Exploring the association between walking, sleep patterns, brain perivascular spaces, and cognitive function in Parkinson's disease

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Abstract

Objective: Perivascular spaces (PVSs) surround cerebral blood vessels and support metabolic waste clearance, that is modulated by sleep. In Parkinson's disease (PD), motor impairments can affect sleep quality. This study explored the interrelationships between sleep disruption, gait difficulties, and cognition in PD, with a specific focus on the potential mediating role of PVS.

Methods: 348 participants from the Parkinson's Progression Markers Initiative (PPMI) were divided into healthy controls (HC), prodromal individuals, and patients with PD. Insomnia levels indicated sleep disruption, and walking difficulties severity were considered. Cognition was measured with the Symbol Digit Modalities (SDM) test. PVS volume fractions in white matter (WM-PVS) and basal ganglia (BG-PVS) were quantified using an automated segmentation pipeline developed by our team. Mediation analyses were conducted to assess whether PVS mediated the relationship between gait impairment and cognition, as well as the relationship between sleep and gait problems. Finally, we examined whether baseline PVS volume fraction predicted disease progression, as indexed by cerebro-spinal fluid (CSF) biomarkers collected after five years.

Results: PVS did not mediate the relationship between gait difficulties and cognition, or between sleep disruption and motor symptoms. Greater walking impairment was directly associated with poorer cognition. Insomnia severity was directly associated with greater walking problems. BG-PVS was negatively associated to cognition. Longitudinally, PVS burden was not predictive of disease progression.

Conclusions: Our findings suggest that sleep and motor impairments independently contribute to cognitive function in PD, but these effects are not mediated by PVS burden.

1. Introduction

Parkinson's disease is a neurodegenerative disorder characterized by motor and non-motor symptoms[1]. Poor sleep [2] and walking difficulties [3] are among the first symptoms of PD, often occurring prior to clinical diagnosis. Increased walking difficulties limit patients' mobility and their ability to engage in beneficial physical activity.

Physical limitations and sleep disturbances significantly affect cognitive performance in PD: gait impairments, especially slower walking speed, are linked to cognitive decline in older adults with PD[4]. Reduced physical activity is also associated with poorer sleep patterns[5], and 60–80% of PD patients experience sleep problems such as insomnia, daytime sleepiness, restless legs syndrome, or rapid eye movement sleep behavior disorder (RBD)[2]. Disrupted sleep contributes to fatigue and cognitive difficulties and may worsen motor symptoms and overall disease progression[2] .

Global cognition was found to be associated with balance problems and walking functions, and to be worsened by chronic sleep disruptions in PD. Sleep disruptions like RBD have also been shown to increase the levels of alpha synuclein in the CSF [6].

Sleep is important for the optimal functioning of the glymphatic system, that is responsible for the clearance of waste molecules from the brain [7]. Poor sleep quality can exacerbate neurotoxins and waste molecules accumulation, altering the glymphatic system normal

functioning[7]. Perivascular spaces, an important component of the glymphatic system, are fluid-filled spaces surrounding blood vessels and serve as a conduit for waste clearance and interstitial fluid dynamics (**Figure 1**) [8]. Previous clinical studies highlighted the role of enlarged PVS in white matter in PD[9] and the association of sleep problems to CSF biomarkers of neurodegeneration[10]. Elevated CSF levels of alpha-synuclein and total Tau (t-Tau) have been associated with increased sleep fragmentation and RBD [11]. Higher levels of PET-measured beta-amyloid have also been linked to greater sleep disturbances in people with RBD, suggesting an association between sleep and abnormal brain protein accumulation[12]. Despite the individual recognition of PVS alterations[9], sleep disturbances[2], and walking abnormalities in PD[4], the interplay among these factors remain underexplored. Understanding how sleep and walking problems may affect PVS integrity and how this contributes to cognitive decline could provide valuable insights into the underlying pathophysiology of PD (**Figure 2**)[13].

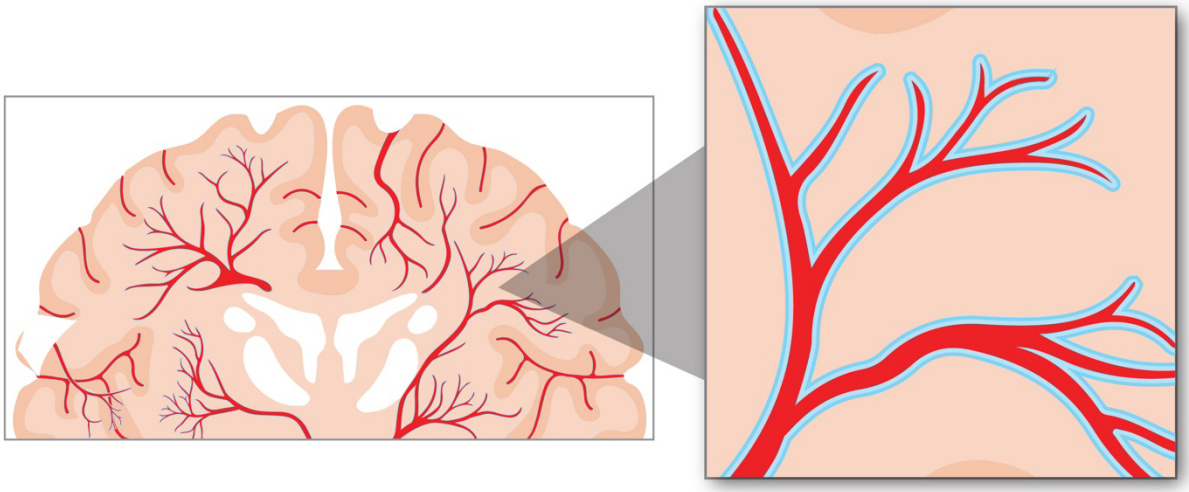

Figure 1: Anatomical representation of perivascular spaces (PVS).

The objective of this cross-sectional study is to investigate the relationship between PVS, sleep quality, and walking difficulties in a cohort composed of healthy controls, prodromal PD individuals, and patients with a clinical PD diagnosis. We *hypothesize* that individuals exhibiting walking difficulties may also experience poorer sleep quality, affecting brain clearance and ultimately impacting cognition.

We first assessed the association between PVS and walking difficulties, accounting for education, age, sex, study group, and body mass index (BMI)[14]. This analysis was also repeated including APOE4 genotype as a covariate, to assess the effect of this genetic information on the relationship between PVS and walking difficulties.

Given our interest in the role of PVS in cognition, particularly in the context of walking and sleep disturbances, we aimed to investigate their potential mediating role across interrelated pathways. First, we hypothesized that PVS mediates the relationship between walking difficulties and cognitive performance. Motor dysfunction has been linked to larger PVS, leading to impaired cerebral blood flow and increased oxidative stress, all of which have been associated with cognitive decline[15,16] . As such, walking difficulties may be an early marker of microvascular pathology, with PVS enlargement serving as a biological intermediary linking motor decline to impaired cognition.

Second, we considered whether PVS mediates the relationship between sleep disturbances and walking difficulties. Poor sleep impairs waste clearance[5], and may initiate a cascade of events —via PVS enlargement— that exacerbates neuroinflammation, affecting brain regions involved in movement coordination[17].

We finally investigated the relationship between PVS and CSF alpha-synuclein at baseline, and beta-amyloid, t-Tau and phosphorylated Tau (p-Tau) after five years. These

biomarkers have been previously considered as markers for cognitive decline progression in PD[13], as well as associated with worsening of motor symptoms in PD[18].

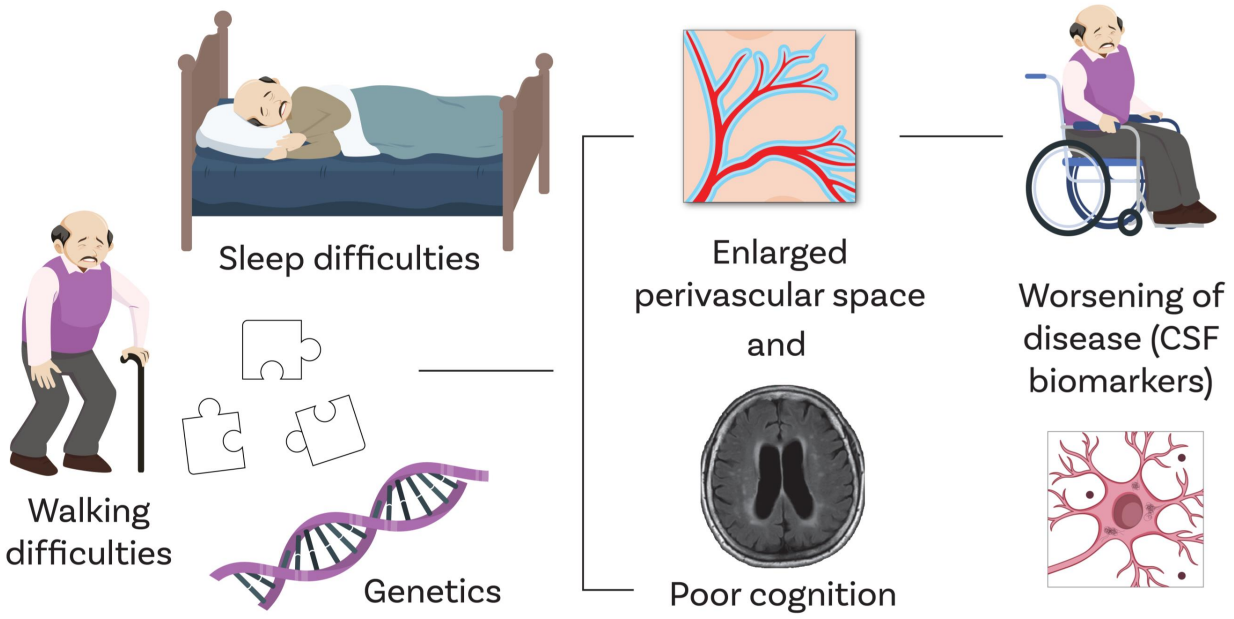

Figure 2: This figure illustrates the current gap in the literature regarding the relationship between motor deficits, sleep disturbances, and their potential influence on PVS, as well as the downstream effects on cognition in Parkinson's disease. Our study aims to investigate these interconnected associations to clarify how walking and sleep problems may be linked to PVS alterations and cognitive functioning in PD.

2. **Materials and Methods**

2.1. Study participants

Participants were from the Parkinson's Progression Markers Initiative (PPMI)[19]. PPMI is a comprehensive international study launched in 2010 to identify and validate biomarkers

for PD progression. The sample used in this study is from the PPMI Clinical, which includes in-person clinical and imaging assessment at fifty multi-international sites.

We used participants baseline data, with an initial sample size of *N*=431. We excluded participants who had missing neuroimaging data (structural T1w images and PVS segmentation output) at baseline and missing data in demographics (age, sex, study group, BMI, education), walking difficulties and sleep scores, resulting in a sample of n=365. Of the 365 participants, 11 participants failed Freesurfer preprocessing. An additional 6 were missing magnetic field scanner information. These 17 participants were excluded, and the pipeline for segmenting the PVS was applied to the remaining 348 participants. The last step of our pipeline removed white matter hyperintensities (WMH), often registered as PVS false positives. Our final sample size was *N*=348, age range 31-82 years old, including 172 PD patients, 117 prodromal cases and 59 HC. Participants were categorized as PD patients if they demonstrated at least asymmetric resting tremor or asymmetric bradykinesia or some combination of bradykinesia, resting tremor and/or rigidity within two years of diagnosis. Prodromal PD cases were defined by olfactory loss or RBD with dopamine transporter (DAT) deficit[19]. HC subjects were clinically ascertained as not having any neurologic dysfunction and Montreal Cognitive Assessment (MoCA)>26. Demographic information is reported in **Table 1**.

Characteristic	Overall, N = 348[1]	Healthy Control, N = 59[1]	Parkinson's Disease, N = 172[1]	Prodromal, N = 117[1]	p-value[2]
AGE	62 (57, 67)	60 (56, 67)	62 (55, 68)	63 (59, 67)	0.11
Body Mass Index	26.4 (23.9, 29.8)	26.7 (24.2, 31.0)	25.9 (23.8, 29.1)	26.4 (24.0, 30.4)	0.2
SEX					0.11
Female	152 (44%)	22 (37%)	70 (41%)	60 (51%)	
Male	196 (56%)	37 (63%)	102 (59%)	57 (49%)	
Education (in years)	16.0 (14.0, 19.0)	16.0 (15.5, 19.0)	16.0 (14.0, 18.0)	18.0 (16.0, 20.0)	<0.001
Symbol Digit Modalities (Total Score)	45 (37, 50)	48 (42, 54)	43 (36, 49)	46 (40, 51)	0.002
Walking Difficulty Group					<0.001
No Walking Difficulty	264 (76%)	57 (97%)	98 (57%)	109 (93%)	
Walking Difficulty	84 (24%)	2 (3.4%)	74 (43%)	8 (6.8%)	
Sleep Group					0.4
Low levels of Insomnia	253 (73%)	47 (80%)	122 (71%)	84 (72%)	
High levels of insomnia	95 (27%)	12 (20%)	50 (29%)	33 (28%)	
Magnetic Field Strength					<0.001
1.5T	159 (46%)	36 (61%)	100 (58%)	23 (20%)	
3T	189 (54%)	23 (39%)	72 (42%)	94 (80%)	

[1] Median (IQR); n (%)
[2] Kruskal-Wallis rank sum test; Pearson's Chi-squared test

Table 1: Demographic information of this study sample. Walking difficulties were assessed with Unified Parkinson's Disease Rating Scale (MDS-UPDRS) II (Item 2.12). Sleep problems were assessed based on the insomnia levels from the MDS-UPDRS I (Item 1.7).

2.2. Neuropsychological and non-motor variables

Walking difficulties were assessed with the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) II (Item 2.12). The MDS-UPDRS is divided into four sections; the first and second parts evaluate non-motor and motor experiences of daily living respectively, the third part focuses on motor examination, and the fourth part assesses motor complications. Item 2.12 ('NP2WALK') expresses walking and balance difficulties level that people experience, represented by a Likert scale, with higher scores reflecting more difficulties (0=normal, 1=slight, 2=mild, 3=moderate, 4=severe).

Sleep problems were assessed from insomnia ratings from the MDS-UPDRS I (Item 1.7), evaluating trouble going to sleep or staying asleep on a scale from 0 to 4, with higher scores indicating worse insomnia. In our analysis, the sleep variable was dummy coded, based on a cut-off value from a previous study [20]: individuals with sleep scores of 0 or 1 were assigned a value of 0, while those with a sleep score of 2 or higher were coded as 1, thus binarizing our participants as good and poor sleepers.

Cognition was assessed for each participant by using the total score of Symbol Digit Modalities (SDM) test, a neuropsychological assessment that measures processing speed, attention, and visual-motor coordination. Participants are given a key that pairs specific symbols with numbers. They must then quickly match symbols to their corresponding numbers. The score is the number of correct symbol-number pairings made within a specified time of 90 seconds. Higher scores indicate better cognitive functioning and faster processing speed. It was previously shown that SDM performs better than other cognitive scales in detecting cognitive impairment in PD[21]. Average scores for the total sample and each subgroup are found in **Table 1**. The PD group showed the lowest SDM median and interquartiles compared to prodromal and HC groups.

2.3. Imaging

Imaging data were acquired according to the PPMI Clinical MRI Technical Operations Manual version 7.0 (2015) described previously[22]. 3D T1-weighted images at baseline were acquired using 1.5 and 3 Tesla scanners, with a slice thickness of 1.5 mm or less

with no interslice gap; voxel size was 1x1x1.2mm. A high-resolution 3D T2 FLAIR sequence was also acquired, with a slice thickness of 1.2 mm, voxel size 1x1 mm in plane resolution, with a total scan time between 20-30 minutes. Additional information can be found in the MRI Technical Operations Manual, Final version 7.0 (2015).

2.3.1. Data processing

Freesurfer (v.5.3) was used for image preprocessing, which included motion correction, image normalization, and skull stripping. Brain volume and parcellation were obtained with the recon-all module on the Laboratory of Neuro Imaging (LONI) pipeline system (https://pipeline.loni.usc.edu) [23,24].

PVS were mapped and segmented from T1w images, following a previously published technique [25,26]. A non-local mean filtering method was applied to denoise the MRI T1w images [27], which defines the similar intensity of a voxel to its neighboring voxels. A Frangi filter was further applied at a voxel level [28] using the Quantitative Imaging Toolkit (QIT) [29], to detect tubular PVS structures on the T1w, and obtaining a probabilistic map of vesselness [28]. To capture true positives and true negatives, a threshold of 0.00003 in WM-PVS was applied to obtain a binary PVS mask for data acquired at 3T, and a threshold of 0.0003 to data acquired at 1.5T. For BG-PVS the threshold of 0.0004 was applied for data acquired at 1.5T and threshold of 0.0001 for 3T data. An example segmentation result is provided in **Figure 3**. The WM-PVS volume fraction was calculated by dividing the total WM-PVS volume by the total white matter volume for each participant. The BG-PVS volume fraction was calculated by dividing the total BG-PVS volume by the basal ganglia volume.

In PPMI, FLAIR data is available, allowing subtraction of WMH as false positives from the PVS segmentation mask. The mask for WMHs was created by combining the WMH masks generated with the Lesion Growth Algorithm[24], which measures the intensity distribution in white matter, grey matter, and CSF on FLAIR and T1-weighted images and identifies WMH voxels as outliers in the signal distribution curve, with neighboring voxels iteratively assigned to the WMH mask by weighing the likelihood of belonging or not to the WMH mask, and the Sequence Adaptive Multimodal Segmentation tool [23], a contrast-adaptive method based on a generative approach in which a forward probabilistic model is inverted to obtain automated and robust segmentations of WMH. Voxels marked as WMH were finally subtracted from the PVS mask to remove false positive PVS [23,24].

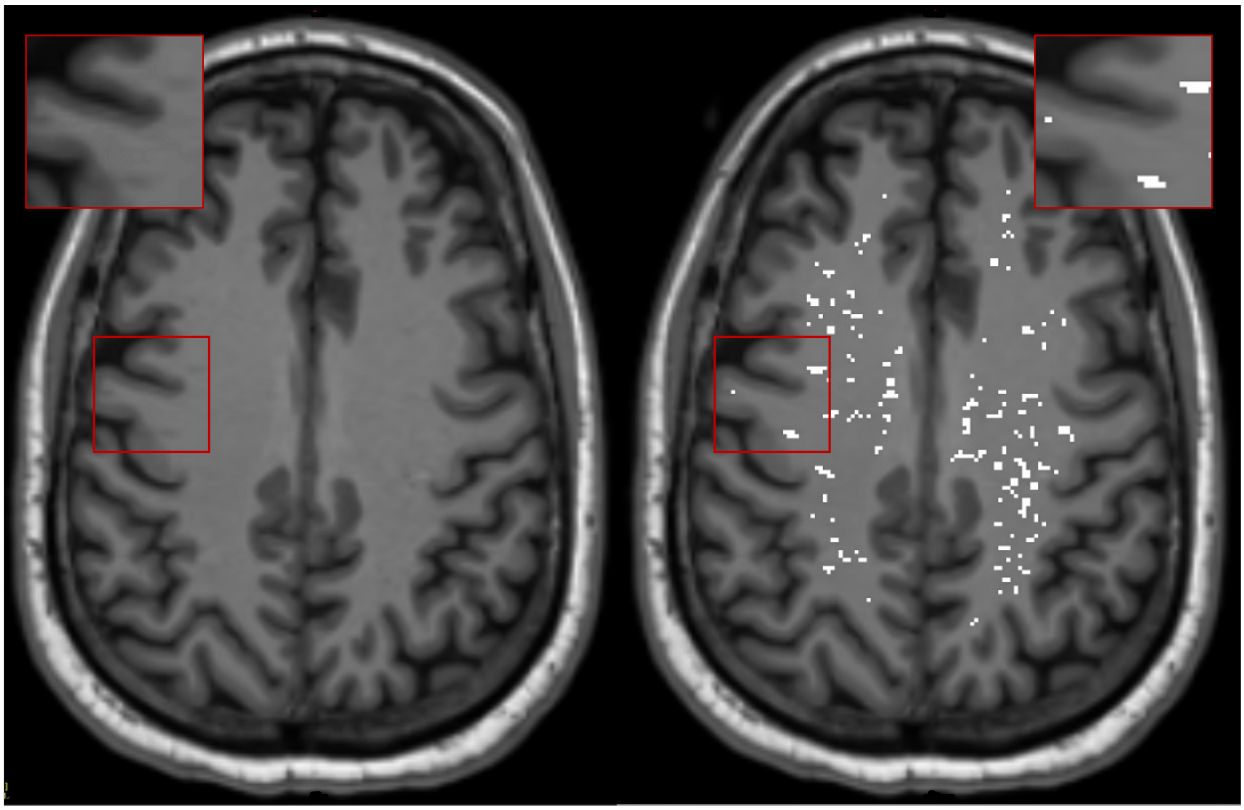

Figure 3: Example of PVS segmentation result in one participant.

2.4. CSF biomarkers

CSF samples, collected according to the PPMI Laboratory Manual procedure, were utilized to measure alpha-synuclein, amyloid-beta, t-tau and tau phosphorylated at the threonine 181 position (p-tau181), using Elecsys electro-chemi-luminescence immunoassays on the cobase 601 platform (Roche Diagnostics) [30,31] as biomarkers of PD progression[13]. While amyloid-beta and both t-Tau and p-Tau were collected longitudinally after 5 years, alpha-synuclein was not collected at that timepoint. As a result, analyses involving alpha-synuclein and PVS were limited to baseline cross-sectional data. The alpha-synuclein essay has a measurement range of 300-7000 pg/mL, the Aβ assay of 200 to 1700 pg/mL, the t-tau assay: 80 to 1300 pg/mL, and the p-tau181 assay: 8 to 120 pg/mL.

2.5. Statistical analysis

2.5.1. Association between walking difficulties and PVS volume fraction

The initial analysis examined the association between walking difficulties and PVS volume fraction (dependent variable), with BMI, age, sex, education, magnetic field strength, study group and sleep scores as covariates. Age, BMI and education were modeled as continuous variables. We used a generalized linear model (GLM) with Gamma distribution and log link to consider the positive skewed distribution of PVS volume fraction.

The population was sub-divided by dummy coding the walking difficulties variable (0=people who had walking score=0, 1=people with any level of walking difficulties – walking score=1,2,3,4) (**Table 1**, variable named 'Walking difficulty group').

We utilized APOE4 genotype data to consider possible confounding on the relationship between PVS and walking difficulties. The APOE4 genotype was added to the model above as a covariate. The genotype information was dummy coded as 0/1, with 0= 'absence of APOE4 allele' and 1='presence of APOE4 allele'. Genetic information was missing for one participant.

2.5.2. Mediation analyses

We conducted a causal mediation analysis separately for WM-PVS and BG-PVS to explore the mediating effect of PVS on the relationship between walking difficulties and cognition, and between sleep and walking deficits. For this purpose, *model 4* in the mediation model of *Preacher and Hayes*[32] was used, testing whether the effect of an independent variable on an outcome is transmitted through a single mediator. This approach estimates both the direct and indirect effects, allowing for the assessment of the mediator's contribution to the total effect.

A second mediation analysis explored the relationship between sleep and walking with PVS as a mediator. The covariates used for each model were BMI, age, sex, education, magnetic field strength, study group, and sleep scores.

2.5.4. Longitudinal analysis with CSF markers

One of our aims was to explore whether the PVS volume fraction could predict the accumulation of CSF biomarkers, including t-Tau, p-Tau, and amyloid-beta, after a 5-year period. We analyzed data from 132 participants (44 HC, 7 prodromal, 81 PD) who had longitudinal CSF biomarker information available. We used linear regression models for

all analyses. Covariates were age, sex, study group, education, sleep, magnetic field strength and BMI. We finally performed a sensitivity analysis to assess scanner magnetic field changes. Statistical significance corresponded to 2-sided *p*-value<0.05.

3. **Results**

3.1. Association of PVS with walking difficulties

We did not find a statistically significant association between WM-PVS volume fraction and walking difficulties (beta(standard error (SE)) =.11(.08), *p*=.16), nor when we included APOE4 in the model as a covariate (beta(SE)=.13(.079), *p*=.10). We also found no statistically significant association between BG-PVS and walking deficits (beta(SE) =.016(.12), *p*=.89), nor when considering APOE4 as a covariate (beta(SE)=.019(.11), *p*=.87).

3.2. Mediation analyses

3.2.1. Model A (Walking-PVS-Cognition)

The mediation analysis revealed a significant direct and total effect of walking on cognition (average direct effect (ADE) estimate (95% confidence interval (CI) *c'*= -3.0067(-5.46, -.49), *p*=.016 and total effect estimate *c*= -3.13(-5.6, -.61), *p*=.012 respectively) (**Figure 4, a**). We found more severe walking difficulties were related to lower cognitive performance, adjusted for age, sex and education effects. WM-PVS was not associated with walking difficulties and did not mediate the relationship between walking and cognition.

When we considered BG-PVS as mediator, results similarly showed statistically significant direct and total effects (ADE *c'*= -3.069 (-5.46, -.54), *p*=.016; *c*= -3.028 (-5.52, -.52), *p*= .017) (**Figure 4, b**). There was no significant association between BG-PVS and walking difficulties, whereas a significant negative association was seen between BG-PVS and SDM scores (estimate b= -5.43, *p*= .01), indicating that higher BG-PVS volume fraction was related to cognitive worsening, which we did not see in the WM-PVS.

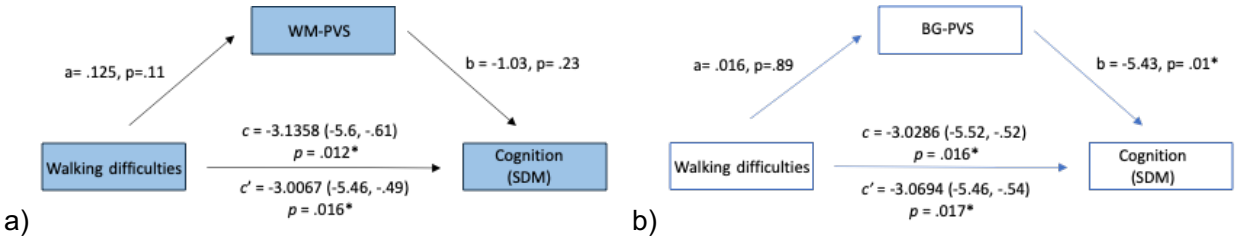

Figure 4: Mediation results testing the relationship between walking difficulties and cognition, with PVS as mediator in a) the total white matter and b) basal ganglia.

3.2.2. Model B (Sleep-PVS-Walking)

When we examined the mediating role of WM-PVS and BG-PVS in the relationship between sleep and walking difficulties, we found that PVS did not mediate such relationship. Results revealed a significant direct effect (ADE *c'*= .106 (.016, .20), *p*=.019) and a significant total effect (*c*=.105 (.01, .19), *p* = .026) in the WM-PVS (**Figure 5, a**). The same was seen when we included the BG-PVS as mediator (ADE *c'*= .11 (.012,.20), *p*=.028 and *c*=.107 (.011, .20), *p*= .028 respectively) (**Figure 5, b**).

A statistically significant positive association between walking difficulties and sleep quality suggests individuals with severe insomnia levels exhibit greater walking deficits.

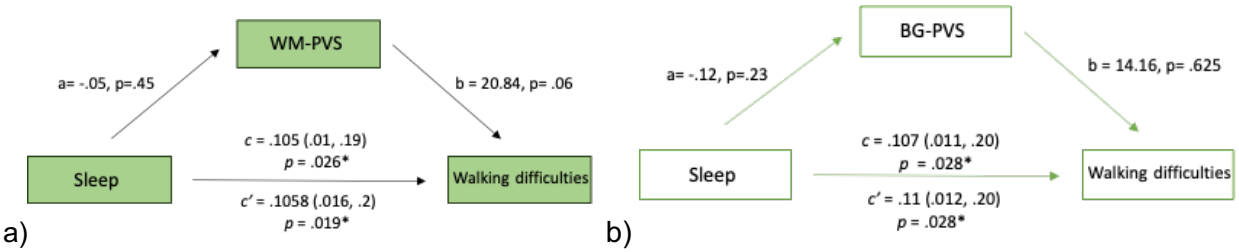

Figure 5: Mediation results testing the relationship between walking difficulties and sleep, with PVS as mediator in the a) total white matter and b) basal ganglia.

We finally performed the same analyses by stratifying our sample based on the study group, (i.e., HC, prodromal PD cases and PD patients), as well as within a combined subgroup of prodromal cases and patients with PD (excluding healthy controls). Results are reported in **Appendix**.

3.3. Baseline PVS and disease progression

The longitudinal analysis results indicated no statistically significant predictive effects of baseline PVS on 5-year CSF biomarkers. With t-Tau, this was seen both in WM-PVS (beta(SE)=84.09(301.74), *p*=.78) and in BG-PVS (beta(SE)=.-85.11(846.88), *p*=.92). Similarly, analyses involving p-Tau and beta-amyloid also revealed no significant predictions.

We also examined the relationship between WM-PVS and BG-PVS burden and baseline alpha-synuclein levels. No significant associations were observed. Longitudinal analyses could not be conducted due to missing alpha-synuclein data at year five in PPMI.

4. **Discussion**

Utilizing the PPMI cohort data, we found that PVS does not mediate the relationship between walking difficulties and cognition and sleep problems. Our results demonstrated a direct relationship between the motor severity and cognitive performance, as well as between insomnia severity and walking deficits, in the whole population and within clinical subgroups. Finally, the longitudinal analysis revealed that baseline PVS does not predict disease worsening 5 years later.

Enlarged PVS may reflect impaired brain clearance, leading to CSF buildup. PVS enlargement has been associated with cerebro-vascular diseases and other conditions in older adults, such as depression, diabetes, and neurodegenerative diseases[33]. Both global and regional PVS volumes were found to be significantly larger in individuals with PD compared to HC[34]. Alongside our results, finding a negative association between BG-PVS and cognitive scores, these observations suggest that PVS changes and waste clearance dysfunction could be linked to various pathologies and act as early markers for cognitive decline.

Our results showed PVS does not mediate the observed relationship between walking problems and cognition, nor between sleep and walking difficulties. Mixed results have

been reported on the association between PVS and cognition. Previous studies showed a negative relationship between PVS volume fraction and cognition[34], whereas others found no association in patients with ischemic stroke[16] or PD [35]. They found that baseline PVS was related to longitudinal motor dysfunction in PD, but not cognitive decline[35], supporting our findings.

The observed direct relationship between walking difficulties and cognitive performance in our cohort builds on previous studies linking motor and cognitive decline in people with PD, especially in attention and frontal-executive function[15]. Greater motor impairment was linked to lower cognitive scores, suggesting that walking and balance problems may contribute to and/or co-occur with cognitive decline. Walking disturbances have been previously recognized as an early marker of cognitive dysfunction in PD, even in prodromal stages of neurodegenerative diseases[15]; mild cognitive impairment and dementia were previously associated with balance problems and gait velocity, as well as with worsening of non-motor symptoms[36].

Our findings indicate that the association between insomnia and walking difficulties is not mediated by PVS volume fraction, suggesting that while sleep disturbances are strongly linked to motor dysfunction, the underlying mechanisms may not primarily involve cerebrovascular changes, specifically PVS dilation. Previous studies have consistently reported a relationship between sleep quality and motor difficulties in PD. Individuals with higher levels of insomnia showed worse gait performance and increased risk of falls[37]; disrupted sleep patterns and reduced slow-wave sleep have been reported to impair

motor function, as sleep restorative effects on regions involved in motor control, such as the basal ganglia, are compromised. The lack of PVS mediation suggests sleep-related motor impairments are linked to other neural disruptions, such as reduced dopaminergic activity and heightened neuroinflammation[6], rather than structural changes in the perivascular pathways.

Our mediation analyses on BG-PVS and WM-PVS revealed that severe walking deficits are directly related to SDM lower scores, and sleep problems are related to greater walking difficulties. These findings suggest that while sleep disturbances contribute directly to motor dysfunction, and motor difficulties appear to be a factor in cognitive decline, PVS did not mediate these effects, as we had hypothesized.

Cognitive decline in PD often manifests as processing speed impairment, captured by SDM. Reduction of walking and motor control are thought to be driven by dopaminergic deficits in the basal ganglia, which also contribute to slower processing speed and attention deficits[38]. The observed negative relationship between BG-PVS and SDM performance highlights that cognitive decline is linked to basal ganglia degeneration (we did not see such association with the WM-PVS). This suggests that region-specific perivascular changes may be more relevant for detecting early cognitive impairments in PD than global PVS burden.

The results of our longitudinal analysis suggest that PVS volume fraction does not predict PD worsening at year 5, as measured by CSF biomarkers. This finding contrasts with the broader hypothesis that glymphatic system alteration may contribute to

neurodegenerative progression in PD[13]. While increased levels of p-Tau and decreased levels of Aβ42 were previously linked to PD cognitive impairment and disease progression [39], the role of PVS enlargement as a predictor of disease worsening remains uncertain. The lack of significant relationship between PVS volume fraction and CSF biomarkers over time is supported by a previous study on the PPMI data that showed no significant correlation between dilated PVS and CSF markers or dopaminergic neuronal degeneration, both at baseline and longitudinally[40]. This suggests that PVS enlargement may not directly reflect the underlying biochemical progression of PD.

Previous literature indicates that CSF biomarkers reflect disease progression in the prodromal and early stages of PD[41]. At these stages, alterations in CSF alpha-synuclein, amyloid-beta, and Tau may signal early neurodegeneration before major motor symptoms appear. Mollenhauer and colleagues found reduced CSF alpha-synuclein levels were associated with more severe motor symptoms and predicted disease worsening in the early phases of PD[41]. Our cohort did not include only PD patients, but also prodromal cases and healthy people, which may explain the absence of a significant longitudinal relationship. Possibly also contributing to our negative finding is that the patients with PD in the PPMI dataset were very mildly affected when recruited, by definition not yet on medications for PD. The first few years of PD progression tend to be quite mild and generally does not include significant cognitive impairment or disability. This likely makes it harder to uncover associations between baseline characteristics and longitudinal change.

One limitation of our study is the lack of alpha-synuclein longitudinal data five years after the initial measurements, preventing a clearer understanding of disease progression and symptom development. Another important consideration is the study's cross-sectional design, which limits our ability to determine the temporal directionality of the associations observed between walking difficulties, sleep disturbances, and PVS enlargement. It remains unclear whether PVS enlargement contributes to motor and sleep impairments, or whether gait and sleep dysfunction influence PVS structure. Additionally, in our analysis of APOE4 genotype, participants were categorized based on the presence or absence of at least one APOE4 allele. Further stratification by number of APOE4 alleles was not performed due to the small number of individuals carrying two APOE4 allele copies. Despite these limitations, our study offers novelty and several strengths. We utilized a semi-automated segmentation method for PVS identification, compared to previous studies that relied on manual counting. Importantly, we further excluded WMH as potential false positives, ensuring a more accurate assessment of PVS volume fraction. Future work should consider additional PVS structural features—such as tortuosity, shape, and size—which may offer more nuanced insights into the clinical relationships between PVS, sleep disturbances, and motor dysfunction in PD. Examining sleep and gait disturbances longitudinally will also be important for understanding how PVS enlargement may influence sleep quality and motor deficits over time and for untangling temporal relationships among walking and sleep impairments. In PD, the integration of behavioral and clinical factors—such as walking difficulties, sleep disturbances, genetics, clinical diagnosis and biological markers—with neuroimaging markers like PVS adds novelty to our study, as previous studies considered these aspects separately. This

multidimensional approach not only deepens our understanding of Parkinson's disease, but highlights the importance of integrating clinical, biological, and structural factors to capture the complexity of disease progression, potentially guiding future therapeutic strategies and personalized care.

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Disclosures

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