. *Mov. Disord.* 35, 2019–2027. doi: 10.1002/mds.28214

Wild, L. B., de Lima, D. B., Balardin, J. B., Rizzi, L., Giacobbo, B. L., Oliveira, H. B., et al. (2013). Characterization of cognitive and motor performance during dual-tasking in healthy older adults and patients with Parkinson's disease. *J. Neurol.* 260, 580–589. doi: 10.1007/s00415-012-6683-3

Wojtala, J., Heber, I. A., Neuser, P., Heller, J., Kalbe, E., Rehberg, S. P., et al. (2019). Cognitive decline in Parkinson's disease: The impact of the motor phenotype on cognition. *J. Neurol. Neurosurg. Psychiatry* 90, 171–179. doi: 10.1136/jnnp-2018-319008

Yang, Q., Nanivadekar, S., Taylor, P. A., Dou, Z., Lungu, C. I., and Horovitz, S. G. (2021). Executive function network's white matter alterations relate to Parkinson's disease motor phenotype. *Neurosci. Lett.* 741:135486. doi: 10.1016/j.neulet.2020.135486

Yogev, G., Giladi, N., Peretz, C., Springer, S., Simon, E. S., and Hausdorff, J. M. (2005). Dual tasking, gait rhythmicity, and Parkinson's disease: Which aspects of gait are attention demanding? *Eur. J. Neurosci.* 22, 1248–1256. doi: 10.1111/j.1460-9568.2005.04298.x

Yogev-Seligmann, G., Giladi, N., Gruendlinger, L., and Hausdorff, J. M. (2013). The contribution of postural control and bilateral coordination to the impact of dual tasking on gait. *Exp. Brain Res.* 226, 81–93. doi: 10.1007/s00221-013-3412-9

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# Oculomotor impairments in *de novo* Parkinson's disease

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**Objective:** Reliable electrophysiological indicators are urgently needed in the precise evaluation of Parkinson's disease (PD). It is still elusive whether oculomotor performance is impaired or has clinical value in early PD. This study aims to explore oculomotor performance in newly diagnosed, drug-naïve PD and its correlation with clinical phenotype.

**Methods:** Seventy-five patients with *de novo* PD, 75 patients with essential tremor (ET), and 46 gender-and age-matched healthy controls (HCs) were included in this cross-sectional study. All subjects underwent oculomotor test *via* videonystagmography. Visually guided saccade latency, saccadic accuracy and gain in smooth pursuit eye movement (SPEM) at three frequencies of the horizontal axis were compared among the three groups. Patients with PD also received detailed motor and non-motor evaluation by serial scales. The association between key oculomotor parameters and clinical phenotypes were explored in PD patients.

**Results:** Both *de novo* PD and ET patients showed prolonged saccadic latency and decreased saccadic accuracy relative to HCs. SPEM gain in PD was uniformly reduced at each frequency. SPEM gain at 0.4Hz was also decreased in ET compared with HCs. However, there was no significant difference of oculomotor parameters between *de novo* PD and ET patients. Furthermore, prolonged saccadic latency was correlated with long disease duration, whereas decreased SPEM gain was associated with severe motor symptoms in *de novo* PD patients.

**Conclusion:** Ocular movements are impaired in *de novo*, drug naïve PD patients; these changes could be indicators for disease progression in PD.

KEYWORDS

**Parkinson's disease, essential tremor, ocular movement, videonystagmography, motor symptom**

# Introduction

Parkinson's disease (PD) is a common neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra, contributing to serial motor and non-motor symptoms. Diagnostic and prognostic biomarkers are pivotal in the precise evaluation of PD, as this disease is usually detected in the late stage when dopaminergic neurons have degenerated completely (Lotankar et al., 2017). Clinical electrophysiological studies demonstrated that PD patients also had various oculomotor abnormalities (Guo et al., 2018). The investigation of oculomotor system by recording eye movements provides valuable information about the pathophysiology of PD. As ocular movements can be measured non-invasively and precisely using an infrared eye tracker system, oculomotor alterations have gained great interest as a potential electrophysiological biomarker for precise evaluation of early PD.

Accumulating evidence since 1983 showed that PD patients exhibited oculomotor deficits in saccadic and smooth pursuit eye movement (SPEM) systems relative to the healthy controls (HCs; White et al., 1983; Jung and Kim, 2019). However, ocular impairments in PD vary depending on disease stage and current medication is one of the major challenges in interpretation of the results. In previous studies, improvement of saccade and SPEM in PD patients during treatment with levodopa has been observed (Bares et al., 2003; Michell et al., 2006; Marino et al., 2010). It was reported that cholinergic therapies may increase saccadic latency and reduce amplitude or gain (Naicker et al., 2017). As a result, it is difficult to determine whether deficits occur because of underlying PD pathology or the anti-parkinsonian medications. It was reported that PD patients in the 'off' medication state may exhibit less fixation stability, longer saccadic latency and decreased SPEM gain compared to normal subjects (MacAskill et al., 2002; Marino et al., 2010; Shaikh and Ghasia, 2019). However, most of these studies were observed in the mild-to-moderate stage of the disease. Few studies have explored this issue with patients in the very early stage of the disease, especially *de novo*, drug-naïve patients (Bares et al., 2003; Marino et al., 2010; Linder et al., 2012).

Essential tremor (ET) has been regarded as a monosymptomatic entity characterized by action tremor involving mainly hands and forearms (Haubenberger and Hallett, 2018). The differential diagnosis of ET and PD can be very challenging, especially in the early course of the disease. Recently, the presence of eye movement disturbances including saccadic and SPEM system were also described in ET patients and cerebellar dysfunction may be the cause (Wójcik-Pędziwiatr et al., 2016). Patterns of abnormal ocular movements were supposed to provide a key for differential diagnosis between these two diseases.

The current study aims to (1) establish whether ocular impairment is present in early PD before the introduction of any medication, relative to HCs and ET patients; (2) analyze the association between oculomotor performance and clinical features in PD.

# Materials and methods

## Subjects

We conducted an observational cross-sectional study from Jan 2017 to Dec 2021. This exploratory study included 75 *de novo* patients with PD, 75 patients with ET and 46 age-and gender -matched HCs. Patients were recruited from the Department of Neurology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Patients with PD were included if they met the Movement Disorder Society criteria (Postuma et al., 2015). None of the patients was under dopaminergic medication or had undergone functional neurosurgery for PD. The diagnosis of ET was made according to the consensus criteria proposed by the Tremor Investigation Group (Deuschl et al., 1998). Overall neurological examinations were conducted by two neurologists and eye movements were visually checked. Individuals with restriction of the eye mobility, red or green color blindness, other chronic or acute brain diseases were excluded. 46 HCs were recruited from the local community. Written informed consents were obtained from all participants. The study was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine.

## Clinical and neuropsychological assessments

For PD patients, motor severity was measured with the modified Hoehn and Yahr (H&Y) stage (1967), Unified Parkinson's Disease Rating Scale part III (UPDRS-III; Richards et al., 1994) and the Freezing of Gait questionnaire (Giladi et al., 2000). Motor subtype (tremor-dominant, akinetic-rigid, mixed) was further defined according to the report from Kang et al. (2005). For ET patients, tremor severity was measured by the Tremor Research Group Essential Tremor Rating Scale (TETRAS; Elble et al., 2012). REM Behavior Disorder Screening Questionnaire (RBDSQ) was used as a screening tool of clinical possible RBD (Nomura et al., 2011).The total burden of non-motor symptoms was measured with Non-Motor Symptoms Questionnaire (NMSQuest; Richards et al., 1994). Olfactory function was assessed by the 16-item odor identification test from Sniffin' Sticks (SS-16; Burghart Messtechnik, Wedel, Germany) as our previous report (Chen et al., 2012). The severity of depressive symptom was assessed using the 17-item Hamilton Rating Scale for Depression (HAMD-17; Hamilton, 1960). Total cognitive function was assessed by Chinese version of Mini-Mental State Examination (MMSE; Katzman et al., 1988) and Montreal Cognitive Assessment Basic (MoCA-BC; Xu et al., 2021).

## Videonystagmography evaluation

All the subjects underwent oculomotor test by a Visual Eyes 4 channel VNG (Micromedical Technologies, USA), which acquired

binocular movement samples at 120 Hz. The video-based eye tracker used the center of the pupil to measure the coordinate of the gaze position. Subjects were seated at a distance of 100 cm in front of the screen and remained seated in darkness for 2 min before testing. Participants were required to keep their heads stationary, while moving their eyes according to instructions on screen.

In the saccade task, subjects were required to fixate a small white target on the central spot (0°). The target was stepped 10° or 20° at intervals greater than 2 s, in pseudorandom directions (right or left). The subject was instructed to visually track the target light as rapidly as possible. Each participant was tested 15 times on each side, across a total of 4 amplitudes (10°, 20°, 30°, or 40°). Saccadic latency was defined as the time between the appearance of the target and the start of the main saccade; whereas saccadic accuracy was regarded as saccadic amplitude/target amplitude×100%. In the smooth pursuit task, the subjects were required to pursue the target when the target started to move along the horizontal axis. Horizontal SPEM was conducted over 4 cycles while tracking targets at three frequencies (0.1, 0.2, and 0.4 Hz). SPEM gain was defined as the mean velocity of eye movement/velocity of the target. The oculomotor parameters: mean saccadic latency, mean saccadic accuracy, and gain of SPEM at three different frequencies were automatically recorded and calculated by the analysis system of the machine. In addition, we also manually checked the results. Misdirected visually guided saccade (VGS) and ocular movements that involving blinking or measurement errors were excluded from analyses.

## Statistical analysis

All analyses were performed with SPSS (version 23.0 for Windows), and figures were generated with GraphPad Prism (version 9.0 for Windows). Continuous variables are expressed as the means ± SD or medians (interquartile ranges (IQR, 25th-75th)). Categorical variables are expressed as frequencies and percentages. To compare categorical data among groups, we applied the chi-square test or Fisher's exact test. Comparisons of means between the two groups were performed using the independent *t* test or non-parametric Kruskal–Wallis test, depending on whether the data were normally distributed or not. We analyzed continuous variables among HCs, ET and PD by one-way analysis of variance (ANOVA). The least significant difference (LSD) method was adopted for *post hoc* analysis. Linear regression analysis by backward was used to determine the independent associated factors of key oculomotor dynamics in *de novo* PD patients. The linear regression variables included age, sex, UPDRS III, MoCA-BC and those with significant difference in univariate analysis (*value of p* <0.1). $\beta$ value and 95% confidence intervals (CIs) were reported accordingly. Diagnostic accuracy was evaluated by receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC), sensitivity and specificity were calculated accordingly for each oculomotor parameter. In all analyses, a two tailed *value of p* <0.05 was considered statistically significant.

# Results

## Demographic and clinical features of *de novo* PD patients and ET patients

The general characteristics and clinical features of patients with *de novo* PD and ET were shown in Table 1. Age, gender, education level and MMSE scores were similar among the three groups, whereas ET patients had an earlier onset age ($p < 0.001$) and longer disease duration ($p < 0.001$). In addition, PD had more non-motor symptom burden as shown with higher scores of NMSQuest ($p = 0.001$), RBDSQ ($p = 0.001$), HAMD-17 ($p = 0.013$), and lower score of SS-16 ($p < 0.001$).

## Oculomotor performances in ET patients and *de novo* PD patients

Both ET and PD group showed prolonged saccadic latency (ET: 209 ms, PD: 210 ms, $p < 0.001$) and decreased saccadic accuracy (ET: 89.2%, PD: 88.4%, $p < 0.05$) relative to HC group (Table 2; Figures 1A,B). Meanwhile, SPEM gain at 0.1 Hz ($p = 0.026$), 0.2 Hz ($p = 0.008$) and 0.4 Hz ($p = 0.004$) was significantly decreased in PD compared with HC group (Table 2; Figures 1C–E). ET patients also had decreased SPEM gain at 0.4 Hz ($p = 0.014$, Table 2; Figure 1E) in comparison with HCs. SPEM gain at 0.4 Hz was not associated with tremor severity in ET as revealed by TETRAS score ($r = -0.230$, $p = 0.103$). There was a trend that ET group had low SPEM gain at 0.1 Hz ($p = 0.062$) and 0.2 Hz ($p = 0.063$), but without statistical significance. No obvious difference was observed in saccadic latency, saccadic accuracy and SPEM gain between the ET and PD group.

We performed the ROC analyses of eye movement parameters as independent factors for detecting PD from HCs. However, the AUC of each parameter was lower than 0.7 (Supplementary Table 1). Combining the saccadic latency, saccadic accuracy and the most significant SPEM gain (0.4 Hz) revealed that the model could significantly distinguish PD from HCs with an 80.4% sensitivity and a 73.3% specificity (AUC = 0.78, $p < 0.001$). Only three eye movement parameters were significant in detecting ET from HCs. The combination of the above three parameters could differentiate ET from HCs with a high sensitivity of 84.8%, but a low specificity of 58.1% (AUC = 0.719, $p < 0.001$; Supplementary Table 2). However, oculomotor performance cannot provide additional benefit to distinguish PD from ET.

## Independent associated factors with key oculomotor dynamics in *de novo* PD patients

The independent factors associated with oculomotor dynamics in *de novo* PD were further investigated by linear regression (Tables 3, 4). We found that prolonged saccadic latency was associated with old age ($\beta = 1.296$, 95% CI: 0.154 to 2.439, $p = 0.027$) and long disease duration

TABLE 1 Demographic and clinical features of *de novo* PD and ET patients.

| Items | HC | ET | PD | *p*-value |
|---|---|---|---|---|
| Number, *n* | 46 | 75 | 75 | |
| Age, years | 62.6 ± 7.9 | 62.9 ± 12.9 | 64.5 ± 8.1 | 0.324 |
| Male, *n* (%) | 29 (63.0) | 42 (56.0) | 40 (53.3) | 0.572 |
| Disease duration, months | NA | 60 (24–120) | 12 (6–24) | [a]**<0.001***** |
| Age at onset, years | NA | 53.5 ± 15.4 | 62.9 ± 8.4 | [a]**<0.001***** |
| Education, years | 9 (9–12) | 9 (9–12) | 9 (9–12) | 0.881 |
| Motor features in PD | | | | |
| Hoehn and Yahr stage ≥ 2 (%) | NA | NA | 38 (50.7) | NA |
| UPDRS-III | NA | NA | 19 (13–29) | NA |
| Motor subtype (%) | | | | |
| Tremor-dominant type | NA | NA | 23 (30.7) | NA |
| Akinetic-rigid type | NA | NA | 44 (58.7) | NA |
| Mixed type | NA | NA | 8 (10.7) | NA |
| FOG-Q | NA | NA | 1 (0–4) | NA |
| Non-motor features | | | | |
| NMSQuest | NA | 4.7 ± 4.8 | 6.9 ± 4.3 | [a]**0.001**** |
| SS-16 | NA | 11 (10–13) | 8 (6–10) | [a]**<0.001***** |
| RBDSQ | NA | 1 (1–3.25) | 3 (1–6) | [a]**0.001**** |
| HAMA-17 | NA | 2.5 (0–5) | 4 (2–9) | [a]**0.013*** |
| MMSE | 27.2 ± 1.5 | 27.7 ± 1.9 | 27.6 ± 2.3 | 0.767 |
| MoCA-BC | NA | 23 ± 3.4 | 22.7 ± 4.6 | [a]0.738 |
| Clinical evaluation in ET | | | | |
| TETRAS | NA | 15.13 ± 5.4 | NA | NA |
| TETRAS-ADL | NA | 11.55 ± 7.4 | NA | NA |

[a]Compared between ET and PD.
HC, healthy control; ET, essential tremor; PD, Parkinson's disease; UPDRS, Unified Parkinson's Disease Rating Scale; FOG-Q, Freezing of Gait-Questionnaire; NMSQuest, Non-motor Symptoms Questionnaire; SS-16, 16-item odor identification test from Sniffin' Sticks; RBDSQ, REM Behavior Disorder Screening Questionnaire; HAMD-17, 17-item Hamilton Rating Scale for Depression; SCOPA-AUT, The Scale for Outcomes in PD autonomic dysfunction; MMSE, Mini Mental State Examination; MoCA-BC, Montreal Cognitive Assessment Basic; TETRAS, Tremor Research Group Essential Tremor Rating Scale; TETRAS-ADL, Tremor Research Group Essential Tremor Rating Scale-Activities of daily living; NA, not applicable.
Values were presented as mean ± SD or median (IQR) or number (proportion).
Statistically significant *p* values were shown in bold. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 2 Oculomotor characteristics in HCs, ET, and *de novo* PD patients.

| Items | HC | ET | PD | *p*-value | | |
|---|---|---|---|---|---|---|
| | Group A | Group B | Group C | A vs. B | A vs. C | B vs. C |
| Number, *n* | 46 | 75 | 75 | | | |
| Saccadic latency, ms | 191.3 ± 18.9 | 209.1 ± 34.9 | 210.4 ± 41.3 | **0.007**** | **0.004**** | 0.820 |
| Saccadic accuracy, 0–100% | 92.2 ± 6.1 | 89.2 ± 6.7 | 88.4 ± 6.8 | **0.019*** | **0.003**** | 0.456 |
| SPEM gain 0.1 Hz, 0–1 | 0.74 ± 0.14 | 0.69 ± 0.17 | 0.68 ± 0.15 | 0.055 | **0.034*** | 0.812 |
| SPEM gain 0.2 Hz, 0–1 | 0.79 ± 0.16 | 0.74 ± 0.17 | 0.72 ± 0.16 | 0.06 | **0.009**** | 0.406 |
| SPEM gain 0.4 Hz, 0–1 | 0.78 ± 0.19 | 0.69 ± 0.19 | 0.67 ± 0.19 | **0.014*** | **0.004**** | 0.614 |

HC, healthy control; ET, essential tremor; PD, Parkinson's disease; SPEM, smooth pursuit eye movements.
Values were presented as mean ± SD.
Statistically significant *p* values were shown in bold.
*$p < 0.05$; **$p < 0.01$.

($\beta$=0.378, 95% CI: 0.054 to 0.703, $p$=0.023) by univariate linear regression analysis. After adjusting confounding factors such as sex, the score of UPDRS III and MoCA-BC, only disease duration was an independent factor of prolonged saccadic latency ($\beta$=0.334, 95% CI: 0.014 to 0.654, $p$=0.041; Table 3).

Furthermore, by univariate and multivariate linear regression analysis, we found that high score of UPDRS III was independently associated with low SPEM gain at 0.1 Hz (multivariate regression: $\beta$ = −0.004, 95% CI: −0.008 to-0.001, $p$=0.025) and 0.4 Hz (multivariate regression: $\beta$ = −0.006, 95% CI: −0.01 to-0.002,

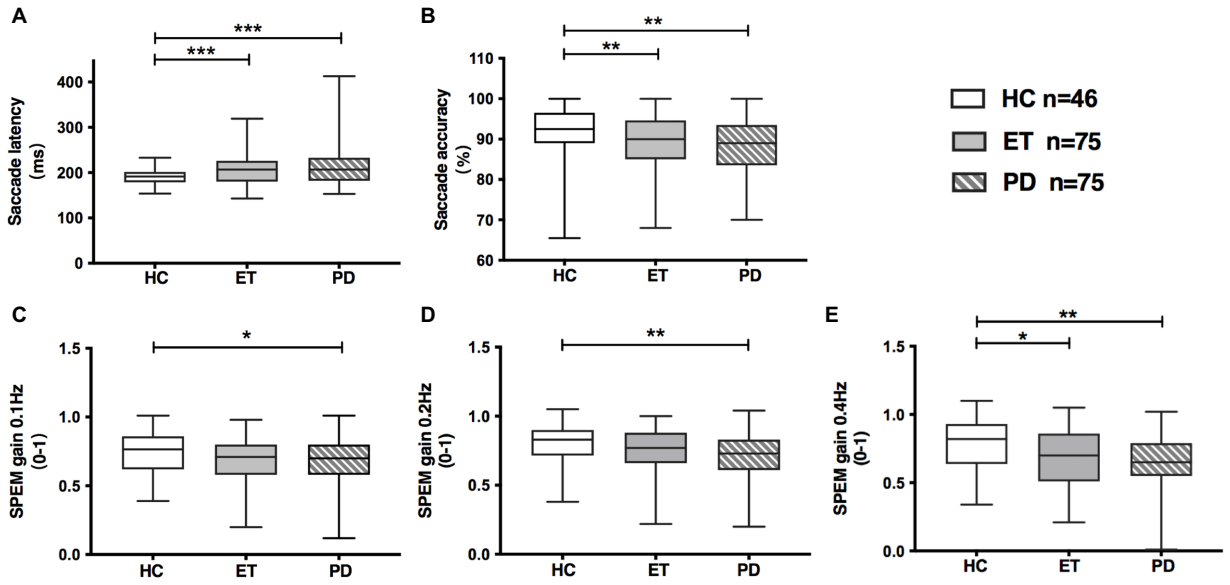


FIGURE 1
Oculomotor parameters in HC, ET and *de novo* PD patients. **(A)** Saccadic latency was increased both in ET and PD group, compared with HC group; **(B)** Saccadic accuracy was decreased both in ET and PD group, compared with HC group; **(C,D)** PD group had decreased gain of smooth pursuit eye movement at 0.1Hz and 0.2Hz, while ET group had marginally decreased gain of smooth pursuit eye movement at 0.1Hz and 0.2Hz, relative to HC group; **(E)** ET and PD patients had decreased gain of smooth pursuit eye movement at 0.4Hz, relative to HCs. Box and whiskers (minimum and maximum) were presented for each group. Middle bars inside boxes represent median, while the edges of boxes indicate first and third quartiles. HC, healthy control; ET, essential tremor; PD, Parkinson's disease; SPEM, smooth pursuit eye movement; *$p$<0.05; **$p$<0.01; ***$p$<0.001.

TABLE 3 Independent associated factors of saccadic latency in *de novo* PD patients.

| Items | Univariate regression | | Multivariate regression | |
|---|---|---|---|---|
| | $\beta$ (95%CI) | $p$-value | $\beta$ (95%CI) | $p$-value |
| Age | 1.296 (0.154, 2.439) | **0.027*** | 1.027 (−0.1, 2.153) | 0.073 |
| Sex | −8.511 (−27.578,10.557) | 0.377 | −7.395 (−25.578,10.788) | 0.420 |
| Disease duration | 0.378 (0.054, 0.703) | **0.023*** | 0.357 (0.039, 0.675) | **0.028*** |
| UPDRS III | 0.607 (−0.131,1.344) | 0.105 | 0.604 (−0.12,1.327) | 0.101 |
| MoCA-BC | 0.275 (−1.818,2.368) | 0.794 | 0.86 (−1.153,2.873) | 0.397 |

PD, Parkinson's disease; CI, confidential interval; UPDRS, Unified Parkinson's Disease Rating Scale. MoCA-BC, Montreal Cognitive Assessment Basic.
Statistically significant $p$ values were shown in bold.
*$p$ < 0.05.

$p$ = 0.008) in *de novo* PD. The association between UPDRS III and SPEM gain at 0.2 Hz was only found in univariate linear regression analysis ($\beta$ = −0.003, 95% CI: −0.006 to-0.0004, $p$ = 0.023), but not in multivariate linear regression analysis (Table 4).

Oculomotor analysis revealed that saccadic latency was not associated with other oculomotor dynamics such as saccadic accuracy or SPEM gain in PD.

## Discussion

This is the first cross-sectional study in China exploring the oculomotor performances in *de novo*, drug naïve PD patients. We observed that newly diagnosed PD patients had prolonged saccadic latency, poorer saccadic accuracy and lower SPEM gain relative to HCs. Impaired oculomotor performances were found to be significantly associated with PD duration and motor severity. No obvious difference in saccadic and SPEM was found between ET and *de novo* PD patients.

Based on prior anatomical and neuroimaging studies (Hikosaka et al., 2000), it was speculated that oculomotor abnormalities may exist in the early stage of PD, even in the prodromal disease stage. However, there have only been a few studies with conflicting results on whether ocular movements are impaired in *de novo* PD (Bares et al., 2003; Marino et al., 2010; Linder et al., 2012;Antoniades et al., 2015; Hanuška et al., 2019). An overview of the comparisons among

TABLE 4 Independent associated factors of smooth pursuit gain in *de novo* PD patients.

| Items | 0.1 Hz | | | | 0.2 Hz | | | | 0.4 Hz | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate regression | | Multivariate regression | | Univariate regression | | Multivariate regression | | Univariate regression | | Multivariate regression | |
| | *β* (95%CI) | *p*-value | *β* (95%CI) | *p*-value | *β* (95%CI) | *p*-value | *β* (95%CI) | *p*-value | *β* (95%CI) | *p*-value | *β* (95%CI) | *p*-value |
| Age | −0.002 (−0.007, 0.002) | 0.295 | −0.001 (−0.006, 0.003) | 0.540 | −0.002 (−0.007, 0.002) | 0.313 | −0.001 (−0.006, 0.004) | 0.580 | −0.003 (−0.008, 0.003) | 0.335 | −0.002 (−0.008, 0.004) | 0.483 |
| Sex | 0.031 (−0.039,0.101) | 0.383 | 0.027 (−0.046,0.1) | 0.462 | 0.023 (−0.052,0.098) | 0.547 | 0.012 (−0.068, 0.093) | 0.760 | 0.035 (−0.053,0.122) | 0.432 | 0.024 (−0.066,0.114) | 0.598 |
| UPDRS III | −0.004 (−0.007,-0.002) | **0.001**** | −0.004 (−0.008,-0.001) | **0.036*** | −0.003 (−0.006,-0.0004) | **0.023*** | −0.002 (−0.006,0.002) | 0.309 | −0.005 (−0.008,-0.002) | **0.002**** | −0.006 (−0.011, −0.001) | **0.012**** |
| FOG-Q | −0.007 (−0.015, 0.001) | 0.080 | 0.003 (−0.008, 0.013) | 0.643 | −0.008 (−0.016, 0.0002) | 0.058 | −0.002 (−0.014, 0.01) | 0.789 | −0.008 (−0.018, 0.002) | 0.097 | 0.005 (−0.009, 0.018) | 0.470 |
| MoCA-BC | 0.004 (−0.003, 0.012) | 0.265 | 0.02 (−0.006, 0.01) | 0.664 | 0.003 (−0.005, 0.011) | 0.451 | 0.001 (−0.007, 0.01) | 0.735 | −0.003 (−0.012, 0.007) | 0.570 | −0.007 (−0.016, 0.003) | 0.183 |
| NMS-quest | −0.008 (−0.016, 0.001) | 0.066 | −0.001 (−0.012, 0.01) | 0.873 | −0.007 (−0.016, 0.002) | 0.124 | 0.001 (−0.013, 0.012) | 0.939 | −0.008 (−0.018, 0.002) | 0.112 | −0.003 (−0.017, 0.011) | 0.696 |
| HAMD17 | −0.006 (−0.012, 0.001) | 0.075 | −0.002 (−0.011, 0.007) | 0.655 | −0.005 (−0.012, 0.002) | 0.181 | −0.003 (−0.012, 0.007) | 0.603 | −0.006 (−0.014, 0.003) | 0.179 | 0.001 (−0.01, 0.012) | 0.874 |

PD, Parkinson's disease; CI, confidential interval; UPDRS, Unified Parkinson's Disease Rating Scale; FOG-Q, Freezing of Gait-Questionnaire; MoCA-BC, Montreal Cognitive Assessment Basic; NMS-Quest, Non-motor Symptoms Questionnaire; HAMD-17, 17-item Hamilton Rating Scale for Depression.
Statistically significant *p* values were shown in bold.
*$p < 0.05$; **$p < 0.01$.

the previous studies is shown in Table 5. Different ocular record tools, sampling rates, saccade tasks and sample size may explain the disparity of the results. We found that *de novo* PD had prolonged saccadic latency, which was consistent with Linder's study (Linder et al., 2012). Also, such kind of patients exhibited lower SPEM gain, as showed in previous two studies (Bares et al., 2003; Marino et al., 2010). However, Linder et al. demonstrated a marginal decrease of SPEM gain in early PD without statistical significance (Linder et al., 2012). The potential reason may be that some patients were under dopaminergic medication in Linder's study. Unlike the current study, Antoniades et al. (2015) and Hanuška et al. (2019) found no discernible difference in saccadic latency between PD and HCs, possibly due to the relatively small sample size. However, these two studies found that anti-saccade appeared to offer a priority over prosaccade in distinguishing early PD from HCs. Further multi-center studies using standardized ocular record tools and tasks will be conducted to validate oculomotor performance in *de novo* PD.

Furthermore, we found that saccadic latency was associated with disease duration and SPEM gain correlated with the motor severity quantified by UPDRS III score in drug naïve PD patients. These findings are consistent with previous studies with patients examined in more advanced stage of the disease (Marino et al., 2007; Terao et al., 2011; Zhang et al., 2018). Neuroimaging evidence has demonstrated that the volume in frontal–parietal regions was reduced and frontal cortex-basal ganglia circuit activity was decreased with disease progression, leading to changes in saccadic parameters (Gorges et al., 2016; Vintonyak et al., 2017; Chen et al., 2022). Anatomical studies have also shown that the basal ganglia may be involved in efficient and automatic SPEM performance(Yoshida and Tanaka, 2009). Neurophysiological recordings in monkeys showed that a subset of neurons in both the external and the internal segments of the globus pallidus modulate SPEM activity (Yoshida and Tanaka, 2009). Significant activation of the caudate nucleus was observed during SPEM in previous imaging studies(O'Driscoll et al., 2000). The SPEM impairments in the current study were in line with the progression of PD motor signs, which may be due to the progressive changes within the basal ganglia. Our results therefore confirmed that the basal ganglia changes in PD patients could impact the operation of cortical and subcortical areas, even at the early stage of the disease. Thus, careful evaluation of the ocular movement performance may provide valuable information for monitoring of the disease.

Similar to previous studies (Gitchel et al., 2013; Wójcik-Pędziwiatr et al., 2016), we found that SPEM in ET patients were abnormal with reduced gain at 0.4 Hz. However, there is much controversy over whether ET patients have eye movement abnormalities in saccadic activity. Our findings are consistent with some previous studies indicating that ET subjects had longer saccadic latency and lower saccadic accuracy than HCs (Gitchel et al., 2013; Wójcik-Pędziwiatr et al., 2017). Two other studies (Helmchen et al., 2003; Trillenberg et al., 2006), however, reported no significant difference in latency of VGSs between the ET and the HC groups. The difference could be explained by the fact that these negative studies only included saccades with a small amplitude (assessed only 10° and 20° saccades). Furthermore, Visser et al. discovered that mean latency in VGSs and anti-saccades performances differed between ET and PD (Visser et al.,

TABLE 5 Serial studies on eye movement evaluations in *de novo* PD patients.

| Study | Sample size, PD/HC | PD treatment | Age and disease duration of PD (mean, years) | VNG machine | Sampling rate | Oculomotor task | Oculomotor characteristics | | Clinical associated factors with oculomotor abnormality in PD |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Saccade | SPEM gain | |
| Bares et al. (2003), Czech Republic | 21/21 | Drug naïve | 59/2.2 | Multichannel Brain-Quick EEG/EMG device (Micromed Ltd., Mogliano, Italy) | / | SPEM | / | Reduced SPEM gain in PD | / |
| Marino et al. (2010), Italy | 10/10 | Drug naïve | 58.5/NA | Vision-based non-intrusive eye tracker | 240 Hz | SPEM | / | Reduced pursuit ocular movements in PD | None |
| Linder et al. (2012), Sweden | 105/38 | Drug naïve or under dopaminergic medication within 3 months | 70/1.2 | VNG Ulmer, Synapsys, Marseille, France | / | Prosaccade & SPEM | Longer saccadic latency in PD | No significant difference | Total axial motor scores were associated with saccadic velocity, precision and SPEM gain |
| Antoniades et al. (2015), UK | 19/20 | Drug naïve | 68/0.67 | Head-mounted oculometer | / | Prosaccade & anti-saccade | No difference in prosaccadic latency, but higher AERs in PD | / | AERs were associated with motor severity |
| Hanuška et al. (2019), Czech Republic | 18/25 | Drug naïve | 62.6/1.6 | Binocular video-based eye tracker (mobile eBT, Eyebrain, Ivry-sur-Seine, France) | 300 Hz | Prosaccade & anti-saccade | No difference in saccadic latency. Higher AERs and lower horizontal prosaccadic gain in PD | / | / |
| The current study (2022), China | 75/46 | Drug naïve | 64.5/1.0 | Visual Eyes 4 channel VNG (Micromedical Technologies, USA) | 120 Hz | Prosaccade & SPEM | Longer saccadic latency in PD | Lower SPEM gain in PD | Prosaccadic latency was associated with disease duration, whereas SPEM gain was associated with motor severity |

HC, healthy control; PD, Parkinson's disease; VNG, videonystagmography; SPEM, smooth pursuit eye movements; AERs, anti-saccadic error rates; NA, not available.

2019), which contradicted our findings. It is possible that this is because our PD patients were at an early stage of the disease, and anti-saccade or more advanced tasks were not further investigated as in Visser's study. As anti-saccade task imposes a higher demand on both cognitive and motor aspects of oculomotor control, future validation studies are needed to investigate the differential value of anti-saccade in ET and *de novo* PD patients.

Although we discovered three ocular parameters with significant difference in separating PD/ET from HCs, the AUC was less than 0.8 in either single or combined parameters (Supplementary Tables1, 2). Furthermore, the ocular parameters could not distinguish ET from *de novo* PD patients. All of these factors made the oculomotor test ineffective as an independent diagnostic tool for the early detection of PD. However, because oculomotor evaluation is a simple, objective, and inexpensive test, it was worth investigating whether combining eye movement evaluation with other clinical features (such as substantia nigra hyperechogenicity) could improve diagnostic of early PD.

The current study has a few limitations that should be mentioned. For starters, because this is a cross-sectional study, only an association between oculomotor parameters and clinical phenotype was discovered. Longitudinal follow-up studies are needed in the future to confirm the importance of ocular movement in the progression of PD. Second, we only have the MMSE for cognitive evaluation in HCs, not the MoCA-BC. Future research should look into how executive dysfunction affects oculomotor performance. Third, in the current study, we used reflexive VGS rather than volitional saccade. Advanced oculomotor tasks (anti-saccade task, for example) in conjunction with fMRI imaging may provide additional clinical value and

insight into the underlying neural basis of PD oculomotor impairments. Fourth, we recruited newly diagnosed, unmedicated PD patients; additional follow-up is required to ensure clinical diagnostic accuracy. Post-mortem studies revealed an 80% accuracy in the initial clinical diagnosis of PD disease (Rizzo et al., 2016). When attempting to correlate an additional diagnostic tool with the disease, this may introduce an additional error.

In conclusion, our Chinese population study confirmed that *de novo* PD patients had oculomotor impairments compared to HCs, primarily with prolonged saccadic latency and decreased SPEM gain. Although these eye movement abnormalities in unmedicated PD could not be distinguished from ET, they were related to disease duration and motor severity in *de novo* PD, implying that these parameters could be used to predict disease progression. In the future, longitudinal follow-up studies with detailed phenotypic evaluation, advanced eye movement tasks, and fMRI imaging will be required.

## 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 the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

M-XZ and QW: conceptualization and formal analysis. M-XZ and WC: methodology and investigation. M-XZ: software and writing—original draft preparation. M-XZ, WC, and QX: validation. M-XZ, QX, YL, LW, and WC: resources. M-XZ, YL, and Y-HJ: data curation. M-XZ, QW, YL, LW, and WC: writing—review and editing. M-XZ and QH: visualization. LZ, Y-RD, J-RL, and WC: supervision. WC: project administration. Y-JC, WC, and J-RL: funding acquisition.All authors contributed to the article and approved the submitted version.

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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.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.985679/full#supplementary-material

## References

Antoniades, C. A., Demeyere, N., Kennard, C., Humphreys, G. W., and Hu, M. T. (2015). Antisaccades and executive dysfunction in early drug-naive Parkinson's disease: the discovery study. *Mov. Disord.* 30, 843–847. doi: 10.1002/mds.26134

Bares, M., Brázdil, M., Kanovský, P., Jurák, P., Daniel, P., Kukleta, M., et al. (2003). The effect of apomorphine administration on smooth pursuit ocular movements in early parkinsonian patients. *Parkinsonism Relat. Disord.* 9, 139–144. doi: 10.1016/s1353-8020(02)00015-9

Chen, W., Chen, S., Kang, W. Y., Li, B., Xu, Z. M., Xiao, Q., et al. (2012). Application of odor identification test in Parkinson's disease in China: a matched case-control study. *J. Neurol. Sci.* 316, 47–50. doi: 10.1016/j.jns.2012.01.033

Chen, J., Zhou, L., Jiang, C., Chen, Z., Zhang, L., Zhou, H., et al. (2022). Impaired ocular tracking and cortical atrophy in idiopathic rapid eye movement sleep behavior disorder. *Mov. Disord.* 37, 972–982. doi: 10.1002/mds.28931

Deuschl, G., Bain, P., and Brin, M. (1998). Consensus statement of the Movement Disorder Society on tremor. Ad Hoc Scientific Committee. *Mov. Disord.* 13, 2–23. doi: 10.1002/mds.870131303

Elble, R., Comella, C., Fahn, S., Hallett, M., Jankovic, J., Juncos, J. L., et al. (2012). Reliability of a new scale for essential tremor. *Mov. Disord.* 27, 1567–1569. doi: 10.1002/mds.25162

Giladi, N., Shabtai, H., Simon, E. S., Biran, S., Tal, J., and Korczyn, A. D. (2000). Construction of freezing of gait questionnaire for patients with Parkinsonism. *Parkinsonism Relat. Disord.* 6, 165–170. doi: 10.1016/s1353-8020(99)00062-0

Gitchel, G. T., Wetzel, P. A., and Baron, M. S. (2013). Slowed saccades and increased square wave jerks in essential tremor. *Tremor Other Hyperkinet Mov (N Y).* 3:tre-03-178-4116-2. doi: 10.7916/D8251GXN

Gorges, M., Müller, H. P., Lulé, D., Pinkhardt, E. H., Ludolph, A. C., and Kassubek, J. (2016). The association between alterations of eye movement control and cerebral intrinsic functional connectivity in Parkinson's disease. *Brain Imaging Behav.* 10, 79–91. doi: 10.1007/s11682-015-9367-7

Guo, L., Normando, E. M., Shah, P. A., De Groef, L., and Cordeiro, M. F. (2018). Oculo-visual abnormalities in Parkinson's disease: possible value as biomarkers. *Mov. Disord.* 33, 1390–1406. doi: 10.1002/mds.27454

Hamilton, M. (1960). A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* 23, 56–62. doi: 10.1136/jnnp.23.1.56

Hanuška, J., Rusz, J., Bezdicek, O., Ulmanová, O., Bonnet, C., Dušek, P., et al. (2019). Eye movements in idiopathic rapid eye movement sleep behaviour disorder: high antisaccade error rate reflects prefrontal cortex dysfunction. *J. Sleep Res.* 28:e12742. doi: 10.1111/jsr.12742

Haubenberger, D., and Hallett, M. (2018). Essential Tremor. *N. Engl. J. Med.* 378, 1802–1810. doi: 10.1056/NEJMcp1707928

Helmchen, C., Hagenow, A., Miesner, J., Sprenger, A., Rambold, H., Wenzelburger, R., et al. (2003). Eye movement abnormalities in essential tremor may indicate cerebellar dysfunction. *Brain* 126, 1319–1332. doi: 10.1093/brain/awg132

Hikosaka, O., Takikawa, Y., and Kawagoe, R. (2000). Role of the basal ganglia in the control of purposive saccadic eye movements. *Physiol. Rev.* 80, 953–978. doi: 10.1152/physrev.2000.80.3.953

Hoehn, M. M., and Yahr, M. D. (1967). Parkinsonism: onset, progression and mortality. *Neurology* 17, 427–442. doi: 10.1212/wnl.17.5.427

Jung, I., and Kim, J. S. (2019). Abnormal eye movements in parkinsonism and movement disorders. *J. Mov. Disord.* 12, 1–13. doi: 10.14802/jmd.18034

Kang, G. A., Bronstein, J. M., Masterman, D. L., Redelings, M., Crum, J. A., and Ritz, B. (2005). Clinical characteristics in early Parkinson's disease in a Central California population-based study. *Mov. Disord.* 20, 1133–1142. doi: 10.1002/mds.20513

Katzman, R., Zhang, M. Y., Ouang Ya, Q., Wang, Z. Y., Liu, W. T., Yu, E., et al. (1988). A Chinese version of the mini-mental state examination; impact of illiteracy in a Shanghai dementia survey. *J. Clin. Epidemiol.* 41, 971–978. doi: 10.1016/0895-4356(88)90034-0

Linder, J., Wenngren, B. I., Stenlund, H., and Forsgren, L. (2012). Impaired oculomotor function in a community-based patient population with newly diagnosed idiopathic Parkinsonism. *J. Neurol.* 259, 1206–1214. doi: 10.1007/s00415-011-6338-9

Lotankar, S., Prabhavalkar, K. S., and Bhatt, L. K. (2017). Biomarkers for Parkinson's disease: recent advancement. *Neurosci. Bull.* 33, 585–597. doi: 10.1007/s12264-017-0183-5

MacAskill, M. R., Anderson, T. J., and Jones, R. D. (2002). Adaptive modification of saccade amplitude in Parkinson's disease. *Brain* 125, 1570–1582. doi: 10.1093/brain/awf168

Marino, S., Lanzafame, P., Sessa, E., Bramanti, A., and Bramanti, P. (2010). The effect of L-Dopa administration on pursuit ocular movements in suspected Parkinson's disease. *Neurol. Sci.* 31, 381–385. doi: 10.1007/s10072-009-0180-1

Marino, S., Sessa, E., Di Lorenzo, G., Lanzafame, P., Scullica, G., Bramanti, A., et al. (2007). Quantitative analysis of pursuit ocular movements in Parkinson's disease by using a video-based eye tracking system. *Eur. Neurol.* 58, 193–197. doi: 10.1159/000107939

Michell, A. W., Xu, Z., Fritz, D., Lewis, S. J., Foltynie, T., Williams-Gray, C. H., et al. (2006). Saccadic latency distributions in Parkinson's disease and the effects of L-dopa. *Exp. Brain Res.* 174, 7–18. doi: 10.1007/s00221-006-0412-z

Naicker, P., Anoopkumar-Dukie, S., Grant, G. D., and Kavanagh, J. J. (2017). Medications influencing central cholinergic neurotransmission affect saccadic and smooth pursuit eye movements in healthy young adults. *Psychopharmacology* 234, 63–71. doi: 10.1007/s00213-016-4436-1

Nomura, T., Inoue, Y., Kagimura, T., Uemura, Y., and Nakashima, K. (2011). Utility of the REM sleep behavior disorder screening questionnaire (RBDSQ) in Parkinson's disease patients. *Sleep Med.* 12, 711–713. doi: 10.1016/j.sleep.2011.01.015

O'Driscoll, G. A., Wolff, A. L., Benkelfat, C., Florencio, P. S., Lal, S., and Evans, A. C. (2000). Functional neuroanatomy of smooth pursuit and predictive saccades. *Neuroreport* 11, 1335–1340. doi: 10.1097/00001756-200004270-00037

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., et al. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* 30, 1591–1601. doi: 10.1002/mds.26424

Richards, M., Marder, K., Cote, L., and Mayeux, R. (1994). Interrater reliability of the unified Parkinson's disease rating scale motor examination. *Mov. Disord.* 9, 89–91. doi: 10.1002/mds.870090114

Rizzo, G., Copetti, M., Arcuti, S., Martino, D., Fontana, A., and Logroscino, G. (2016). Accuracy of clinical diagnosis of Parkinson disease: a systematic review and meta-analysis. *Neurology* 86, 566–576. doi: 10.1212/wnl.0000000000002350

Shaikh, A. G., and Ghasia, F. F. (2019). Saccades in Parkinson's disease: Hypometric, slow, and maladaptive. *Prog. Brain Res.* 249, 81–94. doi: 10.1016/bs.pbr.2019.05.001

Terao, Y., Fukuda, H., Yugeta, A., Hikosaka, O., Nomura, Y., Segawa, M., et al. (2011). Initiation and inhibitory control of saccades with the progression of Parkinson's disease – changes in three major drives converging on the superior colliculus. *Neuropsychologia* 49, 1794–1806. doi: 10.1016/j.neuropsychologia.2011.03.002

Trillenberg, P., Führer, J., Sprenger, A., Hagenow, A., Kömpf, D., Wenzelburger, R., et al. (2006). Eye-hand coordination in essential tremor. *Mov. Disord.* 21, 373–379. doi: 10.1002/mds.20729

Vintonyak, O., Gorges, M., Müller, H. P., Pinkhardt, E. H., Ludolph, A. C., Huppertz, H. J., et al. (2017). Patterns of eye movement impairment correlate with regional brain atrophy in neurodegenerative Parkinsonism. *Neurodegener. Dis.* 17, 117–126. doi: 10.1159/000454880

Visser, F., Bour, L. J., Lee, Y. X., Ten Brinke, T. R., and van Rootselaar, A. F. (2019). Eye movement abnormalities in essential tremor versus tremor dominant Parkinson's disease. *Clin. Neurophysiol.* 130, 683–691. doi: 10.1016/j.clinph.2019.01.026

White, O. B., Saint-Cyr, J. A., Tomlinson, R. D., and Sharpe, J. A. (1983). Ocular motor deficits in Parkinson's disease. II. Control of the saccadic and smooth pursuit systems. *Brain* 106, 571–587. doi: 10.1093/brain/106.3.571

Wójcik-Pędziwiatr, M., Mirek, E., Rudzińska-Bar, M., and Szczudlik, A. (2017). Eye movements in essential tremor patients with parkinsonian and cerebellar signs. *Neurol. Neurochir. Pol.* 51, 299–303. doi: 10.1016/j.pjnns.2017.05.001

Wójcik-Pędziwiatr, M., Plinta, K., Krzak-Kubica, A., Zajdel, K., Falkiewicz, M., Dylak, J., et al. (2016). Eye movement abnormalities in essential tremor. *J. Hum. Kinet.* 52, 53–64. doi: 10.1515/hukin-2015-0193

Xu, Q., Zhou, M., Jiang, C., Wu, L., He, Q., Zhao, L., et al. (2021). Application of the Chinese version of the Montreal cognitive assessment-basic for assessing mild cognitive impairment in Parkinson's disease. *Brain Sci.* 11:1575. doi: 10.3390/brainsci11121575

Yoshida, A., and Tanaka, M. (2009). Neuronal activity in the primate globus pallidus during smooth pursuit eye movements. *Neuroreport* 20, 121–125. doi: 10.1097/WNR.0b013e32831af055

Zhang, Y., Yan, A., Liu, B., Wan, Y., Zhao, Y., Liu, Y., et al. (2018). Oculomotor performances are associated with motor and non-motor symptoms in Parkinson's disease. *Front. Neurol.* 9:960. doi: 10.3389/fneur.2018.00960

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# Assessment of the anti-nociceptive effects of fetal ventral mesencephalic tissue allografts in a rat model of hemi-Parkinson's disease using fMRI

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Extensive studies showed increased subjective pain sensitivity in Parkinson's disease (PD), which appeared to be partially reversed by dopaminergic (DA) treatment. Although cell replacement represents an attractive therapeutic strategy, its potential for PD-related hyperalgesia remains unclear. We investigated re-establishment of DA function *via* allografting exogenic DA cells on pain hypersensitivity in a rat model of PD. We evaluated the anti-nociceptive effects of fetal ventral mesencephalic (rVM) tissue allografts in PD rats after unilateral 6-OHDA-induced toxicity in the medial forebrain bundle. The drug –induced rotation test was used to validate the severity of the nigrostriatal lesion; von Frey and thermal pain tests were employed to evaluate nociceptive function. Nociception-induced cerebral blood volume (CBV) response was measured using a 4.7-T MR system. Finally, the immunohistochemical (IHC) studies were performed and the results were compared with the imaging findings from functional magnetic resonance imaging (fMRI). The grafts significantly improved drug-induced rotation behavior and increased mechanical and thermal nociceptive thresholds in PD rats. The elevation of CBV signals significantly recovered on the grafted striatum, whereas this effect was inhibited by the D2R antagonist eticlopride in each striatum. Quantitative IHC analysis revealed the transplantation markedly increased the numbers of tyrosine hydroxylase immunoreactive cells. Therefore, we concluded transplantation of rVM tissue results in anti-nociceptive effects and improves motor function. Moreover, *in vivo* CBV response confirmed the key role of D2R-mediated pain modulation. Therefore, we demonstrate fMRI as a reliable imaging index in evaluating the anti-nociceptive therapeutic effects of fetal rVM transplantation in the rat model of PD.

KEYWORDS

Parkinson's disease, motor, non-motor, fMRI, neuroimaging, fetal ventral mesencephalic tissue

## Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, and the incidence and prevalence of patients with PD increase steeply with age (Huse et al., 2005). The symptoms of patients with PD may include physical or motor symptoms such as tremors, slowing, stiffening movement, and balance problems (Wasner and Deuschl, 2012). In addition to the motor dysfunction, studies have shown nonmotor symptoms (NMS), such as depression, psychosis, pain, and sleep disturbances, as the important factor for the quality of life of patients with PD (Wasner and Deuschl, 2012). Pain is the most frequent NMS at the onset of disease, and its prevalence increases during the progression of PD (Lee et al., 2006). Pain may precede the onset of motor parkinsonian symptoms and be related to motor fluctuations, early morning dystonia, or secondary causes such as musculoskeletal pain (Quinn et al., 1996). The prevalence of all types of pain is high, but variable. The prevalence of pain is estimated to be between 40 and 85% in patients with PD, with these variations likely due to differences in the study designs, pain assessment methods, or definitions of pain (Cuomo et al., 2019). PD-associated pain is not only a clinically relevant symptom (Djaldetti et al., 2004; Beiske et al., 2009), but is also often overlooked as a consequence of the changes in motor function.

The nigrostriatal pathway has been reported to respond to nociceptive stimuli and also exerts antinociceptive activity (Brooks, 2006). Lesioned nigral dopaminergic (DA) neurons have been proven to exhibit increased sensitivity to pain (Lin et al., 1981; Dieb et al., 2014), whereas activation of DA neurons inhibits the response to pain (Chudler and Dong, 1995). Moreover, clinical evidence and experimental studies have demonstrated the involvement of basal ganglia circuitry in pain modulation. For example, somatosensory information is processed by the basal ganglia *via* several mechanisms, and abnormalities in the basal ganglia affect the encoding of pain (Djaldetti et al., 2004). Moreover, endogenous pain in patients with PD is frequently associated with a higher sensitivity to painful stimuli, and many patients with PD have lower pain thresholds for electrical and heat stimuli (Mylius et al., 2009). Patients at an early stage of the disease also tend to have increased responses to nociceptive stimuli and lower pain thresholds such as enhanced spinal nociceptive reflexes (Zambito Marsala et al., 2011), whereas pain sensory discrimination, e.g., subjective estimation of provoked pain, remains unaltered (Mylius et al., 2009). Enhanced sensitivity to nociceptive stimuli was previously attributed to functional changes at the spinal level (Braak et al., 2007) and within the pain matrix (Gerdelat-Mas et al., 2007), a set of brain areas including the medial pain pathway mainly including the thalamus, the primary and secondary somatosensory cortices (S1 and S2), the anterior/mid cingulate cortex (ACC/MCC) and the insula that consistently respond to painful, as indicated by PET (Brefel-Courbon et al., 2005; Gerdelat-Mas et al., 2007; Mylius et al., 2009). These changes were reversed by dopaminergic treatment, indicating a major role for dopamine depletion in PD-associated pain (Gerdelat-Mas et al., 2007). Taken together, this evidence indicates that numerous circuits at multiple CNS levels are involved in the pain in PD, largely due to anatomical projections of the basal ganglia, which connect specific striatal areas, i.e., the putamen, caudate, and nucleus accumbens (Alexander, 2004).

In the past decade, researchers have undertaken efforts to investigative the mechanisms that underlie the pathology of PD-related pain. In rodents, both systemic administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and intracranial injection of 6-hydroxydopamine (6-OHDA) induce severe lesions of DA neurons in the substantia nigra pars compacta (SNc), and mimic the characteristic pathophysiology of PD. Reduced mechanical and thermal nociceptive thresholds in the hindpaw were demonstrated in these animal models, and this hypersensitivity can be relieved by systemic administration of dopamine receptor agonists (Park et al., 2015; Domenici et al., 2019). Tang et al. (2021) reported that pain hypersensitivity in PD mice was associated with hyperexcitability of superficial dorsal horn neurons, and both were reversed by activation of spinal D2 receptors (Tang et al., 2021). Powerful imaging techniques such as functional magnetic resonance imaging (fMRI) enabled *in vivo* visualization of decreased cerebral blood volume (CBV) in response to nociceptive stimuli in the striatum under normal conditions *via* DA neurotransmission (Chang and Shyu, 2001; Liu et al., 2004; Zhao et al., 2008; Shih et al., 2009). Furthermore, nociception-induced changes in the striatal CBV signals with specificity for DA dysfunction were observed in a rat model of PD, and may be characterized as the pain symptoms of PD (Chen et al., 2013).

Treatment of pain depends on the type of pain and usually requires a multidisciplinary approach. Studies have shown that levodopa (L-dopa) can increase the pain threshold in patients with PD (Brefel-Courbon et al., 2005; Gerdelat-Mas et al., 2007; Schestatsky et al., 2007). Dopamine receptor agonists have also

been demonstrated to relieve nociceptive pain, as described above. Thus, these data suggest that dopamine depletion could contribute to changes in nociceptive processing in PD-related pain, and that dysfunction of the nigrostriatal dopamine system may directly or indirectly affect pain signaling at the spinal cord level. However, the mechanisms how dopamine and its receptors in striatum and spinal cord contribute to pain hypersensitivity in PD remain largely unknown.

Cell therapy in PD have been a major area of research for the last 30 years, with the main focus on using cells to replace the degenerating and lost DA neuronal innervation of the striatum from the nigra (Wijeyekoon and Barker, 2009; Fan et al., 2020). Much experience and some success have been gained from fetal ventral mesencephalic tissue transplants, thus the rapidly advancing field of stem cells may provide attractive alternative options for the treatment of PD (Kordower et al., 1995; Guo et al., 2021). Numerous animal studies have shown that transplantation of DA neurons using either rat ventral mesencephalic (rVM) neural tissue (Weng et al., 2017, 2020), human embryonic stem cells or human fetal ventral mesencephalic neural tissue (Rath et al., 2013; Grealish et al., 2014) improves the motor symptoms in rodent models of PD. However, to our knowledge, no report has validated the potential therapeutic effect of rVM cell transplantation on PD-related nociceptive pain.

One of the major research interests of our group over the past decade has been to investigate the therapeutic effects of transplantation of rVM neural tissue using *in vivo* molecular imaging, i.e., positron emission tomography (PET) using 4-[$^{18}$F]-ADAM targeted serotonin transport or [$^{18}$F] DOPA targeted dopamine synthesis, in 6-OHDA-induced animal PD model (Weng et al., 2013; Chiu et al., 2016; Jhao et al., 2019). Based on the research experience described above, in this study, we hypothesized that transplantation of fetal rVM would beneficially improve the recovery of DA dysfunction in nociceptive impairment. Thus, the aim of this study was to evaluate the feasibility of fMRI as an *in vivo* imaging index for the therapeutic effects of fetal rVM transplantation on PD-related motor and non-motor dysfunction, i.e., pain, in a rat model of PD.

# Materials and methods

## Animals

All animal experiments were approved in advance by the institutional animal care and use committee (NDMC-15-207). Male Sprague Dawley (SD; age, 8-week-old) rats weighing 280–300 g (mean, 290 ± 10 g) from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan) were housed in the Animal Center of the National Defense Medical Center (Taipei, Taiwan), which is certified by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The rats were housed at 23 ± 2°C under a controlled 12-h light/12-h dark cycle. All animals had free access to tap water and a complete pellet diet for 1 week before the experiments began. Only male animals were used in this study to avoid the influence from female reproductive cycles.

## 6-OHDA-induced rat hemi-parkinsonian model

The SD rats were used to establish the parkinsonian model (PD) by lesioning the right medial forebrain bundle (MFB) with 30 μg of 6-hydroxydopamine (6-OHDA; Sigma-Aldrich, Saint Louis, MO, United States) dissolved in 3 μl of ice-cold 0.02% ascorbic acid, as described previously (Weng et al., 2020). Briefly, after anesthetization by intraperitoneal injection of 0.4 ml/kg of 7% chloral hydrate (Riedel-de Haën, Seelze, Germany), the rats were placed in a stereotactic apparatus. The coordinates for the right MFB were 5.3 mm posteriorly, 2.1 mm laterally, and 8.2 mm ventrally from the bregma. The MFB/striatum was lesioned and the left MFB/striatum was used as the unlesioned side/self-control side. Figure 1 shows the conceptual framework of the study with the concept and Figure 2 presents a schematic representation of the study design.

The study described in the manuscript was conducted in the period 2011–2013; however, it should be noted that according to the guideline of the animal study protocol by the Institutional Animal Care and Use Committee guidelines at the National Defense Medical Center, Taipei, Taiwan, R.O.C., the use of chloral hydrate for anesthesia or euthanasia has been restricted in animal study since 2014.

## Motor behavior: Rotation test

Methamphetamine (METH) is an indirect agonist that induces the release of dopamine in the brain (Bustamante et al., 2002). Therefore, we assumed that the right MFB, i.e., the 6-OHDA-lesioned side, would have a much lower response to METH compared to the left, unlesioned MFB. After giving METH, the criteria for the PD model was defined as >300 turns/h (>5 turns/min; Bjorklund and Dunnett, 2019; Jhao et al., 2019). Two weeks after injection of 6-OHDA, 16 6-OHDA-induction and 6 sham control animals were used for METH-induced rotation. The experimental rats were intraperitoneally administered 2.0 mg/kg METH. The recording of rotational behavior was initiated 15 min post-injection and lasted for 60 min.

Based on the results of the rotation test, 12 parkinsonian rats (PD rats) were selected and randomly allocated into PD group: right-side 6-OHDA-lesioned and left-side unlesioned, without treatment ($n = 6$) or PD-rVM group: right-side 6-OHDA-lesioned and left-side unlesioned, and transplanted with rVM tissue ($n = 6$).

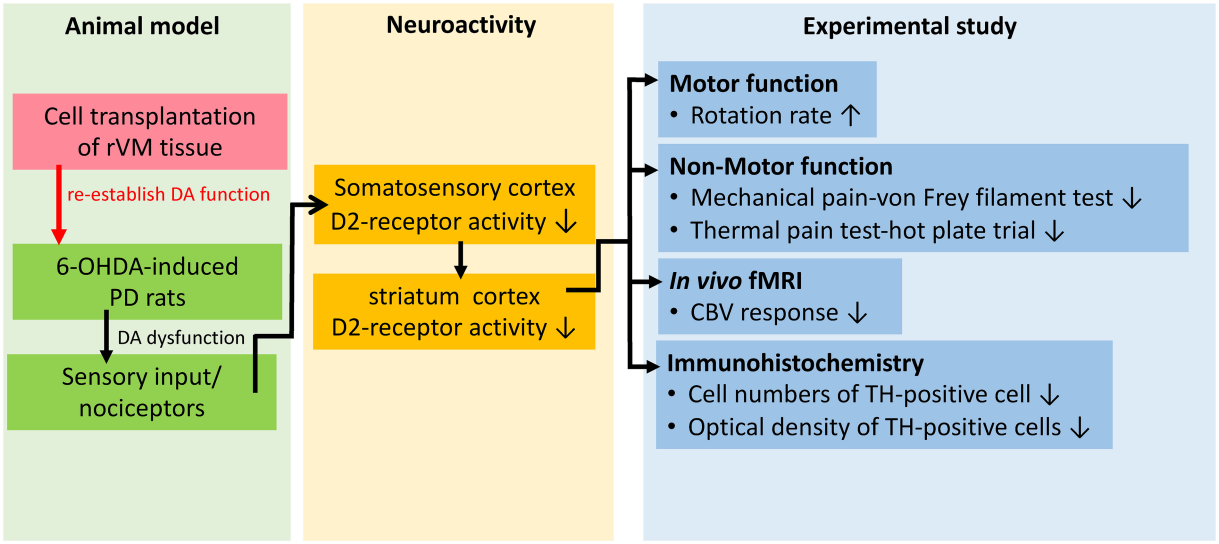


FIGURE 1
Conceptual framework of the study.

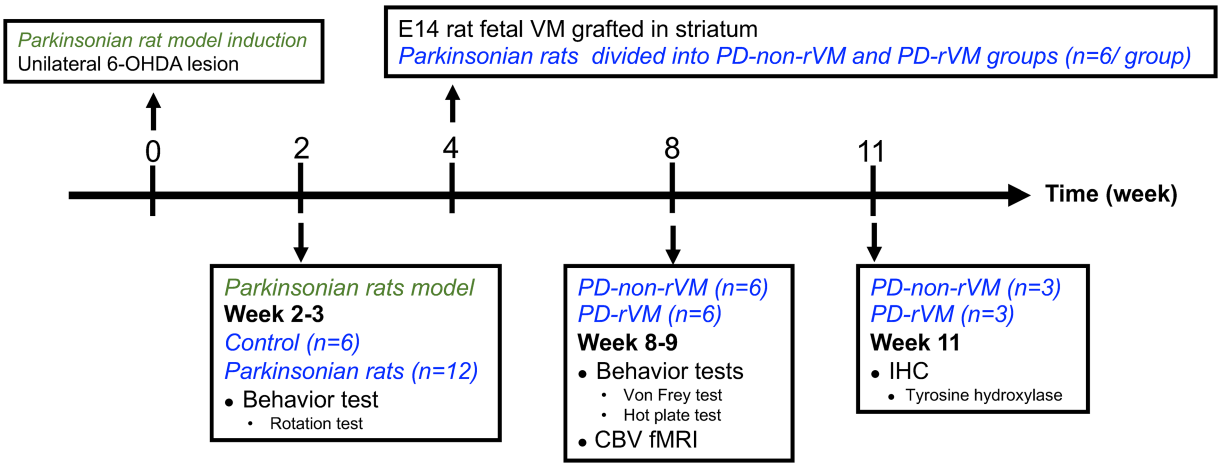


FIGURE 2
Schematic representation of the study design. Unilateral 6-OHDA lesions were induced at week 0 to establish the hemiparkinsonian model. Two weeks later, the animals were subjected to rotation tests; animals that met the PD criteria (>300 turns/h) were randomly allocated to either Group PD (without rVM treatment) or Group PD-rVM (with treatment). Group PD-rVM were transplanted with rVM tissue in week 4. All animals were subjected to behavior tests and CBV fMRI in week 8–9. Immunohistochemistry was performed in week 11.

## Preparation and transplantation of rVM tissue

The PD-rVM group received transplantation/grafts. Briefly, rVM tissue dissected from embryonic E14 rat brains was grafted into the lesioned striatum of the PD-rVM group at week 4, as described by Dunnett, (2000) with some minor modifications (Weng et al., 2017). We ensured the presence of ventral mesencephalon and partial ventral pontine raphe in the dissected tissue slices, which were then placed in Hank's Buffered Salt Solution (Gibco, Grand Island, NY, United States), dissected into small pieces, and transplanted into the ipsilateral striatum using glass microtubes (0.5 mm posterior to the bregma, 2.5 mm lateral to the midline, and 4.5 mm below the dura).

## Pain behavior: Von Frey and hot plate tests

### Mechanical pain: Von Frey filament test

The threshold of mechanical pain was measured in acclimated rodents *via* the up-down method using von Frey filaments on the

plantar surface. Briefly, the rats were placed individually in small cages with a mesh for the von Frey test, and a monofilament (Bio-VF-M; Bioseb, Vitrolles, France) was applied perpendicular to the plantar surface of the hindpaw until the paw buckled. A score ranging between 1.5 and 90 g was assigned in four consecutive positive responses to filaments with decreasing force or three consecutive negative responses to filaments with increasing forces. Nociceptive behaviors, including brisk paw withdrawal, licking, or shaking of the paw, in two trials were considered a positive response. The 50% paw withdrawal threshold (PWT) was determined as described previously (Zhou and Luo, 2015).

## Thermal pain test: Hot plate trial

The hot plate trial was performed 1 day after the von Frey test. Rats were acclimatized for at least 30 min in the test compartment. A heat source (55°C) was positioned on the plantar surface of the hindpaw. Three hot plate trials were conducted, and the mean paw withdrawal latency was recorded. A 20 s cutoff time was used to prevent tissue damage.

## Brain imaging: CBV fMRI

At weeks 8 ~ 9, the rats in the PD and PD-rVM groups ($n = 6$/group) were anesthetized by intravenous injection of 70.0 mg/kg α-chloralose (dissolved in pre-warmed 0.9% saline and 10% polyethylene glycol) for fMRI on a 4.7-T spectrometer (Biospec 47/40; Bruker, Bremen, Germany). A 72 mm volume coil was employed as the radio-frequency transmitter, with a quadrature surface coil on the head as the receiver. Superparamagnetic iron oxide (SPIO; Resovist, Schering, Berlin, Germany) was intravenously administered at 15 mg Fe/kg as the contrast agent to acquire steady-state CBV-weighted fMR images, using previously described settings (Shih et al., 2009, 2012; Chen et al., 2013). Enhanced neural activity increases the influx of SPIO nanoparticles, which leads to a lower signal intensity and represents an increase in regional CBV (Mandeville et al., 2004). A time-series of one hundred gradient-echo images in axial-view were obtained for CBV-weighted fMRI using the following parameters: repetition time, 150 ms; echo time, 15 ms; flip angle, 22.5°; field of view, 2.56 × 2.56 cm; slice thickness, 1.5 mm; acquisition matrix, 128 × 64 (zero-filled to 128 × 128); temporal resolution, 16 s. A total of 100 time-series images were separated equally into five phases (20 fram es for each phase) corresponding to the "off," 'on,' "off," "on," and "off" phases of nociceptive electrical stimulation; the electrical stimulation paradigm is described below.

As shown in Figure 3A, a pair of needle electrodes were used to deliver nociceptive electrical stimulation (10 mA intensity, 3 Hz square wave, 0.5 ms pulse) to the right forepaws of the animals using an A-M Systems model 2,100 constant-current stimulator (Carlsborg, WA, United States). Figures 3B,C presents an example of the correlation map between the CBV signals and the stimulation paradigm. Correlation maps were created by plotting the correlation coefficient (CC) for the CBV signal changes and off–on–off–on–off electrical stimulation paradigm using the cross-correlation method on a voxel-by-voxel basis (Bandettini et al., 1993; Chen et al., 2013). As the experimental conditions affect this correlation, a cut-off point of $r = \pm 0.2$ was empirically selected; this threshold effectively identifies spatial clusters in the striatum and primary somatosensory cortex (S1).

Furthermore, to assess the therapeutic effects of rVM tissue transplantation on DA innervation of the striatum, steady-state CBV weighted fMRI was conducted after intravenous administration of the dopamine D2 receptor antagonist eticlopride (S-(−)-Eticlopride hydrochloride, E101-100MG, Sigma-Aldrich; St. Louis, United States) at a dose of 1.0 mg/kg.

## Immunohistochemistry

At the end of the study period, 6 weeks after cell transplantation, the PD and PD-rVM groups were euthanized for IHC analysis, as described previously (Weng et al., 2017). Briefly, rats were terminally anesthetized with chloral hydrate, perfused with normal saline followed by 4% paraformaldehyde (Sigma-Aldrich), and the brains were excised, post-fixed in 4% paraformaldehyde overnight at 4°C, cryprotected by immersion in 20% sucrose in 0.1 M PBS for 2 days followed by 30% sucrose in 0.1 M PBS for 2 days, and 30-μm-thick coronal sections were obtained using a Leica CM 3050 Cryostat Microtome (Leica Microsystems, Wetzlar, Germany). The sections were rinsed in phosphate-buffered saline (PBS), incubated with 1% hydrogen peroxide (Calbiochem, Torrey Pines, CA, United States) in PBS for 30 min, placed in blocking solution prevents non-specific binding of antibodies to tissue (0.5% Triton X-100 and 3% normal goat serum [Vector, Burlingame, CA, United States] in PBS), and incubated with a primary rabbit recombinant monoclonal anti-TH antibody (1:2,000; Millipore Corporation, Billerica, MA, United States; 4°C overnight), followed by secondary goat anti-rabbit biotinylated IgG antibody (1:200; Vector; 1 h), then avidin-biotin complex (1:200; Vectastain ABC kit, Vector; 60 min), developed in 3,3-diaminobenzidine (0.05%; Sigma-Aldrich) for 6 min, washed thrice with PBS, and mounted on gelatin-coated slides.

To access the number of TH-ir cells, 30 μm striatal micrographs were taken with color CCD camera attached to the confocal microscopy [OPTIPHOT-2 (10×), MICROPHOT-FXA (100× and 200×), Nikon, Tokyo, Japan or Zeiss LSM 880 confocal microscope; ZEISS]. Graft areas were measured by ImageJ, and the density of TH-ir within was expressed as the cell number/mm$^3$.

For semi-quantitative measurement of TH-ir signals, 10× or 100×/200× images in the target and the reference regions (corpus callosum) were taken under the OPTIPHOT-2 microscope or MICROPHOT-FXA microscope (Nikon, Tokyo, Japan), respectively, and converted to 8-bit grayscale images (0–255 gray levels; Mausset-Bonnefont et al., 2003). The optical density (OD) of TH immunoreactivity were semi-quantitively scored using Image-Pro Plus v. 6.0 (Media Cybernetics, Inc., Bethesda, MD, United States), as follows:

OD ratio = (OD of target region − OD of corpus callosum)/OD of corpus callosum.

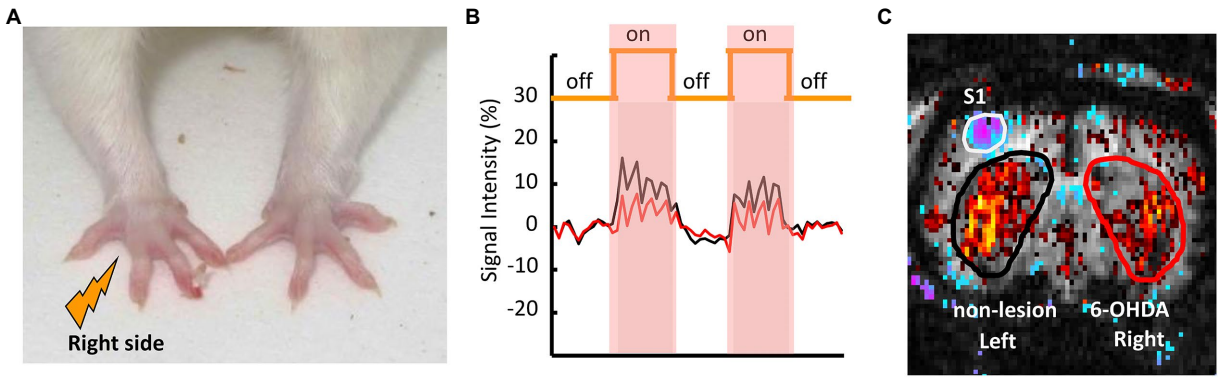


FIGURE 3
Representative fMRI scan of a PD rat and the positions of the ROIs in the 6-OHDA-lesioned and non-lesioned hemispheres. **(A)** Nociceptive electrical stimulation was applied to the right forepaw of the PD rat *via* a pair of needle electrodes. **(B)** The time course of CBV signal intensities during nociceptive stimulation in the left (intact: black) and right (lesioned: red) striata of the PD rat. **(C)** The ROIs for the left non-lesioned striatum are shown in black, and in red for the right 6-OHDA-lesioned striatum.

## Statistical analysis

Data are expressed as mean ± standard deviation. Statistical significances were analyzed using the Student's *t*-test; $^{*}p < 0.05$ was considered significant.

# Results

## Transplantation of rVM tissue improves motor deficits in the parkinsonian rats

The METH-induced rotation test was carried out to evaluate the effects of cell transplantation on DA function in the parkinsonian rats. As shown in Figure 4, a significant increase in net rotation was noted in the parkinsonian rats (14.61 ± 4.89 turns/min, $n = 6$) compared to the normal control group ($n = 6$). However, after rVM transplantation, the PD-rVM group ($n = 6$) exhibited a significant improvement in motor function (2.38 ± 1.20 turns/min, $^{***}p < 0.005$ compared to the PD group).

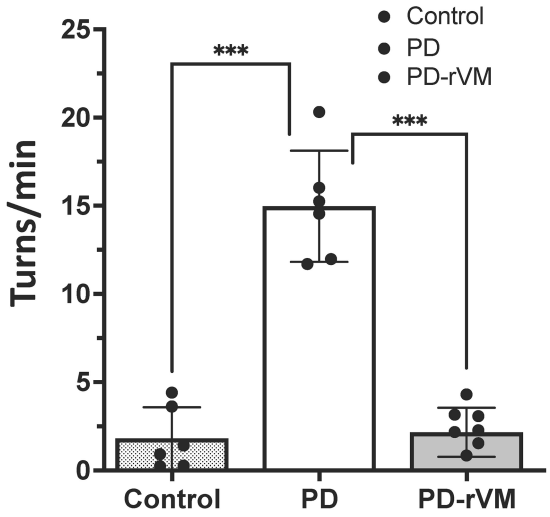


FIGURE 4
METH-induced rotation in 6-OHDA lesioned PD rats. Average turning rates of the control, PD, and PD-rVM groups. Student's *t*-test, $n$=6 per group; $^{***}p$<0.005 compared to control or PD groups.

## Transplantation relieves pain hypersensitivity in parkinsonian rats

The paw withdrawal threshold (PWT) in response to the punctate mechanical stimuli in the Von Frey filament test was measured. In the PD group, a significant lower paw withdrawal threshold was found in the 6-OHDA-lesioned side as compared to that in the unlesioned side ($^{**}p < 0.01$) or to the control group ($^{***}p < 0.005$; Figure 5A). There were no significant differences in the paw withdrawal threshold between the 6-OHDA-lesioned side and unlesioned side in the PD-rVM group; however, the paw withdrawal threshold was significantly higher for the 6-OHDA-lesioned side in the PD-rVM group ($^{*}p < 0.05$) compared to the ipsilesional side in the PD group (Figure 5A).

The response to the thermal stimuli was measured as the latency to paw withdrawal in the hot plate test. In PD group, the latency was much shorter in the 6-OHDA-lesioned side compared to the unlesioned side ($^{***}p < 0.005$) or to the that of control group ($^{***}p < 0.005$). In addition, significantly longer paw withdrawal latencies on the 6-OHDA-lesioned side were revealed in the PD-rVM group when compared to the PD group ($^{***}p < 0.005$, Figure 5B).

## Transplantation of rVM tissue reduces the difference in CBV before and after nociceptive electrical stimulation in parkinsonian rats

Nociceptive electrical stimulation at 3 Hz was applied to the right forepaw during fMRI imaging, and the signal, shown as blue-purple color in left primary somatosensory cortex (S1) was taken as the indication of a successful stimulation. Before applying eticlopride, the dopamine D2R antagonist, the CBV signals in the unlesioned side in PD group were significant higher in the striatum where indicated as red color when compared to the 6-OHDA lesioned side (**$p < 0.01$, Figures 6A,B-pre eti.).

In the group receiving cell transplantation (PD-rVM), stronger CBV signal was still found in the unlesioned side, as compared to that in the 6-OHDA-lesioned side (***$p < 0.005$, Figures 6C,D-pre sti.); however, the difference of blood volume between unlesioned and 6-OHDA-lesioned side was significant lower in the PD-rVM group than that in the PD group (30.5 ± 5.2% vs. 72.9% ± 23.7%, respectively, *$p < 0.05$, Table 1).

## A dopamine D2R antagonist inhibits CBV signals on the 6-OHDA-lesioned side in PD-rVM rats

Next, we used the dopamine D2R antagonist eticlopride to assess whether rVM cell transplantation restores DA function. In the PD group, administration of eticlopride largely inhibited the CBV signals in the 6-OHDA side (*$p < 0.05$, upper-right panel in Figures 6A,B-post eti.). Similar patterns were also observed in the PD-rVM group, as eticlopride significantly inhibited the CBV signals in the 6-OHDA-lesioned side (*$p < 0.05$, lower right panel in Figures 6C,D-post eti).

Moreover, there were significant differences in the CBV in the 6-OHDA-lesioned side between the PD and PD-rVM groups after administration of eticlopride (***$p < 0.005$, Table 1).

## Cell transplantation increases the TH-positive immunoreactivity and cells in hemi-parkinsonian rats

TH staining revealed a low density of residual fibers in the 6-OHDA lesioned striata compared to the unlesioned striata of PD rats (Figure 7, upper panels). TH immunoreactivity in the 6-OHDA-lesioned side was significantly enhanced in the cell transplantation group (PD-rVM; Figure 7, lower panels). Also, stronger TH-positive cell bodies and nerve fibers were found in 6-OHDA lesioned side in striatum of PD-rVM rats than that in PD group (red arrows in the lower right panel of Figure 7).

Furthermore, after cell transplantation, we also found that the number of TH-immunoreactive striatal cells was significantly higher on the lesioned side of PD-rVM group compared to that in the PD group (****$p < 0.0001$, Figure 8A). Semi-quantitative immunohistochemical analysis was performed by normalizing the optical density (OD ratio) of the lesioned side to that of the unlesioned side (100%). Four weeks after cell transplantation, the

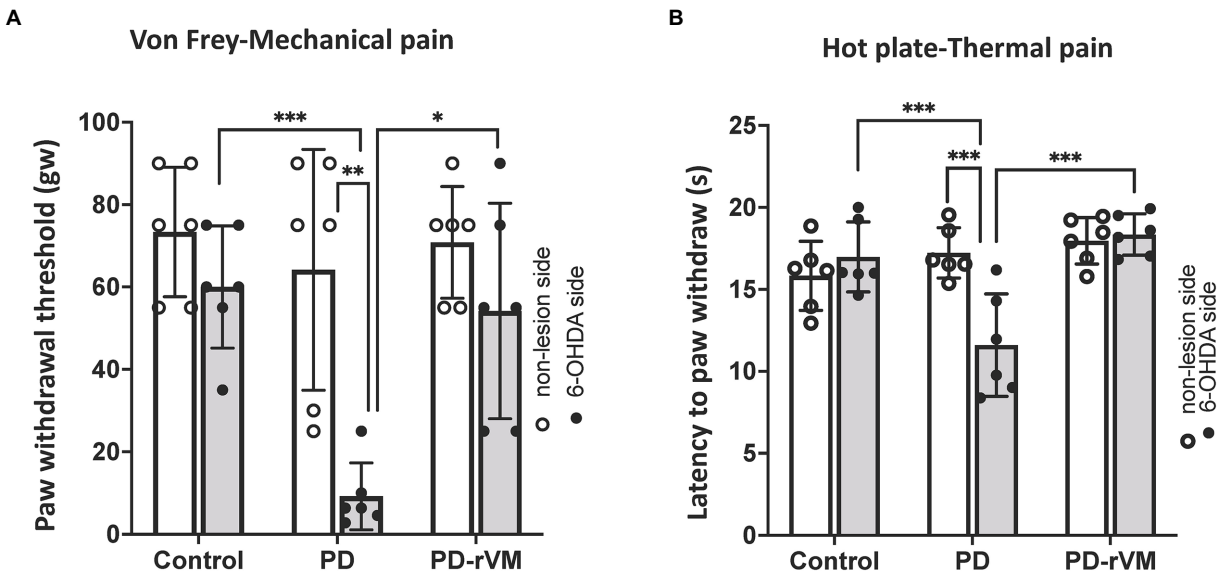


FIGURE 5
Mechanical and thermal pain tests. **(A)** In the von Frey filament test, the average paw withdrawal thresholds were significantly lower on the 6-OHDA lesioned side than the unlesioned side in the PD rats, whereas the PD-rVM group exhibited higher paw withdrawal thresholds to mechanical pain (*$p$<0.05, **$p$<0.01, ***$p$<0.005). **(B)** In the hot plate test, the 6-OHDA-lesioned side of the PD-rVM group showed significantly longer latencies to paw withdraw compared to that of the PD group (***$p$<0.005).

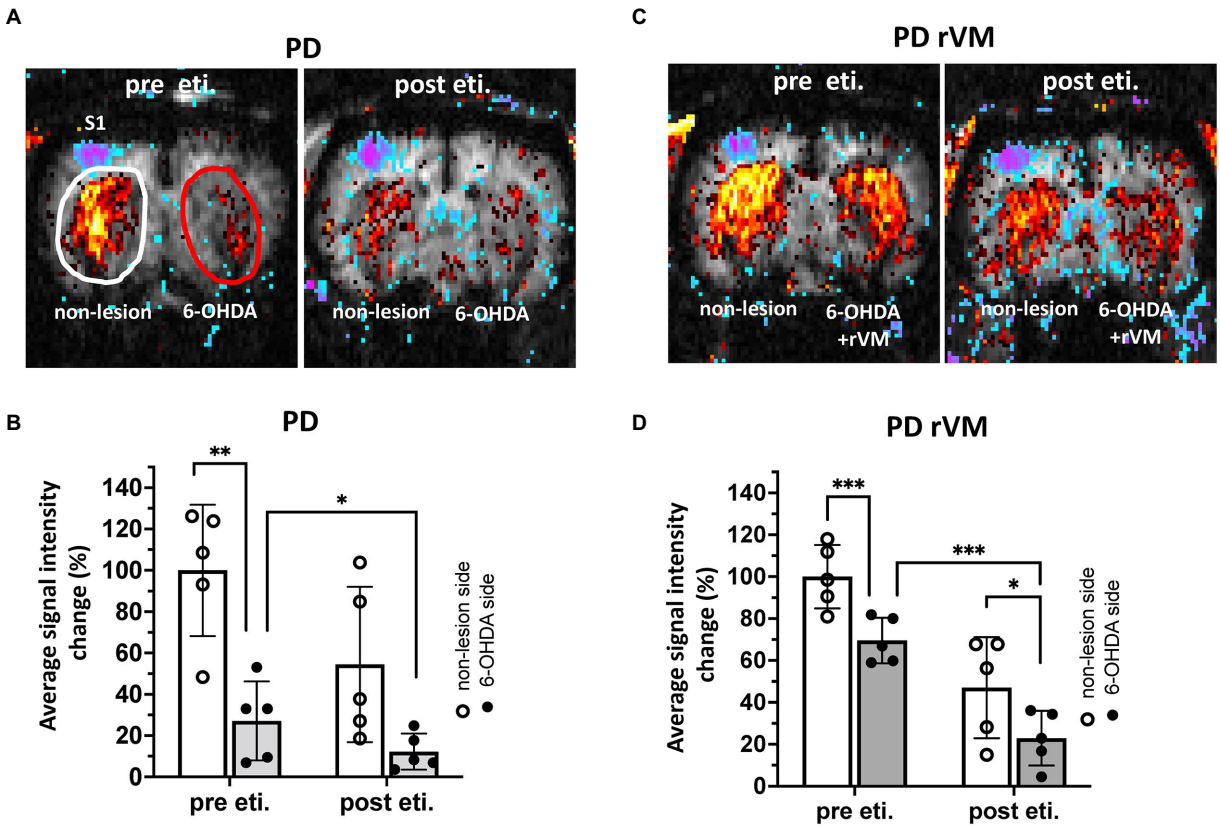


FIGURE 6
*In vivo* fMRI CBV signals in the striatum of 6-OHDA-lesioned PD rats. **(A–C)** The blue-purple signal in left primary somatosensory cortex (S1) where indicated as successful nociceptive electrical stimulation from right forepaw. Representative images of CBV signals was shown in the regions of interest, as white oval denotes non-lesion (left) side and red oval denotes the 6-OHDA side of the striatum. The quantitative results of *in vivo* fMRI CBV signals in 6-OHDA-lesioned PD rats. **(B–D)** Pre eticlopride: In the PD group, the CBV signal on the 6-OHDA-lesioned side was significantly reduced compared to the unlesioned side; however, cell transplantation in the PD-rVM group reduced this difference between the 6-OHDA-lesioned and unlesioned sides from 73 to 30%. **(B–D)** Post eticlopride: Eticlopride significantly reduced the CBV signals in both the PD and PD-rVM groups. *$p$<0.05, **$p$<0.01 and ***$p$<0.005.

TABLE 1 Results of quantitative CBV analysis.

| | PD | | | | PD rVM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Non-lesion | 6-OHDA side | $p$ vs. non-lesion side | Difference between two sides | Non-lesion | 6-OHDA side | $p$ vs. non-lesion side | Difference between two sides | $p$ vs. 6-OHDA side in PD |
| Pre Eti. | 100.0 ± 31.8 | 27.1 ± 19.1 | ** | 72.9 ± 23.7 | 100.0 ± 15.1 | 69.5 ± 10.9 | *** | 30.5 ± 5.2 | * |
| Post Eti. | 54.4 ± 37.7 | 12.2 ± 8.8 | * | 42.2 ± 30.8 | 47.0 ± 24.1 | 22.9 ± 13.1 | * | 24.1 ± 14.8 | *** |
| Pre versus Post eti | ## | # | | | ## | ### | | | |

Data presents as mean ± SD. *$p$ or #$p$ < 0.05, **$p$ or ##$p$ < 0.01, and ***$p$ or ###$p$ < 0.005.

OD values for the 6-OHDA lesioned side were 47% in the PD-rVM rats when normalized to unlesioned side (**$p$ < 0.01, Figure 8B).

## Discussion

In the present study, we used motor and non-motor behavior tests, *in vivo* fMRI, and immunohistochemistry to examine the anti-nociceptive effects of rVM cell transplantation in the striatum following unilateral induction of lesions in DA neurons by micro-injure of 6-OHDA into the right MFB. To the best of our knowledge, this is the first evidence of the anti-nociceptive effects of transplantation of E14 fetal rVM cells based on motor and non-motor behavior tests and *in vivo* fMRI.

We demonstrated that rats with unilateral intrastriatal 6-OHDA-induced PD exhibited profound asymmetry in

motor performance tests, characterized by asymmetric body posture, impaired use of the contralateral forelimb, and sensorimotor orientation defects, i.e., loss of orientating movements elicited by application of sensory stimuli to the body contralateral to the lesion. Moreover, previous reports support the turning rates of the rats with 6-OHDA lesions in the striatum or MFB, as non-linear relationships were observed between turning and the reduction in the DA concentration in the lesioned striatum, loss of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra, and loss of TH-positive innervation in the ipsilateral striatum. These results suggest that at least 40–50% reduction of the nigral TH-positive neurons, striatal TH-positive fibers, and striatal DA levels are required to invoke significant turning (Lee et al., 1996; Kirik et al., 2001; Heuer et al., 2012; Tronci et al., 2012). AMPH-induced rotation was also reported as a sensitive practical and convenient test of functional recovery in cell transplantation, as transplants containing fewer than 500 surviving DA neurons matched well with the number of surviving DA neurons and striatal DA concentration (Brundin et al., 1988; Kirik et al., 2001).

Albeit the usefulness of 6-OHDA-induced PD model was demonstrated by the METH-induced motor function tests in the evidence above and in the current study (Jagmag et al., 2015; Gomez-Paz et al., 2018), quantification analysis of TH-ir striatal and nigral cells, as well as additional motor behavior studies such as gait analysis and forelimb akinesia, provide stronger assessment of the extent of neurotoxin, i.e., MPTP or 6-OHDA in this study, elicited lesions of nigrostriatl DA neurons.

Additionally, we revealed reduced mechanical and thermal detection thresholds in the unilateral hindpaws of the unilateral 6-OHDA-lesioned rats. We also demonstrated the anti-nociceptive effects, i.e., relief of mechanical and thermal pain sensation, of rVM transplantation, which were supported by earlier studies using fetal rVM (Takeda et al., 2014) or chromospheres (Gomez-Paz et al., 2018). However unilateral SNc lesions of DA neurons impaired motor function on the contralateral side (Yoon et al., 2014; Zhou et al., 2019). This finding might partially support the notion that 6-OHDA-induced lesions result in pain hypersensitivity and motor deficits *via* different pathophysiological mechanisms. These observations may also explain why treatments for motor deficits do not produce comparable benefits for pain symptoms in patients with PD.

Noxious electrical somatosensory stimulation reduced bilateral CBV in the caudate putamen (CPu), which is associated with increased neural activity in the region involved in strong vasoactive neurotransmission (Shih et al., 2009). Furthermore, the decreases in striatal CBV modulated by D2-like dopamine receptors (D2R) observed by fMRI represent an important mechanism that occurs during neurovascular processing of nociception (Hagelberg et al., 2004; Martikainen et al., 2005). Moreover, Hsu et al. (2014) reported that changes in negative BOLD fMRI signals during nociceptive-stimulation in PD rats were associated with altered Ca2+ signaling in the DA system (Hsu et al., 2014).

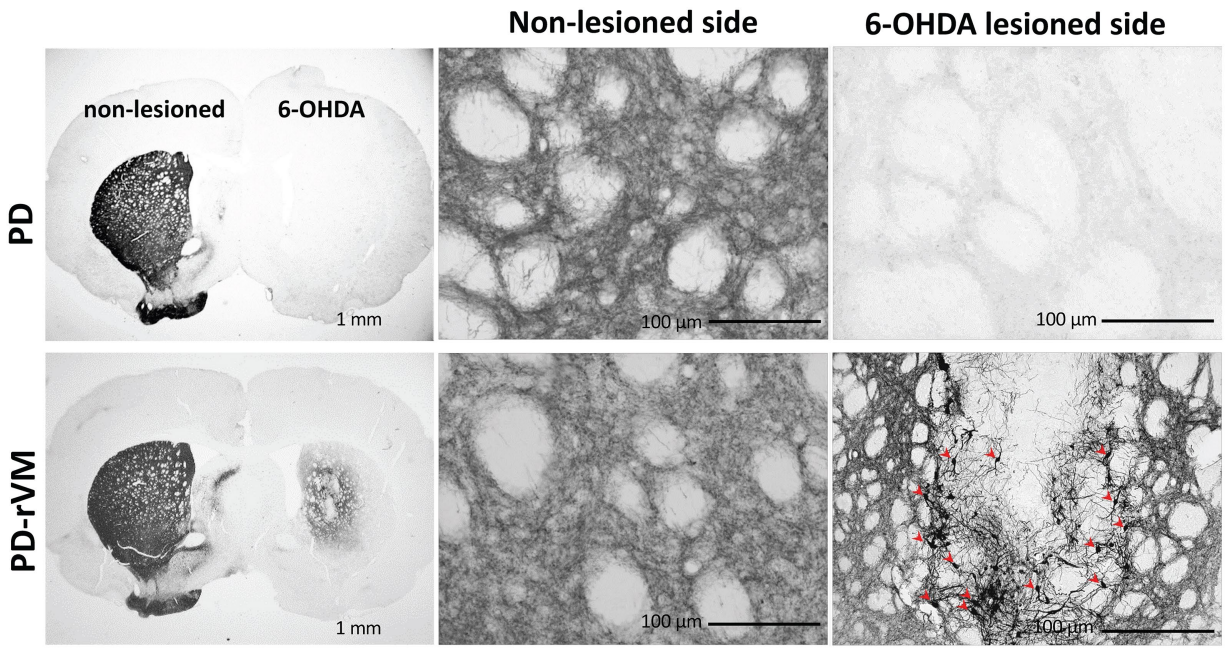


FIGURE 7
Tyrosine hydroxylase immunostaining in the brain of PD rats. In the PD group, the 6-OHDA-lesioned side exhibited much lower TH immunoreactivity than the unlesioned side. In contrast, cell transplantation significantly increased TH immunoreactivity in the PD-rVM group. The TH-positive cells in the striatum on the 6-OHDA-lesioned side of from the PD-rVM group are indicated by red arrows (lower right panel).

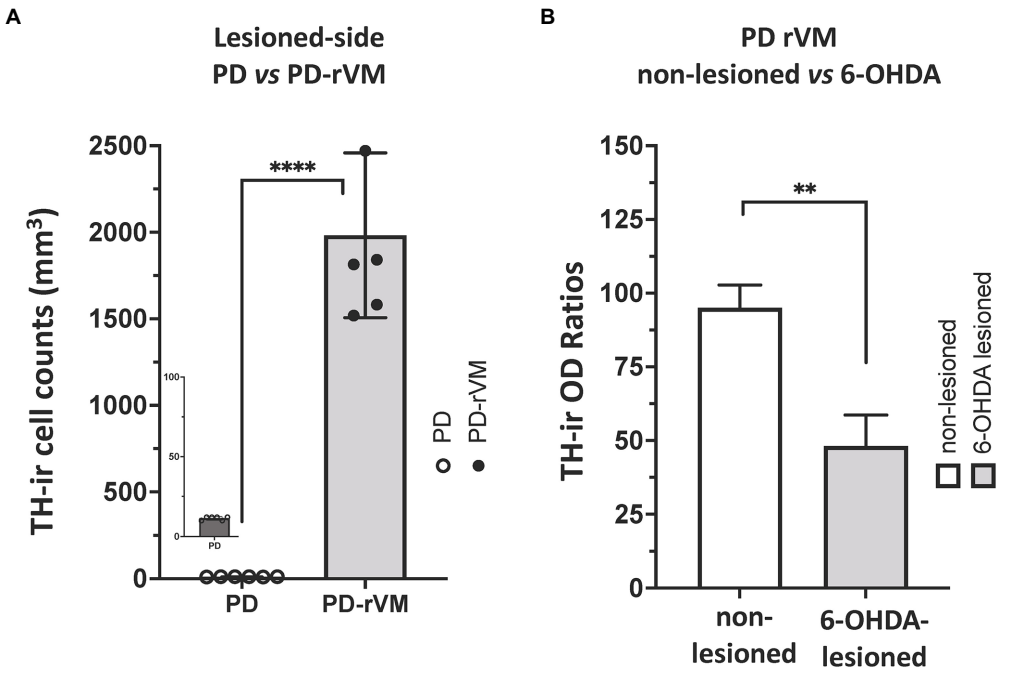


FIGURE 8
Quantitative immunohistochemical analysis of TH immunoreactivity in the striatum of PD rats. **(A)** The number of TH-positive cells was much higher in the lesioned striata of the PD rats receiving cell transplantation (PD-rVM). **(B)** In PD-rVM group, at 4weeks after cell transplantation, TH-ir OD ratios reached to 47% when normalized to unlesioned side (100%). $^{**}p<0.01$, $^{****}p<0.001$.

Lesioning striatal DA neurons increases pain sensitivity (i.e., leads to a lower pain threshold); thus, conversely, recovery of the lesioned neurons may inhibit pain responses (Lin et al., 1984). Therefore, the present results demonstrate that the reduced CBV signals of the lesioned striatum in response to electrical stimulation could be partially explained by DA dysfunction during activation of the nigrostriatal system. Also, administration of the D2R antagonist eticlopride clearly blocked the CBV signals on the unlesioned side. These findings support the suggestion that D2R mediated decreases in CBV play a key role in the anti-nociceptive effects of pain modulation.

Additional to the recovery of behavioral function, our results also showed restored CBV signals in the rVM group, which may be explained by the re-innervation of exogenous rVM cells to dopaminergic fibers (Wakeman et al., 2011). The reduced metabolic demand or loss of cholinergic neurons innervating cortex and microvessels resulted in the reduction of CBV in PD (Globus et al., 1985). Significant region-specific decrease of CBV in the substantia nigra, caudate and putamen in PD patients compared to controls has been evidenced *via* advanced *in vivo* MR or single-photon emission computed tomography (SPECT) imaging (Paez et al., 2020) and the change of regional CBV could be considered as a clinical feature for objective evaluation of disease progression (Taguchi et al., 2019). For rVM-based therapies, there are two major factors for evaluation, i.e., efficiency of exogenous rVM cell transplantation and boosting self-repair of endogenous neural stem cells. In summary, based on our current results, we suggested rVM-based therapies could improve the CBV signal and *in vivo* monitored by using fMRI.

It should be noted that the majority of preclinical validation studies used to assess the therapeutic approaches have been performed in the 6-OHDA rodent model; however, the limitation of 6-OHDA model of PD could be while this is a useful rodent model to assess rVM therapeutic efficiency, it may not reflect the pathological features or progressive status of the disease, such as the abnormal accumulation of misfolded alpha-synuclein (a-Syn) aggregates in different brain areas and its association with neuronal degeneration in PD. Given the fact that Alpha-synuclein (a-Syn) is central to the pathology of PD, mature a-Syn based animal PD models would be valuable to investigate the disease mechanism and therapeutic strategies (Gomez-Benito et al., 2020). Transplantation of human pluripotent stem cells (hPSCs)-derived DA neurons have been reported recently (Chen et al., 2016; Nolbrant et al., 2017). However, controversies do exist surrounding the advantages and disadvantages of different tissue origins for the DA cell replacement therapy in PD. The nature of fetal VM containing high percentage of dopaminergic precursor cells may bypass cell culture or *in vitro* differentiation before transplantation (Laguna and Barker, 2009). Moreover, it appeared that no tumor formation was reported from fetal VM tissue in PD transplantation therapy (Nanni et al., 2007; Laguna and Barker, 2009). However, the access to human fetal tissue from legally terminated embryos and the difficulty to optimize, and standardize the protocols limited the application of fetal VM

tissue-based therapy (Barker and Consortium, 2019; Depierreux et al., 2021).

Nevertheless, with the experiences we established, the future work would be to evaluate the potential of *in vivo* imaging tool with rVM-derived DA neurons in a-Syn-based PD model. Based on the specificity and sensitivity for the DA synthesis in the nigrostriatal pathway, PET molecular imaging of striatal [$^{18}$F]-DOPA uptake has been demonstrated as one of the most reliable tool for the *in vivo* diagnosis of PD, as well as the measurement of terminal loss of dopamine (Nanni et al., 2007; Depierreux et al., 2021). Therefore, [$^{18}$F]-DOPA PET and CBV fMRI coupling imaging could be a promising *in vivo* therapeutic diagnosis tool for hPSCs or D2R target therapy.

The current results showed that unilateral 6-OHDA lesions of the MFB in rats can be used to model specific changes in pain behavior with Von Frey and hot plate tests in PD. By using the contralateral side to control for individual differences and analyzing the CBV with fMRI, it is possible to assess disease progression as a function of changes in anti-nociceptive effects. Chen C V. et al. demonstrated that the hypersensitivity to pain stimulus mainly occurred on the side of the body ipsilateral to the right SN lesion and the reduced responsiveness of the striatal CBV reactions occurred ipsilateral to the right SN lesion (Chen et al., 2013). However, the interpretation of the current results was limited by the lack of sham control, and it would be required to avoid misleading or to have a better understanding between disease status of PD and normal control.

Overall, our data indicate that cell replacement therapy alleviated pain and thermal dysesthesia, and correlated with improved motor function in the PD rats. The fMRI data revealed that the grafted striatum exhibited a recovery of the CBV response to nociceptive stimuli. Moreover, we showed the recovery of the CBV signals in the grafted striatum were inhibited by the D2R antagonist eticlopride, which confirmed that rVM tissue transplantation restored DA cells. In summary, good correlations were observed between the recovery of the nociception-induced CBV responses in the motor and non-motor behavior tests and DA innervation in the IHC studies of the grafted striatum.

## Conclusion

In summary, the present study demonstrates unilateral microinjection of 6-OHDA into the MFB in rats replicates the motor and non-motor deficits of PD. We also show that transplantation of E14 fetal rVM tissue results in anti-nociceptive effects and improves motor function. Moreover, *in vivo* fMRI revealed that the E14 fetal grafts reversed the reductions in the CBV signals observed in the 6-OHDA-lesioned striatum. *In vivo* CBV signaling confirmed the key role of D2R-mediated pain modulation. Therefore, we conclude that rVM tissue transplantation represents a promising therapeutic approach for the treatment of PD-related pain.

## 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 animal study was reviewed and approved by the institutional animal care and use committee of the National Defense Medical Center.

## Author contributions

C-HC and K-HM: conceptualization. C-HC and S-JW: methodology and software. Y-TJ and S-JW: validation. C-HC and SH-H: formal analysis and writing—original draft preparation. W-SH, H-FC, and C-YC: investigation. C-HC, S-JW, and Y-TJ: resources. K-HM and C-HC: data curation and funding acquisition. S-JW, K-HM, Y-TJ, and SH-H: writing—review and editing. C-HC: visualization and project administration. C-CY and K-HM: supervision. All authors contributed to the article and approved the submitted version.

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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.

## Publisher's note

# References


Alexander, G. E. (2004). Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. *Dialogues Clin. Neurosci.* 6, 259–280. doi: 10.31887/DCNS.2004.6.3/galexander

Bandettini, P. A., Jesmanowicz, A., Wong, E. C., and Hyde, J. S. (1993). Processing strategies for time-course data sets in functional MRI of the human brain. *Magn. Reson. Med.* 30, 161–173. doi: 10.1002/mrm.1910300204

Barker, R. A., and Consortium, T. (2019). Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nat. Med.* 25, 1045–1053. doi: 10.1038/s41591-019-0507-2

Beiske, A. G., Loge, J. H., Ronningen, A., and Svensson, E. (2009). Pain in Parkinson's disease: prevalence and characteristics. *Pain* 141, 173–177. doi: 10.1016/j.pain.2008.12.004

Bjorklund, A., and Dunnett, S. B. (2019). The amphetamine induced rotation test: A re-assessment of its use as a tool to monitor motor impairment and functional recovery in rodent models of Parkinson's disease. *J. Parkinsons Dis.* 9, 17–29. doi: 10.3233/JPD-181525

Braak, H., Sastre, M., Bohl, J. R., De Vos, R. A., and Del Tredici, K. (2007). Parkinson's disease: lesions in dorsal horn layer I, involvement of parasympathetic and sympathetic pre- and postganglionic neurons. *Acta Neuropathol.* 113, 421–429. doi: 10.1007/s00401-007-0193-x

Brefel-Courbon, C., Payoux, P., Thalamas, C., Ory, F., Quelven, I., Chollet, F., et al. (2005). Effect of levodopa on pain threshold in Parkinson's disease: a clinical and positron emission tomography study. *Mov. Disord.* 20, 1557–1563. doi: 10.1002/mds.20629

Brooks, D. J. (2006). Dopaminergic action beyond its effects on motor function: imaging studies. *J. Neurol.* 253 Suppl 4:IV8-15. doi: 10.1007/s00415-006-4003-5

Brundin, P., Barbin, G., Strecker, R. E., Isacson, O., Prochiantz, A., and Bjorklund, A. (1988). Survival and function of dissociated rat dopamine neurones grafted at different developmental stages or after being cultured in vitro. *Brain Res.* 467, 233–243. doi: 10.1016/0165-3806(88)90027-2

Bustamante, D., You, Z. B., Castel, M. N., Johansson, S., Goiny, M., Terenius, L., et al. (2002). Effect of single and repeated methamphetamine treatment on neurotransmitter release in substantia nigra and neostriatum of the rat. *J. Neurochem.* 83, 645–654. doi: 10.1046/j.1471-4159.2002.01171.x

Chang, C., and Shyu, B. C. (2001). A fMRI study of brain activations during non-noxious and noxious electrical stimulation of the sciatic nerve of rats. *Brain Res.* 897, 71–81. doi: 10.1016/s0006-8993(01)02094-7

Chen, C. C., Shih, Y. Y., and Chang, C. (2013). Dopaminergic imaging of nonmotor manifestations in a rat model of Parkinson's disease by fMRI. *Neurobiol. Dis.* 49, 99–106. doi: 10.1016/j.nbd.2012.07.020

Chen, Y., Xiong, M., Dong, Y., Haberman, A., Cao, J., Liu, H., et al. (2016). Chemical control of grafted human PSC-derived neurons in a mouse model of Parkinson's disease. *Cell Stem Cell* 18, 817–826. doi: 10.1016/j.stem.2016.03.014

Chiu, C. H., Li, I. H., Weng, S. J., Huang, Y. S., Wu, S. C., Chou, T. K., et al. (2016). PET imaging of serotonin transporters with 4-[(18)F]-ADAM in a parkinsonian rat model with porcine neural xenografts. *Cell Transplant.* 25, 301–311. doi: 10.3727/096368915X688236

Chudler, E. H., and Dong, W. K. (1995). The role of the basal ganglia in nociception and pain. *Pain* 60, 3–38. doi: 10.1016/0304-3959(94)00172-B

Cuomo, A., Crispo, A., Truini, A., Natoli, S., Zanetti, O., Barone, P., et al. (2019). Toward more focused multimodal and multidisciplinary approaches for pain management in Parkinson's disease. *J. Pain Res.* 12, 2201–2209. doi: 10.2147/JPR.S209616

Depierreux, F., Parmentier, E., Mackels, L., Baquero, K., Degueldre, C., Balteau, E., et al. (2021). Parkinson's disease multimodal imaging: F-DOPA PET, neuromelanin-sensitive and quantitative iron-sensitive MRI. *NPJ Parkinsons Dis* 7:57. doi: 10.1038/s41531-021-00199-2

Dieb, W., Ouachikh, O., Durif, F., and Hafidi, A. (2014). Lesion of the dopaminergic nigrostriatal pathway induces trigeminal dynamic mechanical allodynia. *Brain Behav.* 4, 368–380. doi: 10.1002/brb3.214

Djaldetti, R., Shifrin, A., Rogowski, Z., Sprecher, E., Melamed, E., and Yarnitsky, D. (2004). Quantitative measurement of pain sensation in patients with Parkinson disease. *Neurology* 62, 2171–2175. doi: 10.1212/01.wnl.0000130455.38550.9d

Domenici, R. A., Campos, A. C. P., Maciel, S. T., Berzuino, M. B., Hernandes, M. S., Fonoff, E. T., et al. (2019). Parkinson's disease and pain: modulation of nociceptive circuitry in a rat model of nigrostriatal lesion. *Exp. Neurol.* 315, 72–81. doi: 10.1016/j.expneurol.2019.02.007

Dunnett, B. A. (2000). "Dissecting embryonic neural tissues for transplantation," in *Neural Transplantation Methods*. eds. S. B. Dunnett, A. A. Boulton and G. B. Baker (Totowa, NJ: Humana Press).

Fan, Y., Winanto, , and Ng, S. Y. (2020). Replacing what's lost: a new era of stem cell therapy for Parkinson's disease. *Transl. Neurodegener* 9:2. doi: 10.1186/s40035-019-0180-x

Gerdelat-Mas, A., Simonetta-Moreau, M., Thalamas, C., Ory-Magne, F., Slaoui, T., Rascol, O., et al. (2007). Levodopa raises objective pain threshold in Parkinson's disease: a RIII reflex study. *J. Neurol. Neurosurg. Psychiatry* 78, 1140–1142. doi: 10.1136/jnnp.2007.120212

Globus, M., Mildworf, B., and Melamed, E. (1985). Cerebral blood flow and cognitive impairment in Parkinson's disease. *Neurology* 35, 1135–1139.

Gomez-Benito, M., Granado, N., Garcia-Sanz, P., Michel, A., Dumoulin, M., and Moratalla, R. (2020). Modeling Parkinson's disease with the alpha-Synuclein protein. *Front. Pharmacol.* 11:356. doi: 10.3389/fphar.2020.00356

Gomez-Paz, A., Drucker-Colin, R., Milan-Aldaco, D., Palomero-Rivero, M., and Ambriz-Tututi, M. (2018). Intrastriatal Chromospheres' transplant reduces nociception in Hemiparkinsonian rats. *Neuroscience* 387, 123–134. doi: 10.1016/j.neuroscience.2017.08.052

Grealish, S., Diguet, E., Kirkeby, A., Mattsson, B., Heuer, A., Bramoulle, Y., et al. (2014). Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. *Cell Stem Cell* 15, 653–665. doi: 10.1016/j.stem.2014.09.017

Guo, X., Tang, L., and Tang, X. (2021). Current developments in cell replacement therapy for Parkinson's disease. *Neuroscience* 463, 370–382. doi: 10.1016/j.neuroscience.2021.03.022

Hagelberg, N., Jaaskelainen, S. K., Martikainen, I. K., Mansikka, H., Forssell, H., Scheinin, H., et al. (2004). Striatal dopamine D2 receptors in modulation of pain in humans: a review. *Eur. J. Pharmacol.* 500, 187–192. doi: 10.1016/j.ejphar.2004.07.024

Heuer, A., Smith, G. A., Lelos, M. J., Lane, E. L., and Dunnett, S. B. (2012). Unilateral nigrostriatal 6-hydroxydopamine lesions in mice I: motor impairments identify extent of dopamine depletion at three different lesion sites. *Behav. Brain Res.* 228, 30–43. doi: 10.1016/j.bbr.2011.11.027

Hsu, Y. H., Chang, C., and Chen, C. C. (2014). Negative cerebral blood volume fMRI response coupled with ca(2)(+)-dependent brain activity in a dopaminergic road map of nociception. *NeuroImage* 90, 43–51. doi: 10.1016/j.neuroimage.2013.12.028

Huse, D. M., Schulman, K., Orsini, L., Castelli-Haley, J., Kennedy, S., and Lenhart, G. (2005). Burden of illness in Parkinson's disease. *Mov. Disord.* 20, 1449–1454. doi: 10.1002/mds.20609

Jagmag, S. A., Tripathi, N., Shukla, S. D., Maiti, S., and Khurana, S. (2015). Evaluation of models of Parkinson's disease. *Front. Neurosci.* 9:503. doi: 10.3389/fnins.2015.00503

Jhao, Y. T., Chiu, C. H., Chen, C. F., Chou, T. K., Lin, Y. W., Ju, Y. T., et al. (2019). The effect of Sertoli cells on xenotransplantation and Allotransplantation of ventral mesencephalic tissue in a rat model of Parkinson's disease. *Cells* 8:1420. doi: 10.3390/cells8111420

Kirik, D., Winkler, C., and Bjorklund, A. (2001). Growth and functional efficacy of intrastriatal nigral transplants depend on the extent of nigrostriatal degeneration. *J. Neurosci.* 21, 2889–2896. doi: 10.1523/JNEUROSCI.21-08-02889.2001

Kordower, J. H., Freeman, T. B., Snow, B. J., Vingerhoets, F. J., Mufson, E. J., Sanberg, P. R., et al. (1995). Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N. Engl. J. Med.* 332, 1118–1124.

Laguna, G. R., and Barker, R. A. (2009). "Cell therapy in Parkinson's disease," in *Stem Cell Biology in Health and Disease*. ed. T. D. S. Zanker (Berlin, Germany: Springer Nature), 145–154.

Lee, C. S., Sauer, H., and Bjorklund, A. (1996). Dopaminergic neuronal degeneration and motor impairments following axon terminal lesion by instrastriatal 6-hydroxydopamine in the rat. *Neuroscience* 72, 641–653. doi: 10.1016/0306-4522(95)00571-4

Lee, M. A., Walker, R. W., Hildreth, T. J., and Prentice, W. M. (2006). A survey of pain in idiopathic Parkinson's disease. *J. Pain Symptom Manag.* 32, 462–469. doi: 10.1016/j.jpainsymman.2006.05.020

Lin, M. T., Wu, J. J., Chandra, A., and Tsay, B. L. (1981). Activation of striatal dopamine receptors induces pain inhibition in rats. *J. Neural Transm.* 51, 213–222. doi: 10.1007/BF01248953

Lin, M. T., Wu, J. J., and Tsay, B. L. (1984). Effects of kainic acid injections in the striatum on physiologic and behavioral functions in conscious rats. *Exp. Neurol.* 83, 71–83. doi: 10.1016/0014-4886(84)90047-5

Liu, Z. M., Schmidt, K. F., Sicard, K. M., and Duong, T. Q. (2004). Imaging oxygen consumption in forepaw somatosensory stimulation in rats under isoflurane anesthesia. *Magn. Reson. Med.* 52, 277–285. doi: 10.1002/mrm.20148

Mandeville, J. B., Jenkins, B. G., Chen, Y. C., Choi, J. K., Kim, Y. R., Belen, D., et al. (2004). Exogenous contrast agent improves sensitivity of gradient-echo functional magnetic resonance imaging at 9.4 T. *Magn. Reson. Med.* 52, 1272–1281. doi: 10.1002/mrm.20278

Martikainen, I. K., Hagelberg, N., Mansikka, H., Hietala, J., Nagren, K., Scheinin, H., et al. (2005). Association of striatal dopamine D2/D3 receptor binding potential with pain but not tactile sensitivity or placebo analgesia. *Neurosci. Lett.* 376, 149–153.

Mausset-Bonnefont, A. L., De Seze, R., and Privat, A. (2003). Immunohistochemistry as a tool for topographical semi-quantification of neurotransmitters in the brain. *Brain Res. Brain Res. Protoc.* 10, 148–155. doi: 10.1016/S1385-299X(02)00206-4

Mylius, V., Engau, I., Teepker, M., Stiasny-Kolster, K., Schepelmann, K., Oertel, W. H., et al. (2009). Pain sensitivity and descending inhibition of pain in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 80, 24–28. doi: 10.1136/jnnp.2008.145995

Nanni, C., Fanti, S., and Rubello, D. (2007). 18F-DOPA PET and PET/CT. *J. Nucl. Med.* 48, 1577–1579. doi: 10.2967/jnumed.107.041947

Nolbrant, S., Heuer, A., Parmar, M., and Kirkeby, A. (2017). Generation of high-purity human ventral midbrain dopaminergic progenitors for in vitro maturation and intracerebral transplantation. *Nat. Protoc.* 12, 1962–1979. doi: 10.1038/nprot.2017.078

Paez, A. G., Gu, C., Rajan, S., Miao, X., Cao, D., Kamath, V., et al. (2020). Differential changes in arteriolar cerebral blood volume between Parkinson's disease patients with Normal and impaired cognition and mild cognitive impairment (MCI) patients without movement disorder - an exploratory study. *Tomography* 6, 333–342. doi: 10.18383/j.tom.2020.00033

Park, J., Lim, C. S., Seo, H., Park, C. A., Zhuo, M., Kaang, B. K., et al. (2015). Pain perception in acute model mice of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Mol. Pain* 11:28. doi: 10.1186/s12990-015-0026-1

Quinn, N. P., Lang, A. E., and Marsden, C. D. (1996). Painful Parkinson's disease. *Lancet* 1, 1366–1369.

Rath, A., Klein, A., Papazoglou, A., Pruszak, J., Garcia, J., Krause, M., et al. (2013). Survival and functional restoration of human fetal ventral mesencephalon following transplantation in a rat model of Parkinson's disease. *Cell Transplant.* 22, 1281–1293. doi: 10.3727/096368912X654984

Schestatsky, P., Kumru, H., Valls-Sole, J., Valldeoriola, F., Marti, M. J., Tolosa, E., et al. (2007). Neurophysiologic study of central pain in patients with Parkinson disease. *Neurology* 69, 2162–2169. doi: 10.1212/01.wnl.0000295669.12443.d3

Shih, Y. Y., Chen, C. C., Shyu, B. C., Lin, Z. J., Chiang, Y. C., Jaw, F. S., et al. (2009). A new scenario for negative functional magnetic resonance imaging signals: endogenous neurotransmission. *J. Neurosci.* 29, 3036–3044. doi: 10.1523/JNEUROSCI.3447-08.2009

Shih, Y. Y., Chiang, Y. C., Shyu, B. C., Jaw, F. S., Duong, T. Q., and Chang, C. (2012). Endogenous opioid-dopamine neurotransmission underlie negative CBV fMRI signals. *Exp. Neurol.* 234, 382–388. doi: 10.1016/j.expneurol.2011.12.042

Taguchi, S., Tanabe, N., Niwa, J. I., and Doyu, M. (2019). Motor improvement-related regional cerebral blood flow changes in Parkinson's disease in response to antiparkinsonian drugs. *Parkinsons Dis* 2019:7503230. doi: 10.1155/2019/7503230

Takeda, R., Ishida, Y., Ebihara, K., Abe, H., Matsuo, H., Ikeda, T., et al. (2014). Intrastriatal grafts of fetal ventral mesencephalon improve allodynia-like withdrawal response to mechanical stimulation in a rat model of Parkinson's disease. *Neurosci. Lett.* 573, 19–23. doi: 10.1016/j.neulet.2014.05.007

Tang, D. L., Luan, Y. W., Zhou, C. Y., and Xiao, C. (2021). D2 receptor activation relieves pain hypersensitivity by inhibiting superficial dorsal horn neurons in parkinsonian mice. *Acta Pharmacol. Sin.* 42, 189–198. doi: 10.1038/s41401-020-0433-3

Tronci, E., Shin, E., Bjorklund, A., and Carta, M. (2012). Amphetamine-induced rotation and L-DOPA-induced dyskinesia in the rat 6-OHDA model: a correlation study. *Neurosci. Res.* 73, 168–172. doi: 10.1016/j.neures.2012.03.004

Wakeman, D. R., Dodiya, H. B., and Kordower, J. H. (2011). Cell transplantation and gene therapy in Parkinson's disease. *Mt. Sinai J. Med.* 78, 126–158. doi: 10.1002/msj.20233

Wasner, G., and Deuschl, G. (2012). Pains in Parkinson disease--many syndromes under one umbrella. *Nat. Rev. Neurol.* 8, 284–294. doi: 10.1038/nrneurol.2012.54

Weng, S. J., Chen, C. F., Huang, Y. S., Chiu, C. H., Wu, S. C., Lin, C. Y., et al. (2020). Olfactory ensheathing cells improve the survival of porcine neural xenografts in a parkinsonian rat model. *Xenotransplantation* 27:e12569. doi: 10.1111/xen.12569

Weng, S. J., Li, I. H., Huang, Y. S., Chueh, S. H., Chou, T. K., Huang, S. Y., et al. (2017). KA-bridged transplantation of mesencephalic tissue and olfactory ensheathing cells in a parkinsonian rat model. *J. Tissue Eng. Regen. Med.* 11, 2024–2033. doi: 10.1002/term.2098

Weng, S. J., Shiue, C. Y., Huang, W. S., Cheng, C. Y., Huang, S. Y., Li, I. H., et al. (2013). PET imaging of serotonin transporters with 4-[18F]-ADAM in a parkinsonian rat model. *Cell Transplant.* 22, 1295–1305. doi: 10.3727/096368912X658683

Wijeyekoon, R., and Barker, R. A. (2009). Cell replacement therapy for Parkinson's disease. *Biochim. Biophys. Acta* 1792, 688–702. doi: 10.1016/j.bbadis.2008.10.007

Yoon, H. H., Park, J. H., Kim, Y. H., Min, J., Hwang, E., Lee, C. J., et al. (2014). Optogenetic inactivation of the subthalamic nucleus improves forelimb akinesia in a rat model of Parkinson disease. *Neurosurgery* 74, 533–540. doi: 10.1227/NEU.0000000000000297

Zambito Marsala, S., Tinazzi, M., Vitaliani, R., Recchia, S., Fabris, F., Marchini, C., et al. (2011). Spontaneous pain, pain threshold, and pain tolerance in Parkinson's disease. *J. Neurol.* 258, 627–633. doi: 10.1007/s00415-010-5812-0

Zhao, F., Zhao, T., Zhou, L., Wu, Q., and Hu, X. (2008). BOLD study of stimulation-induced neural activity and resting-state connectivity in medetomidine-sedated rat. *NeuroImage* 39, 248–260. doi: 10.1016/j.neuroimage.2007.07.063

Zhou, C., Gu, W., Wu, H., Yan, X., Deshpande, P., Xiao, C., et al. (2019). Bidirectional dopamine modulation of excitatory and inhibitory synaptic inputs to subthalamic neuron subsets containing alpha4beta2 or alpha7 nAChRs. *Neuropharmacology* 148, 220–228. doi: 10.1016/j.neuropharm.2019.01.015

Zhou, C., and Luo, Z. D. (2015). Nerve injury-induced calcium channel alpha-2-delta-1 protein dysregulation leads to increased pre-synaptic excitatory input into deep dorsal horn neurons and neuropathic allodynia. *Eur. J. Pain* 19, 1267–1276. doi: 10.1002/ejp.656

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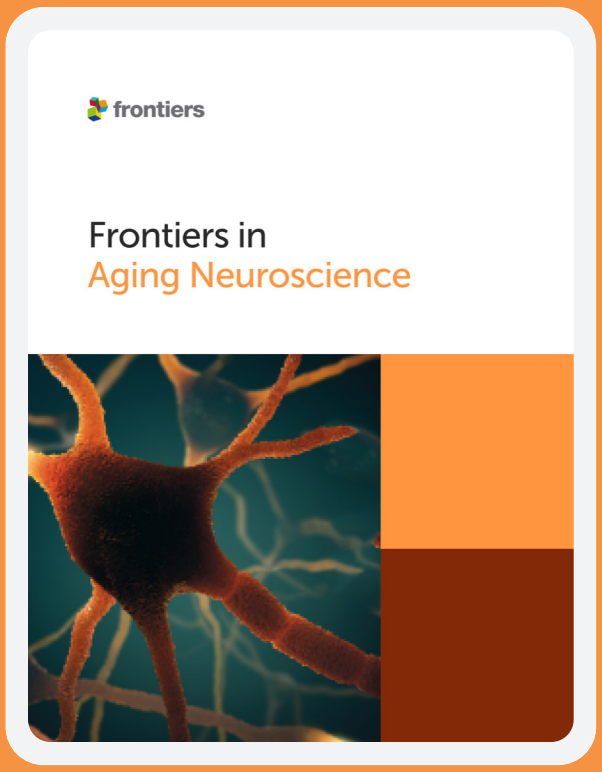


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