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Early detection of neurodegenerative disorders using behavioral markers and new technologies: New methods and perspectives

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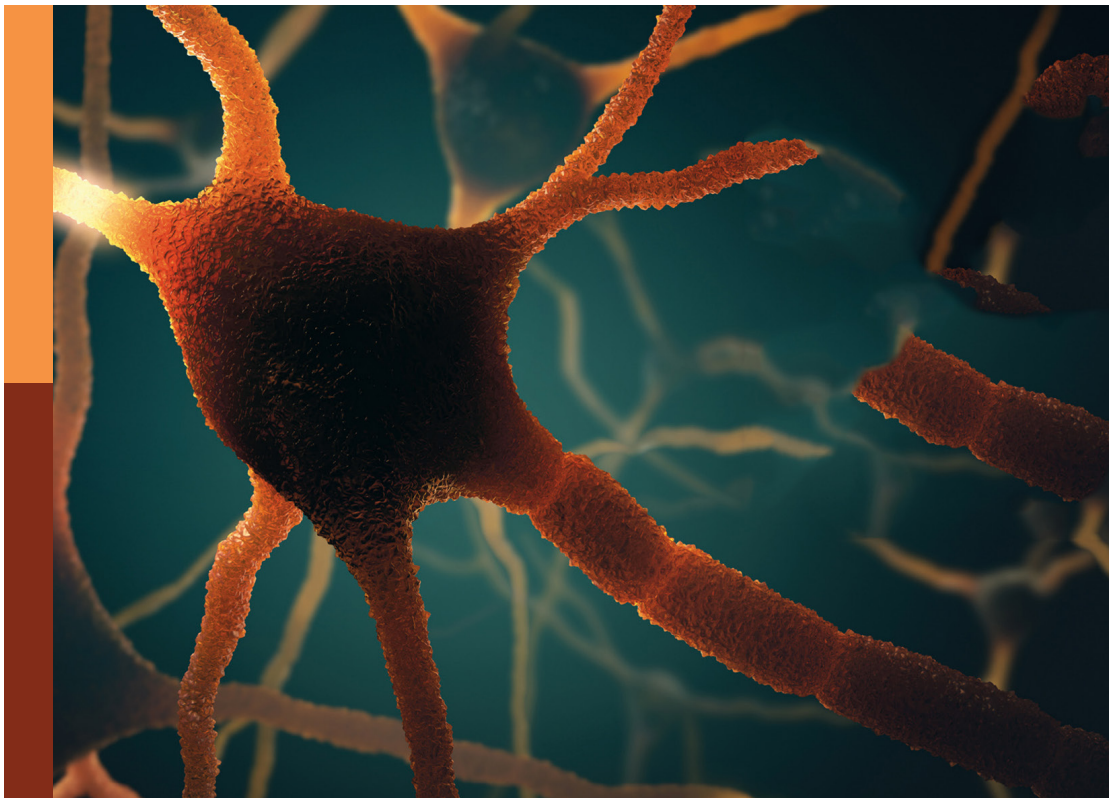

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Early detection of neurodegenerative disorders using behavioral markers and new technologies: New methods and perspectives

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Editorial: Early detection of neurodegenerative disorders using behavioral markers and new technologies: New methods and perspectives

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Editorial on the Research Topic
Early detection of neurodegenerative disorders using behavioral markers and new technologies: New methods and perspectives

Neurodegenerative disorders (ND) are a common cause of mortality and morbidity worldwide, particularly in older people. As life spans continue to increase, the incidence of neurodegenerative diseases is expected to increase as well [World Health Organization (éd.), 2017]. In addition to pathological threats, adults above 60 years show increasing vulnerability to broad decline in memory, attention, and multi-tasking as a matter of normal aging. Indeed, cognitive impairment, with or without ND, can predict imminent motor decline or neuropsychiatric symptoms, such as apathy and depression.

ND, such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), develop progressively over many years. After an asymptomatic stage (only revealed by biomarker evidence), cognitive and neuropsychiatric symptoms start to appear and then worsen over time, until they lead to a loss of autonomy in activities of daily living. Multi-domain interventions (targeting simultaneously multiple areas, such as cognition, lifestyle, and physical activity) are showing promising results in delaying ND progression (Kivipelto et al., 2018; Meng et al., 2022). The earlier and more personalized the intervention, the more promising the results are hypothesized to be (Devos et al., 2021; Solomon et al., 2021; Röhr et al., 2022). For this reason, detecting ND in its early stages is an important clinical and research challenge.

Biomarkers can predict risk for AD, PD, and other ND, several years before the clinical symptoms appear. For instance, biomarkers of amyloid β pathology (low CSF Aβ42 or increased CSF Aβ40–Aβ42 ratio; increased tracer retention in amyloid PET) and biomarkers of tau pathology (increased phosphorylated tau in CSF; increased tracer retention in tau PET) can characterize AD pathology before the appearance of clinical symptoms (Dubois et al., 2021). Also, α-synuclein species, lysosomal enzymes, markers of amyloid and tau pathology, and neurofilament light chain biomarkers from CSF and blood reflect the

pathophysiology of Parkinson's disease and are providing promising preliminary results for the early diagnosis (Parnetti et al., 2019). Furthermore, low baseline Aβ42 in the CSF of non-demented PD patients predicts development of cognitive impairment over time (Leaver and Poston, 2015). However, lumbar puncture and PET imaging are invasive, expensive, and realized only in specialized clinical settings. For these reasons, there is growing interest in finding new approaches that can be employed to investigate early signs of cognitive, motor, and behavioral decline that can be indicative of potential ND, and can thus help to identify people who should be tested with the more specialized biomarker indices. In order to be helpful, these technological approaches must be non-invasive, rapid to administer and safe to apply outside of specialized clinics. Basic research with wearable sensors, new Information and Communication Technologies (such as automated video and audio-analyses), Virtual Reality video games, smartphone or tablet applications and olfactory tests have recently shown promise to reveal changes in subjects' abilities and behaviors, which in turn can support the clinician in early identification of subtle disorders (Robert et al., 2016; Maremmani et al., 2018).

In this Research Topic we collected nine papers that examined specific non-invasive tools, methods or technologies applied in assessments of cognitive and/or motor performance, or neuropsychiatric symptoms, in cohorts of older adults with ND or with diminished cognitive function.

Subjective reports

Pang et al. showed the interest of validated subjective cognitive complaints for dementia screening. Specifically, in a large sample of community-dwelling older adults, they demonstrated that combining a reliable single-question assessment for subjective cognitive decline with an objective tool (such as the Montreal Cognitive Assessment battery-MoCA, Nasreddine et al., 2005) can efficiently discriminate dementia patients from healthy older adults in the community, suggesting the potential of self-reports for large-scale screenings.

Neuroimaging and psychophysiological measures

In terms of PD screening, Chang et al. showed that resting state EEG characteristics extracted by Holo-Hilbert Spectral Analysis and processed with machine learning algorithms are important markers for the diagnosis of PD, in particular showing a reduction of β bands in frontal and central regions, and reduction of γ bands in central, parietal, and temporal regions in PD patients. Also, these characteristics are positively correlated with the depression severity (i.e., θ and β bands values in all brain regions). Similarly, Ma et al. found that the incidence of multiple step saccades in their visually guided reactive saccade task could be a complementary biomarker for the early diagnosis of PD. This approach provides for an easy assessment of ND through eye-tracking.

Kinematics

Assessing motor performance and action kinematics is relevant not only for PD detection, but can be employed also to screen for mild cognitive impairment, especially in dual-task conditions. Ali et al. found that kinematic gait parameters of knee peak extension angle during a dual task performance (walking + story recall) were sensitive enough to discriminate individuals with MCI from healthy controls.

Measuring the global level of motor activity and sleep using actigraphy recorded over 7 days, Cai et al. found that diurnal vector magnitude and total time in bed correlated negatively with apathy severity in patients with Cerebral Small Vessels Disease. This confirms that objective motor indicators can be relevant also for the assessment of neuropsychiatric symptoms, such as apathy and depression.

Brain oxygenation and autonomic biomarkers

Other non-invasive biomarkers are also starting to show promising results. For instance, Li et al. showed that objective indicators of brain oxygenation status and cerebral autoregulation function (assessed using near-infrared spectroscopy technology and a non-invasive blood pressure device) can reflect cognitive function, and correlate with the level of cognitive decline in older adults assessed using the MoCa.

In their review, Barthelemy et al. highlighted the importance of monitoring autonomic biomarkers (such as heart rate variability) parameters, in addition to classical cardiovascular risk factors, to increase the prediction of stroke.

Genetics and plasma biomarkers

Tung et al. confirmed the interest of combining genetic (Apolipoprotein E-ApoE polimorfism) and clinical information to detect cognitive decline and optimize interventions. Interestingly, across comorbidities, functional gastrointestinal disorder was the strongest predicting factor for dementia in ε4 allele carriers.

Finally, Liang et al. investigated the predictive ability of preoperative plasma biomarkers along with cerebral oxygen saturation for the incidence of post-operative cognitive dysfunction in older patients with MCI. They drafted recommendations on the cerebral oxygen saturation level based on Aβ-42 status to reduce the risk of post-operative cognitive disfunction.

Taken together, these results corroborate the idea that new technologies, non-invasive sensors, and machine learning algorithms can complement and support traditional assessment, and their results converge with traditional biomarker evidence. The important developments from this topic should motivate near term research to examine which markers type of screening should be employed for a patient depending the type of risk factors. Furthermore, large-scale studies are necessary to test the convergent validity compared to traditional biomarkers.

Author contributions

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

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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Evaluating the Different Stages of Parkinson's Disease Using Electroencephalography With Holo-Hilbert Spectral Analysis

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Electroencephalography (EEG) can reveal the abnormalities of dopaminergic subcortico-cortical circuits in patients with Parkinson's disease (PD). However, conventional time-frequency analysis of EEG signals cannot fully reveal the non-linear processes of neural activities and interactions. A novel Holo-Hilbert Spectral Analysis (HHSA) was applied to reveal non-linear features of resting state EEG in 99 PD patients and 59 healthy controls (HCs). PD patients demonstrated a reduction of β bands in frontal and central regions, and reduction of γ bands in central, parietal, and temporal regions. Compared with early-stage PD patients, late-stage PD patients demonstrated reduction of β bands in the posterior central region, and increased θ and δ2 bands in the left parietal region. θ and β bands in all brain regions were positively correlated with Hamilton depression rating scale scores. Machine learning algorithms using three prioritized HHSA features demonstrated "Bag" with the best accuracy of 0.90, followed by "LogitBoost" with an accuracy of 0.89. Our findings strengthen the application of HHSA to reveal high-dimensional frequency features in EEG signals of PD patients. The EEG characteristics extracted by HHSA are important markers for the identification of depression severity and diagnosis of PD.

Keywords: electroencephalography, Holo-Hilbert spectral analysis, machine learning, Parkinson's disease, depression

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease affecting the brain, predominantly pigmented nuclei in the midbrain, brainstem, cerebral cortex and olfactory tubercle (Braak et al., 2003). Other than motor symptoms, PD also presents cognitive symptoms, which usually occur during more advanced stages of disease or may coincide with motor symptoms if there is a disruption of fronto-striatal circuits (Marsden, 1982; Cooper et al., 1991). Although

the neurodegeneration of PD occurs mainly in subcortical structures, dopaminergic cortical-subcortical circuits between the basal ganglion, thalamus, and frontal lobes are also affected (Alexander et al., 1986). The disruption of these circuits leads to specific cognitive deficits in patients with PD.

The activity of cortical neurons averaged over the cortex can be illustrated using electroencephalogram (EEG) (Nunez et al., 2001). EEG signals can be considered as brain-computer interface systems (BCI), as EEG-based intelligent BCI enables the uninterrupted monitoring of fluctuations in human cognitive states and is beneficial for healthcare support and research in various fields. This system registers the capability of human brain interaction with the environment and advanced technology via machine learning algorithms. EEG signals directly measure cortical electrical activity with high temporal resolution (Ramadan and Vasilakos, 2016) and is the most largely used non-invasive modality for both real-world BCIs and clinical use (Schalk et al., 2004). With comparatively high signal quality, reliability and mobility compared to other imaging approaches, EEG devices collect signals in non-overlapping frequency bands, where their powerful intra-band connection reflects distinct behavioral states (Zhang et al., 2017), and present diverse corresponding features and motifs. Moreover, the temporal resolution is exceedingly high, up to the millisecond level, with minimal risk compared to other invasive and non-invasive modalities. Nonetheless, a drawback of the EEG is the low spatial resolution within signals ensuing from the limited number of electrodes. Hence, this obligates consideration of the inferior signal-to-noise ratio since objective factors like environmental noise and subjective factors like fatigue status could contaminate the EEG signals. Thus, a broad category of unsupervised learning algorithms for signal enhancement, namely blind source separation, estimates original sources and parameters of a mixing system and removes artifact signals, including eye blinks and movement (Sweeney et al., 2012). Independent component analysis (ICA) is the most widely used blind source separation (BSS) method as it decomposes observed signals into independent components and restructures clean signals by eradicating independent components comprising artifacts (Gu et al., 2021). Machine learning has been incorporated into EEG signals' analysis, and is a subset of computational intelligence comprising numerous research areas. Machine learning depends on general patterns of reasoning via computer systems to investigate a specific task without providing obvious coded instructions. In supervised learning, it divides the data into two subsets during the learning process: a training set (i.e., dataset to train a model) and a test set (i.e., dataset to test the trained model). Supervised learning can be used for classification and regression by applying what has been learned in the training stage using labeled examples to test the new data (i.e., testing data) to classify types of or predict future events. Contrariwise, unsupervised learning is utilized when the data used for training are neither classified nor labeled (Kasabov, 2001). In EEG-based BCI applications, numerous model types have been used and developed for machine learning, where prominent families of models comprise linear classifiers, neural networks, non-linear Bayesian classifiers, nearest neighbor classifiers, and classifier combinations (Kotsiantis et al., 2006). To apply machine learning algorithms to EEG data, EEG signals must be pre-processed and their features extracted from raw data, including frequency band power and connectivity features between two channels (Daly et al., 2012). The training data used to train the classifier and test data for estimating the classifier belong to the same feature space and follow the same probability distribution (Gu et al., 2021).

Following the above, studies show that EEG is useful in identifying alterations in electrical activity in the brains of PD patients. Han et al. (2013) analyzed EEG signals in patients with PD and healthy controls (HCs), and found increased powers in θ and δ bands, and reduced powers in the α and β bands. Benz et al. (2014) discovered significant differences in EEG activity between patients with PD and Alzheimer's disease (AD), with more pronounced slowing of EEG in patients with PD compared to AD group (Benz et al., 2014). Babiloni et al. (2011) mapped eye-closed resting state EEG (rsEEG), and found abnormal alterations of δ bands at central regions, as well as θ and β bands at posterior cortical regions. Cao et al. (2021) used event-related spectral perturbation analysis to investigate EEG spectral dynamics induced by different walking phases and distinguished EEG signals throughout the transition from walking to voluntary stopping from those during the transition to involuntary stopping caused by freezing of gait (Cao et al., 2021). However, EEG signals in the above studies were only inspected visually based on a set of qualitative rules with subjective interpretations (Ebersole and Pedley, 2003). The non-linear and non-stationary processes of neural activities and interactions cannot be fully revealed with conventional time-frequency analysis based on linear Fourier and Wavelet transforms (Huang et al., 2016). Development of a new models for EEG signal analysis is thus necessary to detect information about neuronal firings and their interactions, including cross-scale coupling of neural networks through synchronizations, resonance, phase locking, and amplitude modulations (AM). Fuzzy models, which apply fuzzy rules, fuzzy logic, and fuzzy measure theory (i.e., fuzzy integrals) to a fuzzy inference system, are better for processing non-linear and non-stationary EEG signals in BCI research (Gu et al., 2021). As such, this has been widely used in entropy analysis to measure the dynamic complexity of signals, and is a crucial and urgent development as the state of complexity in humans is significantly affected by health. Cao and Lin (2018) demonstrated that inherent fuzzy entropy (Inherent FuzzyEn) and its multiscale version, which utilized empirical mode decomposition (EMD) and fuzzy membership function (i.e., exponential function), addresses the dynamic complexity in EEG data (Cao and Lin, 2018). This method was also applied successfully in investigating the extraction of repetitive steady-state visual evoked potentials to investigate EEG complexity change in patients with migraine (Cao et al., 2020).

Adhering to this, recent studies systematically demonstrate that the Holo-Hilbert spectrum analysis (HHSA) can reveal dimensional and non-linear characteristics of EEG signals in the domain of visual perception (Nguyen et al., 2019; Juan et al., 2021) and working memory (Liang et al., 2021), and outperformed conventional linear analytical methods (i.e., Fourier and Wavelet analyses). HHSA is an innovative investigation instrument based

on EMD and Hilbert Huang Transformation (Huang, 1998; Huang et al., 2016) which delivers an informational and high-dimensional frequency illustration of data from non-stationary and non-linear processes. This comprehensive method permits the investigation of the carrier and AM frequencies, as well as their interactions in neuronal oscillations. This approach is particularly important to further elucidate differences in non-linear neural processing of the envelope in AM signals in PD patients and HCs, thus providing potential neurodegenerative signals within the cortex of patients with PD.

Through this analysis, we desire to detect a decrease in higher frequency and increase in lower frequency powers, as indicated in previous reports (Tanaka et al., 2000; Kotini et al., 2005; Bosboom et al., 2006; Moazami-Goudarzi et al., 2008). Differences in the rsEEG of PD patients could also yield an impact on their cognitive or psychiatric status (Soikkeli et al., 1991; Caviness et al., 2007). Hence, in this study, we analyzed eye opening and closing rsEEG in age- and sex-matched patients with PD and HCs using the HHSA as this method can divulge the non-linear and non-stationary processes of neural activities and interactions of the rsEEG in both groups. We then looked for whether there were any association of the rsEEG with clinical assessments in both groups, and correlated the HHSA results with clinical and psychiatric scale scores. The HHSA features extracted from EEG signals were further analyzed by machine learning algorithms to generate a predictive model to distinguish between PD patients and HCs.

MATERIALS AND METHODS

Patient Recruitment

This is a cross-sectional study were patients were recruited during 2018/07/01 to 2020/12/31 in Chang Gung Memorial Hospital-Linkou Medical Center in Taiwan. Patients were diagnosed with PD according to the UK Brain Bank criteria for PD Demographic information, Levodopa Equivalent Daily Dose (LEDD) (Tomlinson et al., 2010), the Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz, 2003) and Hoehn and Yahr (H&Y) stage (Hoehn and Yahr, 1967) were recorded for each patient. All patients underwent a battery of neuropsychological assessments including the Mini-Mental State Examination (MMSE) (Tombaugh and McIntyre, 1992), Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), Clinical Dementia Rating (CDR) (Morris, 1993), Beck Depression Inventory II (BDI-II) (Beck et al., 1996), Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1986), Activities of Daily Living (ADL) (Lawton and Brody, 1969), the Parkinson's Disease Questionnaire (PDQ-39) (Jenkinson et al., 1995), and Neuropsychiatric Inventory Questionnaire (NPI) (Cummings, 1997). Patients with PD (H&Y stage 1–2) were defined as those in early stage (EPD), while those with H&Y stage greater than 2 were classified as those in late stage (LPD). Sex- and age-matched HCs were randomly recruited from neurology outpatient clinics. All subjects had no systemic infection, chronic renal failure, cardiac or liver dysfunction, malignancies, autoimmune diseases, stroke, or neurodegenerative diseases other than PD. Diagnoses were determined by two experienced neurologists in movement disorders (K. H. Chang and C. M. Chen) who were blinded to both EEG and neuropsychiatric results.

Electroencephalography Acquisition Protocol

Electroencephalography data acquisition was performed using the Brain Products GmbH amplifier (Brain Amp) with a 32-channel EEG cap (EASYCAP) according to the international 10–20 system. Both caps were saturated with Ag/AgCl gel and placed on all participants' heads. The whole 10-min for both eye-closed and eye-opened resting EEG were digitized at a 2,500 Hz (5 PD) and 5,000 Hz (59 HCs, 95 PD) sampling rate without any online filters. The reference was the average of electrodes at the two sides of the mastoid (A1 and A2) or POZ. Two pairs of bipolar electrodes were also mounted to detect eye movements with the VEOU and VEOL electrodes placed above and below the left eye, respectively, with the HEOR and HEOL electrodes positioned adjacent to the canthus of each eye. The impedances of all channels were maintained below 5 kΩ.

Electroencephalography Recording, Preprocessing, and Denoising

Electroencephalography recordings were all downsampled to 2500 Hz and re-referenced to the frontal cephalic (Fz) channel, and further standardized to 26 channels (FP1, FP2, F7, F3, Fz, F4, F8, FC1, FC2, FC5, FC6, C3, Cz, C4, CP1, CP2, CP5, CP6, P3, P7, Pz, P4, P8, O1, Oz, O2). The data were then fragmented into consecutive epochs of 8000 ms. EEG epochs with ocular, muscular, and other artifacts were preliminarily identified and excluded by a computerized automatic procedure using ICA. The HHSA was then used to compute the power spectrum of each trial.

Holo-Hilbert Spectral Analysis for Electroencephalography Recordings

Holo-Hilbert spectral analysis is an analytical method derived from Hilbert-Huang Transform (HHT) for analyzing complex signals such as EEG (Huang et al., 1998, 2016; Nguyen et al., 2019; Liang et al., 2021). HHT was achieved by using EMD and estimating instantaneous frequency by Hilbert transform. The EMD decomposed data into a finite number [$\sim\log_2$ (length of data)] of intrinsic mode functions (IMFs) and generated a high-resolution time-frequency spectral representation (**Figures 1A–E**).

In EMD, each IMF is obtained by a sifting process with the following properties: (1) the number of local extrema (including local maxima and local minima) and the number of zero-crossings must either be equal or differ by up to 1; and (2) the mean value of the envelope estimated by local maxima and local minima should be zero. Based on EMD, the advanced HHSA is achieved using a process of two-layer EMD of natural signals and high-dimensional spectral representation. In the current study, both the first and second layer EMD were performed using an improved complete ensemble EMD with adaptive noise (CEEMDAN) method for obtaining the first and second layer IMFs (Colominas et al., 2012, 2014; Tsai and Liang, 2021).

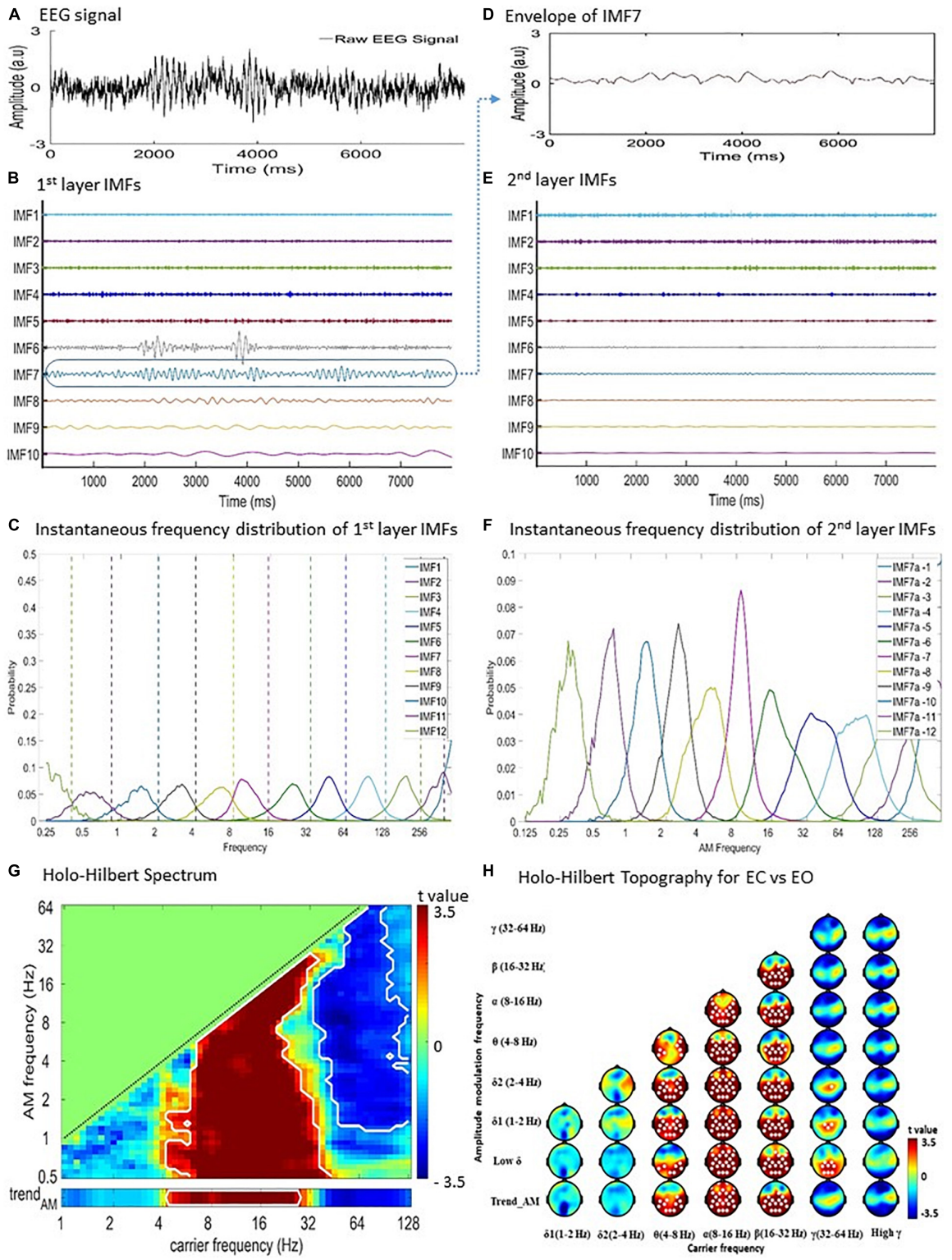

FIGURE 1 | Holo-Hilbert Spectral Analysis (HHSA) for the EEG recordings. Diagram of two-layer ensemble empirical mode decomposition (EEMD) of resting EEG data. **(A)** Raw EEG signal from a single subject at a single channel. **(B)** The first layer EEMD decomposes the raw signal into 12 intrinsic mode functions (IMFs). **(C)** The instantaneous frequency distribution of first layer IMFs denoting the frequency ranges represented by each IMF. **(D)** To illustrate the second layer EEMD, the envelope of IMF7 was extracted. **(E)** Subsequent application of EEMD on the IMF7 envelope produces the second layer IMFs. **(F)** Instantaneous frequency distribution of the second layer IMFs designating the frequency ranges represented by each IMF. **(G)** Holo-Hilbert spectrum of the carrier wave modulated by the envelopes. **(H)** Summated topographical maps of AMs of the carrier wave (0.5–64 Hz) by envelopes (1–128 Hz) frequencies. EC, eye closing; EO, eye opening.

Compared with the original EMD or ensemble EMD method, the improved CEEMDAN method has characteristics of less mode-mixing, lower reconstruction error (i.e., the noise residual within IMFs) (Wu and Huang, 2009), and higher consistency of frequency distribution ranges in the order of IMFs for different noisy signals (Colominas et al., 2014; Tsai and Liang, 2021). The steps of the two-layer CEEMDAN are described as follows:

Apply the first layer CEEMDAN to data of each EEG channel to decompose the data into a collection of IMFs (**Figure 1B**). The first layer EMD can be expressed as:

$$x(t) = \sum_{j=1}^{n} c_j(t) + r_n = \sum_{j=1}^{n} a_j(t)\cos\theta_j(t) + r_n,$$

in which the signal $x(t)$ is decomposed into n IMFs, and the jth IMF, $c_j(t)$, is further expressed as $a_j(t)\cos\theta_j(t)$, where $a_j(t)$ is the amplitude function (AF) achieved using a cubic spline algorithm, $\theta_j(t)$ is the phase function (PF) obtained using a direct quadrature (DQ) transform (Huang et al., 2009), and r_n is the final residue (i.e., trend) without any oscillatory characteristics. The instantaneous frequency (IF) of the jth IMF is obtained by taking the time derivative of the phase function, $\theta_j(t)$.

Perform the second layer CEEMDAN on the AF (i.e., envelope) of each IMF acquired from the first layer EMD (see **Figures 1D,E**), given as:

$$a_j(t) = \sum_{k=1}^{l_2} a_{jk}(t)\cos\Theta_{jk}(t) + R_{jl_2},$$

where the jth first layer IMF's AF, $a_j(t)$, is decomposed into l_2 second layer IMFs, and each second layer IMF is further expressed as $a_{jk}(t)\cos\Theta_{jk}(t)$, where $a_{jk}(t)$ is the second layer AF, $\Theta_{jk}(t)$ is the second layer PF, and R_{jl_2} is the second layer final residue without rhythmic characteristics. Therefore, these second layer IMFs expand each first layer AF in terms of rhythmic AMs from small to large time scales. The nested form of the entire two-layer CEEMDAN are:

$$x(t) = \sum_{j=1}^{n}\left[\sum_{k=1}^{l_2} a_{jk}(t)\cos\Theta_{jk}(t) + R_{jl_2}\right]\cos\theta_j(t) + r_n.$$

By taking the time derivative of the second layer PF, $\Theta_{jk}(t)$, we obtained the instantaneous "AM frequency" (**Figure 1F**). To highlight the concept of instantaneous "AM frequency" derived from the second layer CEEMDAN, the original IF obtained from the first layer CEEMDAN will be referred to as the instantaneous "carrier frequency" when it is represented in a spectrum.

Given that all the oscillatory information was obtained, such as the first and second layers of AF, instantaneous frequency, and instantaneous AM frequency (including instantaneous phase, and instantaneous AM phase), the spectral representation can be achieved as follows:

(A) The AM power (i.e., square of the second layer AF) of each second layer IMF for every specific time point is projected to the spectrum according to the instantaneous AM frequency of the second layer IMF, and the instantaneous frequency of its corresponding first layer IMF, resulting in the 3D HHS. The coordinate of "carrier frequency" is consistent with the frequency coordinate in conventional time-frequency spectrograms.

(B) Take the marginal sum/mean of the 3D HHS (1) over the AMF axis (or a specific range of the AMF axis, **Figure 1E**); (2) over the time axis (or a specific window of the time axis); or (3) over the carrier frequency axis (or a specific range of the carrier frequency axis, **Figure 1C**). This will result in the 2D time-carrier frequency, carrier-AM frequency, or time-AM frequency marginal HHS, respectively. This optional step could be tailored to specific research interests. For the current resting EEG study, the marginal sum is taken over the entire time axis to produce the 2D carrier-AM frequency marginal HHS.

In the 2D carrier-AM frequency marginal HHS, AMF should be lower than carrier frequency (i.e., $\frac{d\Theta_{jk}}{dt} < \frac{d\theta_j}{dt}$) because for any given IMF the rhythmic amplitude variations (i.e., AMs) should be slower than its corresponding carrier wave. Therefore, AM power can only exist below the carrier-AM frequency "equi-frequency" line on the HHS (**Figure 1G**). In the present study both the carrier and AM frequencies are $\log_2$-scaled. The lowermost AM frequency bin, denoted as "trend$_{AM}$," is positioned at the bottom of the HHS, separated from other higher AM frequency bins. The spectral power in the trend$_{AM}$ bin signifies the "unmodulated" power estimated by the trend (i.e., the last component) of each second-layer EMD. Both AM and carrier frequency bins are categorized according to physiological frequencies as following: low δ (0.5–1 Hz), δ1 (1–2 Hz), δ2 (2–4 Hz), θ (4.0–8.0 Hz), α (8–16 Hz), β (16–32 Hz), low γ (32–64 Hz), and high γ (64–128 Hz) (Buzsáki and Watson, 2012). The topography of the amplitude-frequency modulation is then plotted using the summation of overall activities at all sensors for respective bands of carriers and AM frequencies (**Figure 1H**). All HHSA analyses were performed using customized MATLAB (MathWorks) scripts.

Statistical Analysis

For results visualization, the time dimension of the spectral power was summed to produce a two-dimensional Holo-Hilbert spectrum (AM frequency bins × carrier frequency bins). In this spectrum, the y-axis represents AM frequency and the x-axis refers to carrier frequency. All trials were then averaged and the data from each group were merged as one dataset. Subsequently, the averaged and merged data of each group was rescaled by the log ratio to the average of all timepoints to elevate the homogeneity to fit a normal distribution for further statistical analysis.

For statistical comparisons, differences of the eye-closed and eye-open condition within groups were examined using paired t-test, whereas differences between groups were examined using independent t-tests. A two-tailed cluster-based non-parametric permutation test (CBnPP test under $p < 0.05$ with 5,000 permutations) was conducted on the multichannel HHSA spectra

TABLE 1 | Clinical characteristics of patients with Parkinson's disease (PD) in early (EPD) and late (LPD) stages, and healthy controls (HC).

	HC	PD		
	(*n* = 59)	EPD (n = 80)	LPD (n = 19)	Total (n = 99)
Sex (female/male)	31/28	39/41	9/10	48/51
Age (years)	66.59 ± 8.03	65.26 ± 10.76	72.42 ± 9.38	66.65 ± 10.85
Duration (years)		5.88 ± 8.20	13.5 ± 5.52	7.37 ± 8.30
Hoehn and Yahr stage		1.58 ± 0.54	2.89 ± 0.46	1.84 ± 0.75
LEDD (mg)		467.09 ± 435.39	1351.63 ± 659.26	642.16 ± 599.59
Antidepressants (%)	1 (1.69)	2 (2.50)	1 (5.79)	3 (3.03)
Antipsychotics (%)	1 (1.69)	0	2 (10.53)	2 (2.02)
UPDRS-total	1.63 ± 2.20	28.92 ± 16.93*	79.20 ± 39.88*#	40.18 ± 28.68*
UPDRS-part III	0.56 ± 1.26	17.51 ± 9.33*	41.79 ± 15.78*#	22.21 ± 14.47*
MMSE	29.64 ± 9.06	27.36 ± 3.85*	22.11 ± 6.66*#	26.35 ± 4.94*
CDR	0.22 ± 0.25	0.32 ± 0.24	0.66 ± 0.37*#	0.38 ± 0.3*
ADL	99.92 ± 0.65	99.63 ± 1.55	70.0 ± 28.28*#	93.94 ± 16.92*
MoCA	27.86 ± 2.39	24.53 ± 5.73*	18.0 ± 8.67*#	23.27 ± 6.85*
BDI-II	1.68 ± 2.89	6.23 ± 4.83*	16.21 ± 7.14*#	8.14 ± 6.62*
HAM-D	1.63 ± 2.73	5.05 ± 3.62*	10.89 ± 6.40*#	6.17 ± 4.84*
PDQ-39	6.10 ± 8.24	23.20 ± 18.25*	68.11 ± 33.69*#	31.82 ± 28.16*
NPI	0.54 ± 1.72	1.89 ± 2.71*	7.58 ± 7.47*#	2.98 ± 4.61*

**Statistically significantly different in comparison with HC.*
#Statistically significantly different in comparison with PD in early stage.
ADL, Activities of Daily Living; BDI-II, Beck Depression Inventory II; CDR, Clinical Dementia Rating; HAM-D, Hamilton Depression Rating Scale; LEDD, Levodopa Equivalent Daily Dose; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NPI, Neuropsychiatric Inventory Questionnaire; PDQ-39, Parkinson's Disease Questionnaire; UPDRS, Unified Parkinson's Disease Rating Scale.

(channels × AM frequency bins × carrier frequency bins) for multiple comparisons correction (Maris and Oostenveld, 2007). The neighboring distance between two EEG sensors was defined as 75 mm with 5000 permutations for each test. Though unconventional compared to the Bonferroni or false discovery rate (FDR), it is recognizably efficient for multiple comparison errors (Maris and Oostenveld, 2007). This was done for both NC and PD groups, and further for EPD and LPD groups.

Pearson's correlation analysis was performed to analyze the linear dependence between two variables. Sex distribution was analyzed using a χ^2 test. Each set of data was expressed as mean ± standard deviation. All *P*-values were two-tailed, and $P < 0.05$ was considered significant.

Classification, Feature Extraction and Selection for Machine Learning

Electroencephalography components were extracted from each AM of 26 electrodes, where the ratio between two EEG components was used as a feature. The total number of features was 172,640. Afterward, a correlation analysis was conducted on features, which retained one distinct feature from a cluster of features with a correlation greater than 0.95. Subsets of 100 features from the tens of thousands of remaining features were applied to the LogitBoost algorithm to select the three most prioritized features from every subset. This procedure was iterated until the number of features was reduced to 3, crucial for the function in the final model. In this study, seven common algorithms were employed: LogitBoost, Bagging (Bag), Gentle adaptive boosting (GentleBoost), Decision tree (Tree), support vector machine (SVM), Naïve Bayes and K-Nearest Neighbor, all of which were implemented via the MATLAB software. The models' performance estimation was further analyzed using receiver operating characteristic (ROC) curves to determine the area under the ROC curve (AUC), and values of sensitivity, specificity, precision, F1 measure, and accuracy.

RESULTS

Demographic Features of Parkinson's Disease Patients

This study recruited 99 patients with PD and 59 cognitively normal subjects as HCs (**Table 1**). Patients with PD demonstrated significantly higher scores in CDR (PD: 0.38 ± 0.3, HC: 0.22 ± 0.25, $P = 0.001$, Cohen's $d = 0.58$), BDI-II (PD: 8.14 ± 6.62, HC: 1.68 ± 2.89, $P < 0.001$, Cohen's $d = 1.26$), HAM-D (PD: 6.17 ± 4.84, HC: 1.63 ± 2.73, $P < 0.001$, Cohen's $d = 1.16$), PDQ-39 (PD: 31.82 ± 28.16, HC: 6.10 ± 8.24, $P < 0.001$, Cohen's $d = 1.24$), and NPI (PD: 2.98 ± 4.61, HC: 0.54 ± 1.72, $P < 0.001$, Cohen's $d = 0.70$), compared with HCs. MMSE (PD: 26.35 ± 4.94, HC: 29.64 ± 9.06, $P < 0.001$, Cohen's $d = 0.45$) and MoCA (PD: 23.27 ± 6.85, HC: 27.86 ± 2.39, $P < 0.001$, Cohen's $d = 0.90$) were significantly lower in PD patients compared with HCs. LPD patients were older (EPD: 65.26 ± 10.76 years, LPD: 72.42 ± 9.38 years, $P = 0.002$, Cohen's $d = 0.71$), had a longer disease duration (EPD: 5.88 ± 8.20 years, LPD: 13.5 ± 5.52 years, $P < 0.001$, Cohen's $d = 1.10$), greater scores for UPDRS (EPD: 28.92 ± 16.93, LPD: 79.20 ± 39.88,

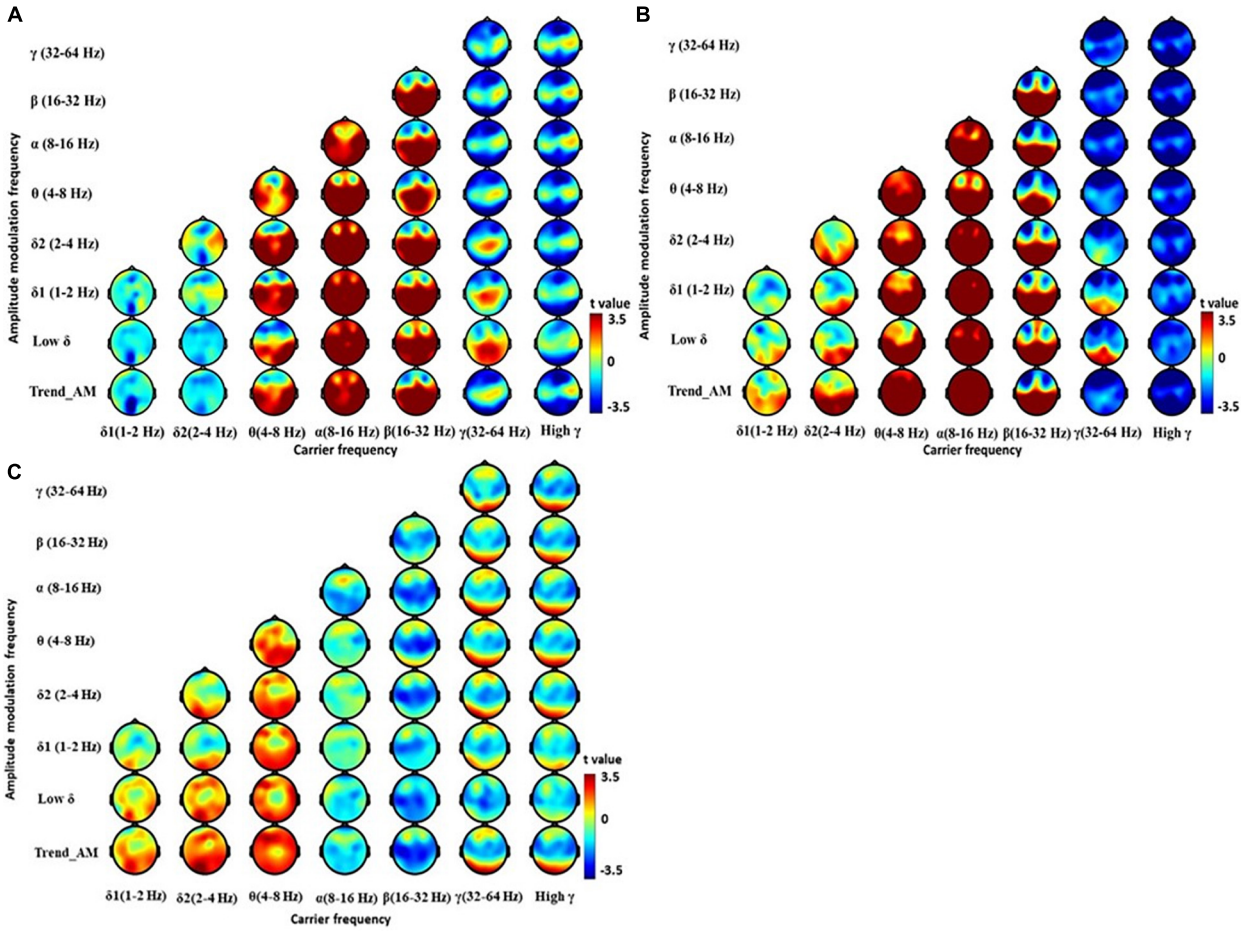

FIGURE 2 | Electroencephalography power difference between PD and HC using Holo-Hilbert Spectrum Analysis (HHSA). Holo-Hilbert topography (HHT) in the eyes-closed minus the eyes-open condition based on cluster-based non-parametric permutations in the **(A)** HC, **(B)** PD, and **(C)** PD minus HC. The color bar denotes *t*-statistics ranging from blue (–3.5) to red (+3.5).

$P < 0.001$, Cohen's $d = 2.17$) and Hoehn and Yahr stage (EPD: 1.58 ± 0.54, LPD: 2.89 ± 0.46, $P < 0.001$, Cohen's $d = 2.61$), and greater LEDD (EPD: 467.09 ± 435.39 mg/d, LPD: 1351.63 ± 659.26 mg/d, $P < 0.001$, Cohen's $d = 1.58$) compared with EPD patients. CDR (EPD: 0.32 ± 0.24, LPD: 0.66 ± 0.37, $P < 0.001$, Cohen's $d = 1.09$), BDI-II (EPD: 6.23 ± 4.83, LPD: 16.21 ± 7.14, $P < 0.001$, Cohen's $d = 1.64$), HAM-D (EPD: 5.05 ± 3.62, LPD: 10.89 ± 6.40, $P < 0.001$, Cohen's $d = 1.12$), PDQ-39 (EPD: 23.20 ± 18.25, LPD: 68.11 ± 33.69, $P < 0.001$, Cohen's $d = 1.66$), and NPI (EPD: 1.89 ± 2.71, LPD: 7.58 ± 7.47, $P < 0.001$, Cohen's $d = 1.01$) were significantly greater in patients with LPD compared to those with EPD. Patients with LPD displayed lower MoCA scores (EPD: 24.53 ± 5.73, LPD: 18.0 ± 8.67, $P < 0.001$, Cohen's $d = 0.89$) and ADL scores (EPD: 99.63 ± 1.55, LPD: 70.0 ± 28.28, $P < 0.001$, Cohen's $d = 1.48$) compared to those with EPD. Antidepressants were prescribed in three (3.03%) patients with PD and one (1.69%) HC, respectively. Two (2.02%) patients with PD and one (1.69%) HC were treated with antipsychotics.

Electroencephalography Features of Parkinson's Disease Patients by Holo-Hilbert Spectral Analysis

Holo-Hilbert spectral analysis showed significant differences in spectral powers between the PD and HC group (**Figure 2** and **Supplementary Table 1**). In the HC group, θ and β bands were dispersed from frontal to occipital regions. Reduced γ bands were observed at frontal and occipital regions (**Figure 2A**). Although PD group demonstrated spread of energy into θ bands in frontal, central, parietal, and occipital regions similar to HC (**Figure 2B**), the increased power of θ bands were dispersed to pre- and lateral-frontal regions (**Figure 2C**). δ2 bands spreading to central, parietal, temporal, and occipital regions were also noted in the PD group. Compared to the HC group, PD patients demonstrated reductions of β bands in frontal and central regions (**Figure 2C** and **Supplementary Table 1**). Reduced γ bands, particularly in relatively high amplitude frequencies, were also seen in central, parietal, and temporal regions of PD patients. These results suggest an increase of

slowing resting state brain activity into θ and δ2 frequency domains, and reduction of brain activity in β and γ frequency domains, in PD patients.

Electroencephalography Features Between Early- and Late-Stage Parkinson's Disease Patients

Holo-Hilbert spectral analysis showed significant differences between the EPD and LPD group (**Figure 3** and **Supplementary Table 2**). Compared with HCs, EPD patients demonstrated dispersed θ and δ bands particularly in relatively low AM frequencies from lateral frontal to occipital regions, and reduced β and γ bands in central and temporal regions (**Figure 3A**). LPD patients demonstrated increased θ bands from the central frontal to occipital regions, dispersed δ bands in occipital regions, decreased α bands in central and temporal regions, decreased β bands in central, parietal, and occipital regions, and reduced γ bands in the central region (**Figure 3B**). Compared with EPD group, LPD patients demonstrated reduction of β bands in the posterior central region, and increased θ and δ2 bands in left parietal region (**Figure 3C** and **Supplementary Table 2**). These results suggest that LPD patients showed further reduction of fast resting state brain activity in β frequency domains, and an increase of slowing resting state brain activity in θ and δ2 frequency domains, as compared with EPD patients.

Correlations Between Electroencephalography and Clinical/Neuropsychiatric Features

We further correlated HHSA features with clinical and neuropsychiatric scale scores, where significant results were shown with HAM-D scores (**Figure 4**). HAM-D scores were significantly positively correlated with β bands in central, parietal, and occipital regions in PD patients, with an r value up to and more than 0.7 (**Figure 4B**). A subgroup analysis showed HAM-D was significantly positively correlated with δ1 and δ2 bands in central regions of EPD patients (**Figure 4C**). HAM-D and activity from θ to β bands in most of brain regions were significantly positively correlated in LPD patients (**Figure 4D**). These correlations were not observed in HCs (**Figure 4A**). Other clinical and neuropsychiatric scales were not correlated with HHSA features of EEG. These results showed fast and slow brain activities, particularly in central, parietal, and occipital regions, could be associated with depressive moods of patients with PD.

Machine Learning Classification Using Electroencephalography and Neuropsychiatric Features in Parkinson's Disease Patients

We further selected the three most prioritized HHSA features (FZ, AM frequency 1–2 Hz, Carrier frequency 128–256 Hz; F8, AM frequency 2–4 Hz, Carrier frequency 4–8 Hz; C3, AM 8–16 Hz, Carrier frequency 32–64 Hz) that demonstrated significant differences between PD and HC to 7 machine learning algorithms. The sample sizes were 94 for training (PD: 59, HC: 35), with 10-fold cross validation, and 64 for testing (PD: 40, HC: 24). **Figure 5** shows the results of applying the training data to each algorithm, with the best result appearing in the "Bag" algorithm with an AUC of 0.90, followed by "LogitBoost" with an AUC of 0.89, and "GentleBoost" with an AUC of 0.88, and AUC of other algorithms were all greater than 0.7. The application of each algorithm to testing data showed that "Bag" demonstrated the highest level of accuracy (0.81), followed by "Tree" (0.80), "LogitBoost" (0.79) and "SVM" (0.74) (**Table 2**). These results support the potential of implementing machine learning algorithms with HHSA features of EEG as diagnostic tools for PD.

DISCUSSION

Using the HHSA, we decomposed the EEG signals to produce frequency bands that reflect the natural rhythmic activity of large neural populations. PD patients demonstrated a reduction of β bands in frontal and central regions, and reduction of γ bands in central, parietal, and temporal regions. Compared with EPD patients, LPD patients demonstrated further reduction of β bands in the posterior central region, and increased θ and δ2 bands in left parietal regions. Fast and slow resting state brain activity in the central parietal and occipital regions were positively correlated with HAM-D scores. Machine learning algorithms using three prioritized HHSA features demonstrated good performance when differentiating between PD and HCs, strengthening the application of HHSA in PD diagnosis.

The concept of fuzzy sets (Zadeh, 1965) reliably addresses complexity via the FuzzyEn measure (Chen et al., 2007, 2009) and hence delivers stronger relative reliability and more accurate complexity compared to other entropy-based evaluations (Chen et al., 2009), validating its powerful application to short time series with noise impurity. As EEG signals typically display complex variabilities, indefinite disruption, and great levels of non-linearity and non-stationarity, and other dynamic information (Costa et al., 2005), studying dynamic complexity via entropy better elucidates complex systems (Chen et al., 2007) and potentiates its application clinically (Yang et al., 2013). Patients with Alzheimer's disease exhibit EEG slowing, reduced complexity of EEG signals, and perturbations in EEG synchrony (Dauwels et al., 2010). These results submit dynamic complexity as a potential bio-signature to monitor health conditions. With high non-linear and non-stationary brainwaves in EEG, especially superimposed trends in signals, the estimation of entropy-based analysis could impact the data by increasing its standard deviation. Thus, to eliminate trend oscillations, the inherent functions (i.e., IMFs) extracted from the EMD are deemed an effective filter for reducing superimposed trends in signals (Huang et al., 1998), as seen in the HHSA. Similarly, the HHSA establishes its advantage in its ability to adapt to EEG signals in time sequences. The HHSA method complements the deficits of traditional spectral analysis and provides a complete informational illustration of non-linear and non-stationary data via the nested EMD

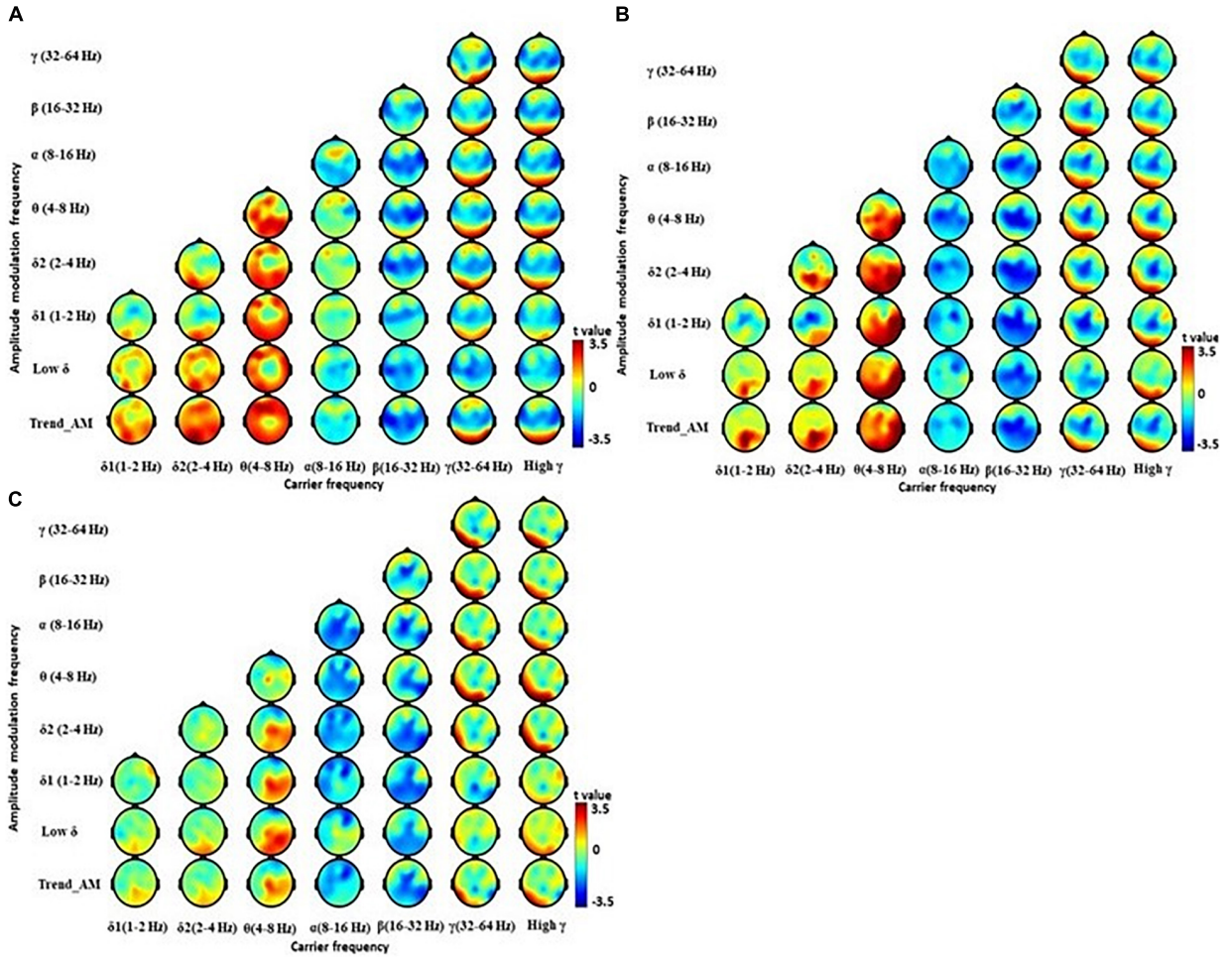

FIGURE 3 | Electroencephalography power difference between PD at early (EPD) and late stage (LPD) using Holo-Hilbert Spectrum Analysis (HHSA). Holo-Hilbert topography (HHT) in the eyes-closed minus the eyes-open condition based on cluster-based non-parametric permutations in the **(A)** EPD minus healthy control (HC), **(B)** LPD minus HC, and **(C)** LPD minus EPD. The color bar denotes *t*-values ranging from blue (–3.5) to red (+3.5).

and Hilbert–Huang transform (HHT) approach to identify intrinsic amplitude and frequency modulations within non-linear systems (Huang et al., 2016). For non-linear processes, the data contains both amplitude and frequency modulations (intra-mode and inter-mode) engendered via two processes: linear additive or non-linear multiplicative processes. To handle multiplicative processes, extra dimensions in the spectrum are necessary to account for disparities in both the amplitude and frequency modulations concurrently. The HHSA competently accommodates both the additive and multiplicative processes, intra- and inter-mode, stationary and non- stationary, linear and non-linear interactions (Huang et al., 2016). The spectral analysis divulges time-dependent fluctuations and explicitly a measure of the degree of non-linearity within each IMF through the intra-wave frequency variations (Huang, 2014). A core benefit for decomposing the time series into IMFs is that all additive and multiplicative interactions can be separated, extracted and quantified by the first and second layer EMD and HHSA (Huang et al., 1998, 2016; Wu and Huang, 2009). The HHSA can thus methodically define, elucidate and enumerate the linear and non-linear intra- and inter-mode interactions and unfetters spectral analysis from restrictions imposed by Fourier, wavelet or HHT. Since EEG complexity can distinguish patients from health controls, the HHSA is also a promising application for healthcare solutions in the real world.

Overall, the results we obtained concur with previous reports (Tanaka et al., 2000; Kotini et al., 2005; Bosboom et al., 2006; Moazami-Goudarzi et al., 2008) as PD patients exhibited generalized EEG slowing. Recently, Cao et al. (2020) revealed the adaptability of the brain to its environment during visual stimulation using multiscale inherent fuzzy entropy (Cao et al., 2020). The behavioral features of brain electrical activity that decreases in response due to repeated visual stimulation is defined as habituation, and reflects robustness of the brain system (Thompson and Spencer, 1966; Groves and Thompson, 1970). By computing brain complexity in its habituation toward SSVEPs (Cao et al., 2020), they objectively estimated the complexity measure of physiological signals that reveals the

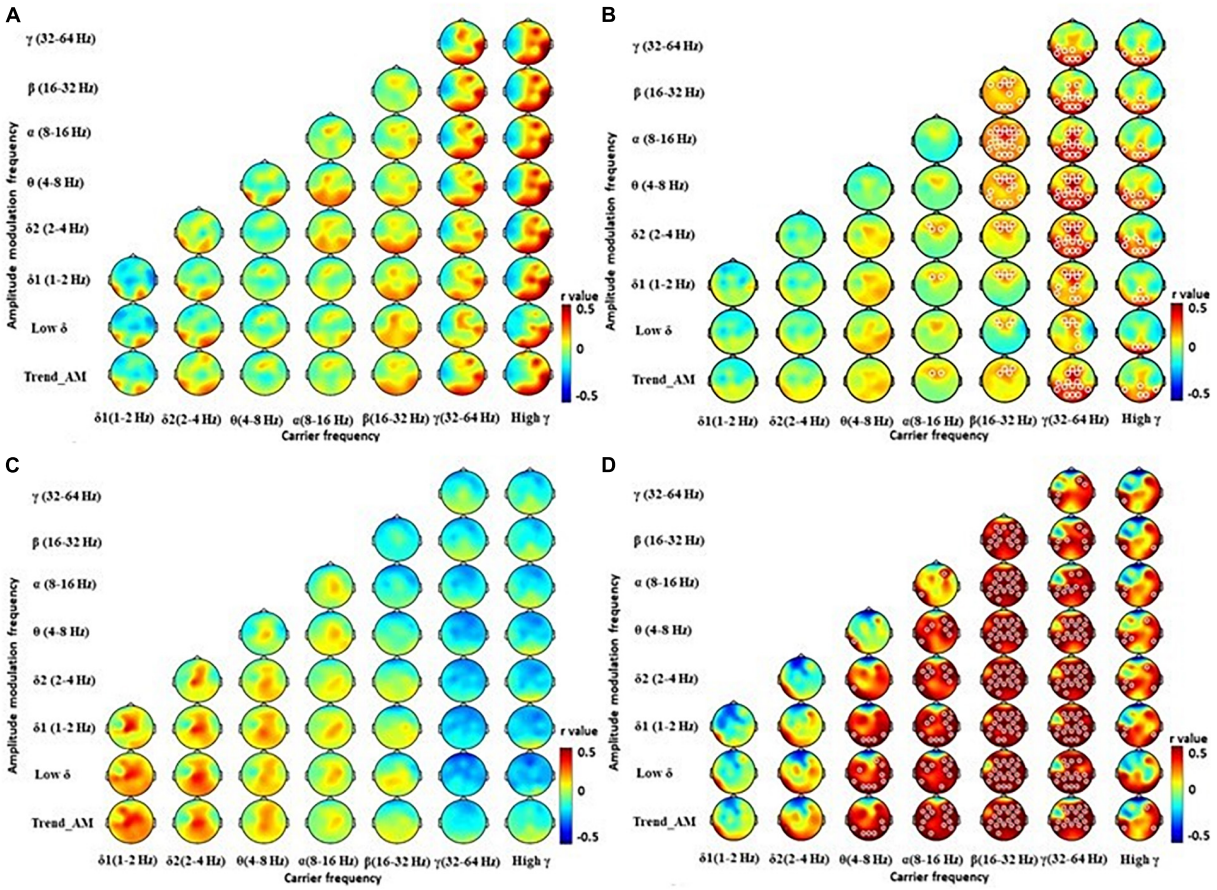

FIGURE 4 | Correlation between powers of Holo-Hilbert Spectrum Analysis (HHSA) and Hamilton Depression Rating Scale (HAM-D). The contrasted HHSA for correlation between HAM-D and **(A)** healthy controls, **(B)** patients with Parkinson's disease (PD), **(C)** PD patients at early stage (EPD), **(D)** PD patients at late stage (LPD). The white circles indicate that contrast on those EEG channels is statistically significant ($P < 0.05$, cluster permutation test, two-tailed). Color notations depict the *r* value of correlations (shown up to 0.05 for easier visualization purposes).

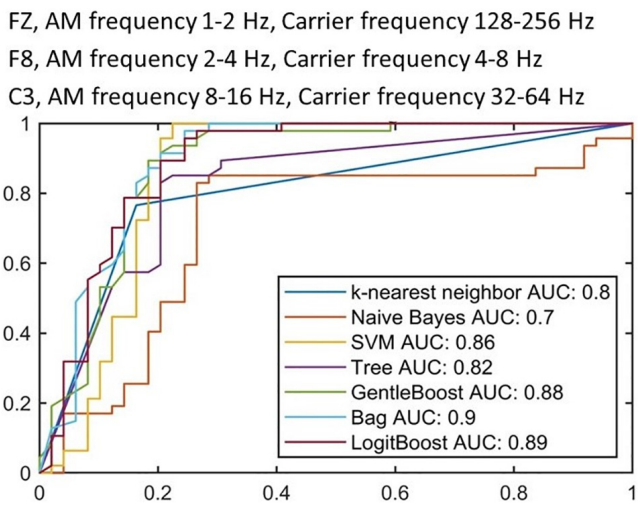

FIGURE 5 | Receiver operating characteristic curves from training stage with 10-fold cross validation. Each ROC curve represents a candidate algorithm (the AUC of all algorithms are higher than 0.7).

TABLE 2 | Performance evaluation of classification algorithms deploying PD and HC using features extracted via different analytic methods.

	LogitBoost	Bag	GentleBoost	Tree	SVM	Naïve Bayes	K-Nearest Neighbor
Sensitivity	0.85	0.85	0.90	0.80	0.60	0.20	0.65
Specificity	0.70	0.75	0.60	0.75	0.87	0.95	0.80
Precision	0.70	0.75	0.70	0.80	0.80	0.80	0.79
F1 measure	0.74	0.80	0.77	0.80	0.69	0.32	0.70
Accuracy	0.79	0.81	0.79	0.80	0.74	0.60	0.70

robustness of brain systems (Gao et al., 2021) with an essential measure in the crucial features of non-linear neuro-dynamics (Gao et al., 2011). Diseased systems are known to show lower entropy values compared to healthy systems (Takahashi et al., 2010; Cao and Lin, 2018; Cao et al., 2019) and decreased complexity may epitomize reduced brain system integrity, while elevated complexity strongly correlates with stable and accurate behavioral performance (Lippé et al., 2009). Cao et al. (2020) found that EEG complexity increases with increasing visual stimulus times, postulating a strong ability of the brain to tolerate perturbations that ensues in functional or structural systemic modification (Cao et al., 2020). Humans are also able to stop reacting to a stimulus that is no longer biologically relevant (Thompson and Spencer, 1966; Groves and Thompson, 1970), but rather habituate to repeated visual stimulus that no longer have effects. This habituation performance is a form of adaptive behavior, and reflects the robustness of brain systems. In our results using the HHSA, the differences between controls and patients plausibly agree with Cao et al. (2020), as the slowing of EEG in PD patients during the resting state also indicates decreased complexity (Babiloni et al., 2011; Yang et al., 2013). Thus, PD patients may also have decreased habituation since the robustness of their brain systems are compromised.

Parkinson's disease patients largely exhibited a reduction in higher frequency β and γ bands. This occurred with increment in lower frequency θ and δ bands. In patients with PD, the extensive decline of dopamine leads to abnormal oscillatory activity within the thalamus, further affecting oscillations within the cortex (French and Muthusamy, 2018). The pathophysiologic oscillations of the thalamus (i.e., thalamocortical dysrhythmia) (Jeanmonod et al., 2001), occurs due to thalamic over-inhibition. The lack of dopamine input to the basal ganglion causes the globus pallidus output nucleus to be abnormally active (Levy et al., 2000), and exerts over-inhibition on the ventral lateral and ventral anterior nucleus, through the pallido–thalamic tract (Magnin et al., 2000; Anderson et al., 2003). This overinhibition results in hyperpolarization and deactivation of calcium T-channels in thalamic neurons, and generates low-threshold calcium spike bursts in an inter-burst frequency of ∼4 Hz (Steriade et al., 1990; Jeanmonod et al., 1996; Llinás, 2014). The anatomical and functional coupling between the thalamus and cortex produces high coherence between these structures (Van Horn and Sherman, 2007), yielding overproduction of θ activity in the cortex. Our study consistently showed increased θ bands in the cortex of patients with PD (**Figure 2C**). The increased brain activity in δ bands, particularly in LPD patients (Soikkeli et al., 1991; **Figure 3C**), denotes further widespread slowing of activity in PD, which is a marker of bradyphrenia (Brown, 2003; Rowland et al., 2015) as well as cognitive decline and dementia (Bosboom et al., 2006; Stoffers et al., 2007).

The consistent finding of reduced β and γ bands in frontal, central, parietal, and temporal regions in patients with PD (**Figure 2C**), probably originates from the unilateral sensorimotor cortex (Stancák and Pfurtscheller, 1996; Doyle et al., 2005). This spreads to bilateral sensorimotor regions at movement onset (Neuper et al., 2006), starting from 1000 ms prior to movement onset. This suppression of β bands is likely sustained if the effector is moving (Wheaton et al., 2009). Notably, treating PD patients with levodopa significantly increases β bands, suggesting abnormal β bands (Melgari et al., 2014) as a possible biomarker of motor impairment in patients with PD. A prominent γ band provides a signature of engaged networks. In the sensory cortex, γ bands increase with sensory drive (Henrie and Shapley, 2005), and with a broad range of cognitive phenomena, including perceptual grouping (Tallon-Baudry and Bertrand, 1999) and attention (Fries et al., 2001). The role of reduced γ bands in patients with PD warrants further study.

Correlation analyses with clinical scales revealed significant strong positive correlations only in HAM-D. No other correlation was found between θ bands with overall PD severity (UPDRS, H&Y stage), or cognitive examinations (CDR, MMSE, MoCA). Further studies will be needed to explore the pathophysiological and clinical roles of δ activity in patients with PD. As for the correlation with depression, this is largely reflected by activities in the thalamocortical and cortico-cortical circuits due to altered EEG oscillations (Fingelkurts and Fingelkurts, 2015). In the resting state, depression is associated with increased β bands. Increased β and θ bands are also reported in depressed patients with attentional deficits (Li et al., 2016). Our study identified a positive correlation between β bands and HAM-D scores, clarifying a role of β bands in the severity of depression (**Figure 4B**). We also found that patients with PD in different stages may demonstrate different correlations between EEG signals and the severity of depression. A positive correlation between δ bands in the central region and HAM-D scores were observed in EPD patients (**Figure 4C**), while global θ and β bands were positively correlated with HAM-D scores in LPD patients (**Figure 4D**). These findings indicate different pathophysiological mechanisms of depression present in patients with PD at different stages. Given that LEDD in LPD patients was significantly higher compared to EPD patients, effects of anti-parkinsonian medications on EEG patterns should be considered.

The introduction of machine learning algorithms in EEG analysis provides a potentially easy, accessible, and affordable technique to support the diagnosis of PD. However, the measurement protocols, number of channels, data preprocessing, and feature selection remain inconsistent. Vanneste et al. (2018) applied SVM subsequent to EEG signals processing with standardized low-resolution brain electromagnetic tomography, and found nine featured EEG signals in 31 PD patients and 264 HCs and found an accuracy of 0.94. However, the model performance may have been overestimated due to the imbalance of patients with PD and HCs. Yuvaraj et al. (2018) extracted 13 features in eyes-closed EEG signals in 20 PD patients and 20 HCs by high order spectra. Utilizing the SVM according to these features achieved an accuracy greater than 0.99. However, the relatively small number of subjects raise concerns of overfitting and inadequate generalization. Our method of machine learning considered the fact that EEG signals are irregular and mobile, hence exhibits unpredictability during the classification performance (Abbass et al., 2014). Transfer learning can manage data that violate this hypothesis through manipulating knowledge acquired while learning a given task for solving a different but related task. This obliterates the need to calibrate from the initiating point, yields less noise for transferred information, and depends on prior usable data to proliferate dataset size. By using fuzzy-rule based classification systems, sensible rules can be developed to process EEG activities based on knowledge of neurophysiology and neuroscience, and are therefore explicable. The extraction of intrinsic EEG activities from a neuro-fuzzy model similar to ours considers the fact that EEG signals are non-linear and non-stationary (Jang et al., 2005). A fuzzy inference system (FIS) automatically extracts fuzzy "If-Then" rules from the data and describes which input feature values correspond to which output category (Fabien et al., 2007), permitting the advantage of flexible boundary conditions for BCI applications, EEG pattern classification, and interpreting what the FIS has learned (Sugeno, 1993). Hence, this provides better domain accommodation interpretability and signal processing capability that are particularly advantageous for handling non-linear and non-stationary EEG signals. In PD, Oh et al. (2020) proposed an EEG-based deep learning approach with a convolution neural network (CNN) architecture as a computer-aided diagnostic system and established its possibility in clinical usage for PD detection (Oh et al., 2020). Dunne et al. (2016) presented a specific class of recurrent neural network (RNN) structure termed echo state networks (ESNs) to differentiate EEG signals collected from patients with random eye movement sleep behavioral disorder who ultimately developed PD or Lewy Body Dementia and healthy controls (Dunne et al., 2016). Cao et al. (2020) used inherent fuzzy entropy to study repetitive SSVEPs for analyzing EEG complexity change between migraine phases, while employing the AdaBoost classification with an accuracy of 0.81 $\pm$ 0.06 and AUC of 0.87 for differentiating interictal and preictal phase of migraine (Cao et al., 2020). In our study, a relatively large number of patients and HCs were recruited, and limited features were selected to avoid overfitting, adding to the consideration that we incorporated features from the second layer EMD into our method, additional features that can be used for the classification are introduced. The specific electrodes were F8 (AM 2–4 Hz with FM 4–8 Hz), FZ (AM 1–2 Hz with FM 128–256 Hz) and C3 (AM 8–16 Hz with FM 32–64 Hz). The Bag algorithm demonstrated the best accuracy (0.81) compared with other algorithms (**Table 2**), while the ROC showed an AUC of 0.90 by Bag, followed by 0.79 by GentleBoost and LogitBoost (**Figure 5**). These findings suggest the potential application of HHSA in preprocessing EEG signals for further diagnosis of PD by machine learning algorithms. Further validation by larger cohorts and refinement of feature extraction methods would be important to improve the performance of these models.

Although our study consolidates the role of HHSA in identification of EEG features in patients with PD, there are some limitations. The numbers of LPD and PDD patients are relatively small. The EEG signals could be affected by use of medications, such as anti-parkinsonian, antidepressants, and anti-psychotics. The single-center nature of our studies lacks external validity. Future multi-center studies with a large number of patients will be required to incorporate our findings into clinical practice.

CONCLUSION

Our HHSA method for decomposing and characterizing PD EEG signals permitted the differentiation between matched normal controls and PD patients. Furthermore, the HHSA was sensitive in detecting tendencies toward depression corresponding with a hyperstable regulation of arousal. Features extracted from the HHSA also enabled the distinction of PD from normal controls, specifically in the F8, FZ and C3 electrodes. Further validation will be needed using larger cohorts to refine feature extraction methods to improve the performance of these models, especially to differentiate between the different stages and existence of PD induced dementia.

DATA AVAILABILITY STATEMENT

Data will be made available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study involved human participants and was reviewed and approved by the Institutional Review Boards of the Chang Gung Memorial Hospital (ethical license nos: 201801049A3 and 201801051A3). Informed consent was collected from all subjects involved in the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

K-HC, C-MC, and C-HJ: conception, organization, resources, and finalize the manuscript. Y-SL, Y-RW, IF, W-KL, M-LC, NH, and C-HJ: performing EEG and their analysis. K-HC, C-MC, H-CW, and S-NL: recruited patients, examined patients and controls. IF, C-HJ, W-KL, and NH: statistical analysis. K-HC:

funding acquisition. K-HC and C-HJ: writing of the first draft. All authors: review and critique. C-MC: supervision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.832637/full#supplementary-material

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Identifying Cognitive Impairment in Elderly Using Coupling Functions Between Cerebral Oxyhemoglobin and Arterial Blood Pressure

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Background: This study aimed to assess brain oxygenation status and cerebral autoregulation function in subjects with cognitive dysfunction.

Methods: The Montreal Cognitive Assessment (MoCA) was applied to divide the subjects into three groups: cognitive impairment (Group CI, 72.50 ± 10.93 y), mild cognitive impairment (Group MCI, 72.02 ± 9.90 y), and normal cognition (Group NC, 70.72 ± 7.66 y). Near-infrared spectroscopy technology and a non-invasive blood pressure device were used to simultaneously measure changes in cerebral tissue oxygenation signals in the bilateral prefrontal lobes (LPFC/RPFC) and arterial blood pressure (ABP) signals from subjects in the resting state (15 min). The coupling between ABP and cerebral oxyhemoglobin concentrations (Δ [O_2Hb]) was calculated in very-low-frequency (VLF, 0.02–0.07 Hz) and low-frequency (LF, 0.07–0.2 Hz) bands based on the dynamical Bayesian inference approach. Pearson correlation analyses were used to study the relationships between MoCA scores, tissue oxygenation index, and strength of coupling function.

Results: In the interval VLF, Group CI ($p = 0.001$) and Group MCI ($p = 0.013$) exhibited significantly higher coupling strength from ABP to Δ [O_2Hb] in the LPFC than Group NC. In the interval LF, coupling strength from ABP to Δ [O_2Hb] in the LPFC was significantly higher in Group CI than in Group NC ($p = 0.001$). Pearson correlation results showed that MoCA scores had a significant positive correlation with the tissue oxygenation index and a significant negative correlation with the coupling strength from ABP to Δ [O_2Hb].

Conclusion: The significantly increased coupling strength may be evidence of impaired cerebral autoregulation function in subjects with cognitive dysfunction. The Pearson correlation results suggest that indicators of brain oxygenation status and cerebral

autoregulation function can reflect cognitive function. This study provides insights into the mechanisms underlying the pathophysiology of cognitive impairment and provides objective indicators for screening cognitive impairment in the elderly population.

Keywords: cognitive dysfunction, coupling function, near-infrared spectroscopy, arterial blood pressure, tissue oxygenation index

INTRODUCTION

As the population ages, dementia has become a global public health concern (Kisa et al., 2018). It is estimated that 115 million people will be living with dementia by 2050, which is a great challenge affecting families, communities, and health care systems around the world (Prince et al., 2015). Mild cognitive impairment is thought to lie on a functional continuum between normal cognitive aging and the earliest signs of dementia (Rombouts et al., 2005). Studies have indicated that early interventions in the early stages of dementia (i.e., mild cognitive impairment) present an opportunity to improve or maintain cognitive function to slow the trajectory of cognitive decline (Livingston et al., 2017). Without an intervention, the neurodegenerative process will cause irreversible atrophy (Raz et al., 2016). Accurate identification of cognitive dysfunction is a prerequisite to receiving these interventions. Therefore, the establishment of boundaries between normal aging and dementias using reliable, sensitive, quantitative, and objective criteria is essential for improved clinical outcomes.

Cerebral autoregulation is a protective mechanism that maintains cerebral blood flow at a relatively constant level despite fluctuations of cerebral perfusion pressure (Beek et al., 2008). Cerebral autoregulation is a frequency-dependent phenomenon that allows rapid ABP changes (<0.2 Hz) to be transmitted to cerebral blood flow, whereas slow ABP changes are filtered (Numan et al., 2014; Claassen et al., 2016). Impaired cerebral autoregulation leads to a greater dependence of cerebral blood flow on blood pressure, leaving brain tissue unprotected against the potentially harmful effects of blood pressure fluctuations (den Abeelen et al., 2014). It has been evidenced that cerebral autoregulation function is altered or impaired in patients with a variety of conditions such as diabetes (Hu et al., 2008), Parkinson's disease (Vokatch et al., 2007), and stroke (Xiong et al., 2017). Recent research suggested an interrelationship between Alzheimer's disease pathology, radiographic markers of cerebral hypoperfusion, and cerebral autoregulation (Brickman et al., 2015; Zhou et al., 2019). Therefore, it can be hypothesized that cerebral perfusion and cerebral autoregulation are altered in subjects with cognitive dysfunction.

Cerebral autoregulation assessment requires accurate and continuous measurements of cerebral blood flow (Lam et al., 2019). Near-infrared spectroscopy (NIRS) is a non-invasive neuroimaging technique that allows the continuous measurement of tissue oxygenation and hemodynamic parameters in the cerebral (Kozlová, 2018). The attributes of NIRS such as portability, tolerance of motion artifacts, and use in patients with pacemakers and metal implants have made this technique particularly suitable for the analysis of cerebral autoregulation in the elderly population (Addison, 2015). Kainerstorfer et al. (2015) demonstrated the reliability of non-invasive measurement of cerebral autoregulation in microvascular systems using NIRS. Currently, NIRS has been used to observe cerebral autoregulation in patients with subarachnoid hemorrhage (Budohoski et al., 2016), acute neurological injury (Rivera et al., 2017), and sepsis patients (Eleveld et al., 2021). Therefore, in the present study, NIRS was employed to investigate cerebral autoregulation function in subjects with cognitive dysfunction.

In past decades, various methods have been adopted for the non-invasive assessment of cerebral autoregulation in the resting state based on spontaneous fluctuations in blood pressure and cerebral blood flow. Of all the available methods to do this, transfer function analysis is the most frequent method reported in the literature to quantify cerebral autoregulation (Meel-van den Abeelen et al., 2014). Nevertheless, the cerebral autoregulation parameters calculated by transfer function analysis do not seem to differentiate between subjects with cognitive dysfunction (Gommer et al., 2012; Tarumi et al., 2014). Close attention has recently been dedicated to the study of coupling functions based on dynamical Bayesian inference, which has been used in the assessment of cerebral autoregulation function in patients with stroke and hypertension (Su et al., 2018; Li et al., 2021). A great advantage of the Bayesian method is its ability to simultaneously detect time-varying synchronization, the directionality of coupling, and time-evolving coupling functions, even in the presence of noise (Stankovski et al., 2014). This study aimed to investigate the potential of the coupling function method based on dynamic Bayesian inference for the assessment of cerebral autoregulation in subjects with cognitive dysfunction.

In this study, NIRS and non-invasive blood pressure devices were used to simultaneously measure cerebral oxygenation signals in the prefrontal cortex (PFC) and arterial blood pressure (ABP) signals from subjects in the resting state. Coupling function between the ABP and cerebral oxygenation signals was established based on dynamical Bayesian inference. Very-low-frequency (VLF, 0.02–0.07 Hz) and low-frequency (LF, 0.07–0.20 Hz) oscillation of oxyhemoglobin has shown to be robust parameter for evaluating cerebral autoregulation (Kainerstorfer et al., 2015; Eleveld et al., 2021). Spontaneous oscillations in the VLF interval are mainly associated with hemodynamic fluctuations that originate from spontaneous cortical neural activity, and the spontaneous oscillations in the LF interval are believed to reflect vasomotor and sympathetic activity (Vermeij et al., 2014). In the present study, cerebral autoregulation function in elderly subjects with cognitive dysfunction was assessed by coupling functions in the VLF and LF bands

and compared with those in healthy elderly controls. Pearson correlation analysis was used to study the relationships between montreal cognitive assessment (MoCA) scores and indicators of cerebral autoregulation function. In addition, we investigated the relationship between MoCA scores and brain oxygenation status. This study provides insights into the mechanisms underlying the pathophysiology of cognitive impairment and provides objective indicators for screening cognitive impairment in the elderly population.

METHODS

Participants

This study was performed in senior centers and the Rehabilitation Hospital, National Research Center for Rehabilitation Technical Aids. The trial was registered with the Chinese Clinical Trial Registry (registration no. ChiCTR2100053043). Written informed consent was obtained from the participants before the study. When the subject had difficulties understanding the informed consent due to cognitive dysfunction, their family provided content. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

A total of 166 volunteers were initially enrolled in this study (**Figure 1**). The target group was right-handed elderly individuals aged > 50 years. The exclusion criteria during the initial enrollment were neurological illness and traumatic brain injury with any known cognitive consequences. The subjects were grouped according to the MoCA scores, which was administered by trained personnel. The subjects with MoCA scores 26 or above were categorized as normal cognition (Group NC); those with scores between 15 and 25 were categorized as mild cognitive impairment (Group MCI); and those with scores of 14 or less were categorized as cognitive impairment (Group CI).

Data Collection

After recording basic information and MoCA scores, cerebral oxygenation and ABP signals were simultaneously collected. All measurements were non-invasive and safe for the subjects. After 5 min of rest, 15 min recordings were made for (1) ABP that were continuously measured with finger-pulse photoplethysmography at a sampling rate of 1,000 Hz (CNAP™ Monitor 500, CNSystems Medizintechnik AG, Graz, Austria); (2) cerebral oxygenation data that were measured via NIRS (ECO-N17-C25L, Enginmed Bio-Medical Electronics, Suzhou, China) at a sampling rate of 20 Hz. Each sensor of the ECO-N17-C25L consisted of a light-emitting diode and two PIN diodes. The light-emitting diode component worked with three-wavelengths (760, 810, and 840 nm) and served as the source of emitted light, whereas the PIN diodes served as the detectors. The distances between the light source and the two detectors were 30 and 40 mm. The differential spacing of the receiving detectors provided spatial resolution to distinguish signals from cerebral and extracerebral tissue. The probes were positioned over the PFC area (LPFC/RPFC) and then wrapped around the forehead with an elastic bandage to block ambient light.

Signals and Preprocessing

The ECO-N17-C25L used a spatially resolved spectroscopy algorithm to calculate the concentration changes in oxygenated and deoxygenated hemoglobin concentrations (Δ [O_2Hb] and Δ [HHb], respectively) compared with their original values in human tissue. It has been shown that this algorithm is little influenced by either background absorption or overlying tissues (Teng et al., 2006; Han and Zhang, 2016). The tissue oxygenation index (TOI) is an indicator that characterizes the brain oxygenation status, which directly reflect the dynamic balance between oxygen supply and consumption in regional tissue (Jin et al., 2021). The TOI value can be derived from the ratio of tissue oxygenated hemoglobin concentrations to total hemoglobin concentration in blood flow within venous, arterial, and cerebral cortical tissue, where the total hemoglobin concentration is the sum of the [O_2Hb] and [HHb] concentrations (Naulaers et al., 2007). Mathematically, TOI (%) can be expressed as follows:

$$\mathrm{TOI} = \frac{\mathrm{O_2Hb}}{\mathrm{O_2Hb} + \mathrm{HHb}} \times 100\% \quad (1)$$

Moving average and cubic spline interpolation methods were used to eliminate noise-like abrupt spikes and motion artifacts in the NIRS signal, respectively (Scholkmann et al., 2010). The window width of the moving average method was 5 s. To achieve a uniform time basis, the raw ABP signal was downsampled to 20 Hz.

Data Analysis

In the present study, the cerebral autoregulation function was assessed by investigating interactions between cardiac oscillations and slow oscillations in the cerebral. An overview of the modeling of the interaction between brain activity and ABP is shown in **Figure 2**. In the first step, the phase time series of NIRS and ABP signals were extracted by continuous wavelet transform. In the second step, the interactions between extracted components were studied by a coupled-phase-oscillator model based on dynamical Bayesian inference. Finally, the coupling direction and coupling strength were calculated to quantify the coupled systems. All these methods were explained below.

Dynamic Phase Extraction for Coupling Function Analysis

In the present study, the continuous wavelet transform was used to extract spontaneous oscillations of NIRS and ABP signals in various characteristic frequency bands. The continuous wavelet transform is a time–frequency analysis method, which uses the logarithmic scale for the frequency, thus low frequencies have higher resolutions. The continuous wavelet transform is given by the equation:

$$W(s,t) = \frac{1}{\sqrt{s}} \int_0^\infty \psi\left(\frac{u-t}{s}\right) g(u)\, du \quad (2)$$

where $W(s, t)$ is the wavelet coefficient, $g(u)$ is the time series, and ψ is the mother wavelet, scaled by the factor s and translated in time by t. The complex Morlet wavelet $\psi(u) = \frac{1}{\sqrt[4]{\pi}} e^{-i2\pi\mu} e^{-\mu^2/2}$ (with i the imaginary unit) was chosen to be the

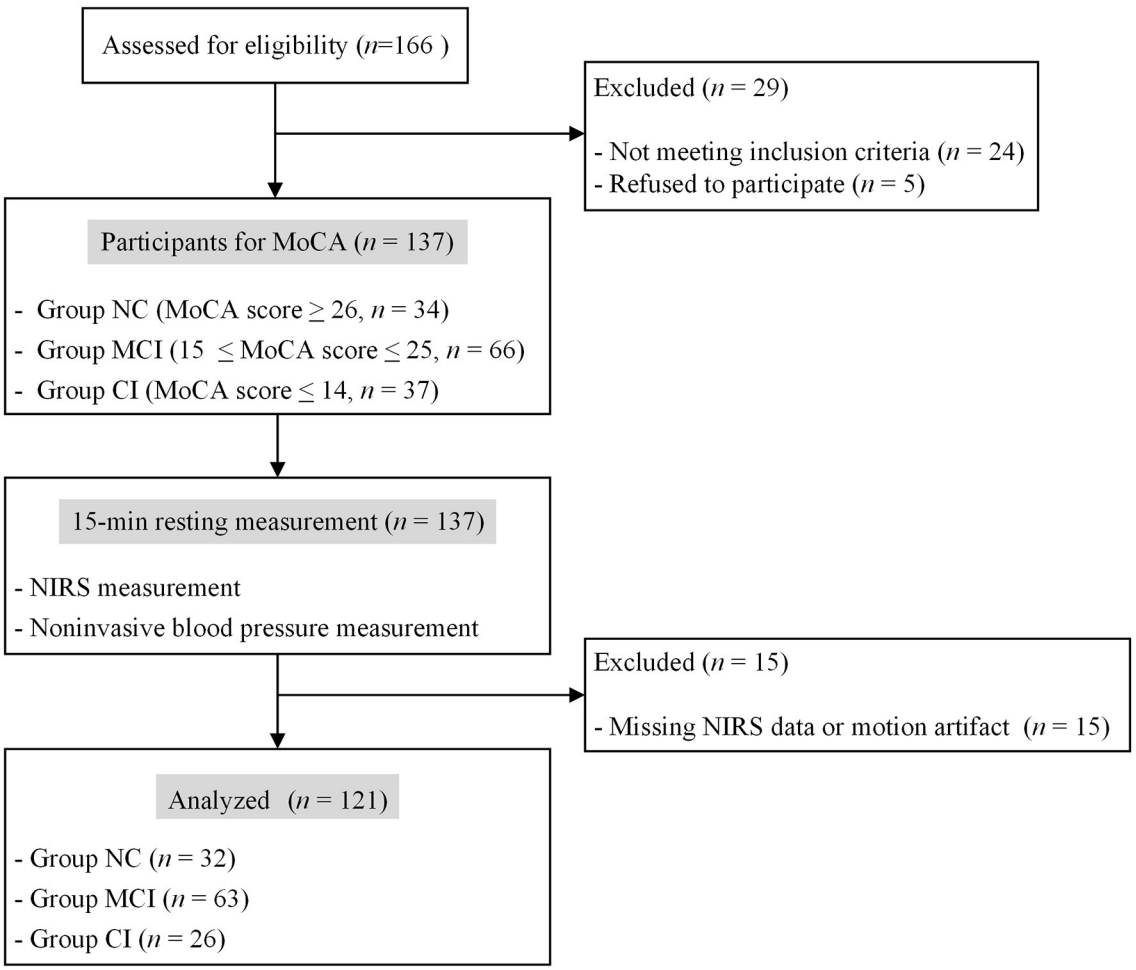

FIGURE 1 | Flow chart of participant inclusion.

mother wavelet because it maximizes joint time-localization and frequency resolution (Stefanovska et al., 1999).

The phases of Δ [O_2Hb] signal ($\phi_{\Delta[O2Hb]}$) was extracted in the VLF (0.02–0.07 Hz) and LF (0.07–0.2 Hz) range. The phase extraction of the heart activity from the ABP signal (ϕ_{ABP}) was 0.6–2 Hz (Stefanovska, 2007). The signals extracted from these intervals are periodic, enabling the underlying oscillatory processes and their interactions to be studied effectively through phase dynamics, and leading to extraction of phase-to-phase cross-frequency couplings (Stankovski et al., 2017a).

Coupling Functions Using Dynamical Bayesian Inference

The interactions were modeled with cross-frequency coupling based on dynamical Bayesian inference. Coupling functions prescribe the physical rule specifying how the inter-oscillator interactions occur. To learn about influence of each oscillator on the others, the system was decomposed into a group of phase oscillators which interact. Their decomposition can describe the functional contribution from each separate subsystem within a single coupling relationship (Stankovski et al., 2016). This system can be defined by two differential stochastic equations (Stankovski et al., 2017b):

$$\dot{\phi}_m(t) = w_m(t) + q_m(\phi_n, \phi_m, t) + \delta_m(t) \tag{3}$$

with $m = 1$, $n = 2$. where w_m and ϕ_m are the natural frequency and phase of oscillator m, $\delta_m(t)$ is Gaussian white noise, and $q_m(\phi_n, \phi_n, t)$ is the coupling function describing the influence of oscillator n on the phase of oscillator m.

The theorem of dynamical Bayesian inference is summarized in Stankovski et al. (2012):

$$p_\chi(\mathcal{M}|\mathcal{X}) = \frac{\ell(\mathcal{X}|\mathcal{M})\, p_{prior}(\mathcal{M})}{\int \ell(\mathcal{X}|\mathcal{M}) p_{prior}(\mathcal{M})\, d\mathcal{M}} \tag{4}$$

where $p_\chi(\mathcal{M}|\mathcal{X})$ is the conditional probability of observing the data $\mathcal{X}$ given the hypothesized parameters $\mathcal{M}$. $p_{prior}(\mathcal{M})$ is the probability of $\mathcal{M}$ before observing the data $\mathcal{X}$. $p_\chi(\mathcal{M}|\mathcal{X})$ is known as the posterior probability–the probability that the hypothesized parameters are correct given $\mathcal{X}$ and the prior probability $p_{prior}(\mathcal{M})$.

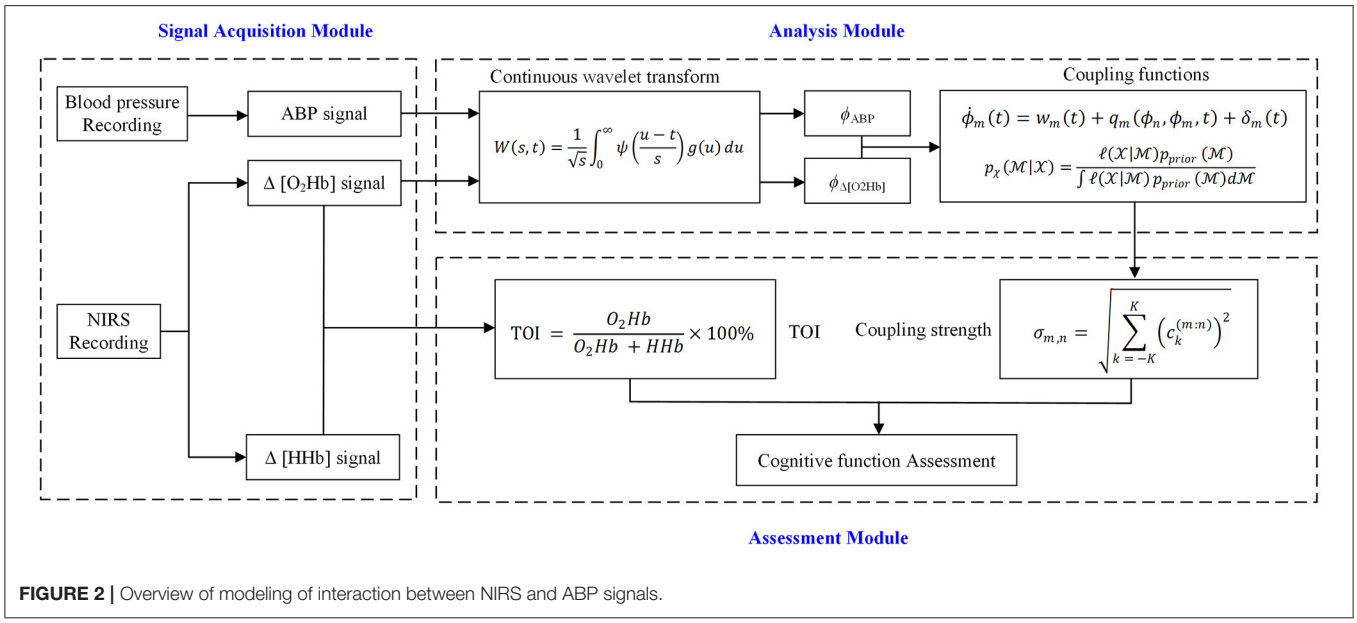

FIGURE 2 | Overview of modeling of interaction between NIRS and ABP signals.

Quantitative Measures

To simplify quantitative comparisons obtained results, the coupling strength and coupling direction are calculated. The coupling strength gives a quantitative measure of the information flow between the coupled systems and is an important indicator to characterize the magnitude and the extent of the coupling relationship (Stankovski et al., 2017b). A higher coupling strength value indicates that the fluctuations in one oscillation are more direct in transferring amplitude changes to the other. The coupling direction represents the predominant direction of the coupling function. The strength $CS_{m,n}$ of the coupling from the oscillator m to n is defined as:

$$CS_{m,n} = \sqrt{\sum_{k=-K}^{K}\left(c_k^{(m:n)}\right)^2} \quad (5)$$

The directionality index CD represents the predominant direction of the coupling function, which is defined as (Stankovski et al., 2012):

$$CD(t) = \frac{CS_{n,m} - CS_{m,n}}{CS_{n,m} + CS_{m,n}} \quad (6)$$

If $CD \in [-1, 0]$ ($CD \in [0, 1]$), the n (m) drives the m (n). The result of the CD value calculated from Equation (6) is >0. Therefore, only the coupling functions in the direction from ABP to Δ [O_2Hb] in the VLF and LF interval were discussed in the present study.

Statistical Analysis

Age, body mass index, sex, blood pressure, and MoCA scores are expressed as the means and standard deviation. The Kolmogorov–Smirnov and Levene tests were applied to test variance normality and homogeneity of the data at the group level. Significant intergroup differences in TOI and coupling strength were assessed by one-way ANOVA. Bonferroni's t-test was used for the intergroup pairwise comparisons. Three comparisons between the groups were designed (Group NC vs. Group MCI, Group NC vs. Group CI, and Group MCI vs. Group CI). Therefore, the corrected statistical significance was defined as $p < 0.0167$ ($p < p_{original}/3$). The associations between MoCA scores, TOI, and coupling strength were assessed by Pearson's correlation analysis. A difference of $p < 0.05$ was considered statistically significant. Receiver–operator characteristic analysis with Youden's J statistic was used to test the sensitivity and specificity and determine the optimal threshold value for the TOI and cerebral autoregulation indices to differentiate subjects with mild cognitive impairment from those with normal cognition.

RESULTS

Demographic and Cognitive Test Results

The demographic characteristics and cognitive test results for each group are shown in **Table 1**. The demographics of Groups NC, MCI, and CI, including age, sex, body mass index, and blood pressure were not significantly different among the three groups. The groups did have significantly different MoCA scores.

Group-Dependent Variation in TOI

The TOI in the left and right PFC (LTOI/RTOI) of each subject was calculated by averaging the TOI values in the time domain over the acquisition period (15 min). The averaged TOI of the bilateral PFC was expressed as Mean TOI. An example of the typical curves of original NIRS data and TOI was shown in **Supplementary Material**. **Figure 3** shows the comparison of the TOI values among the three groups. The result shows that the

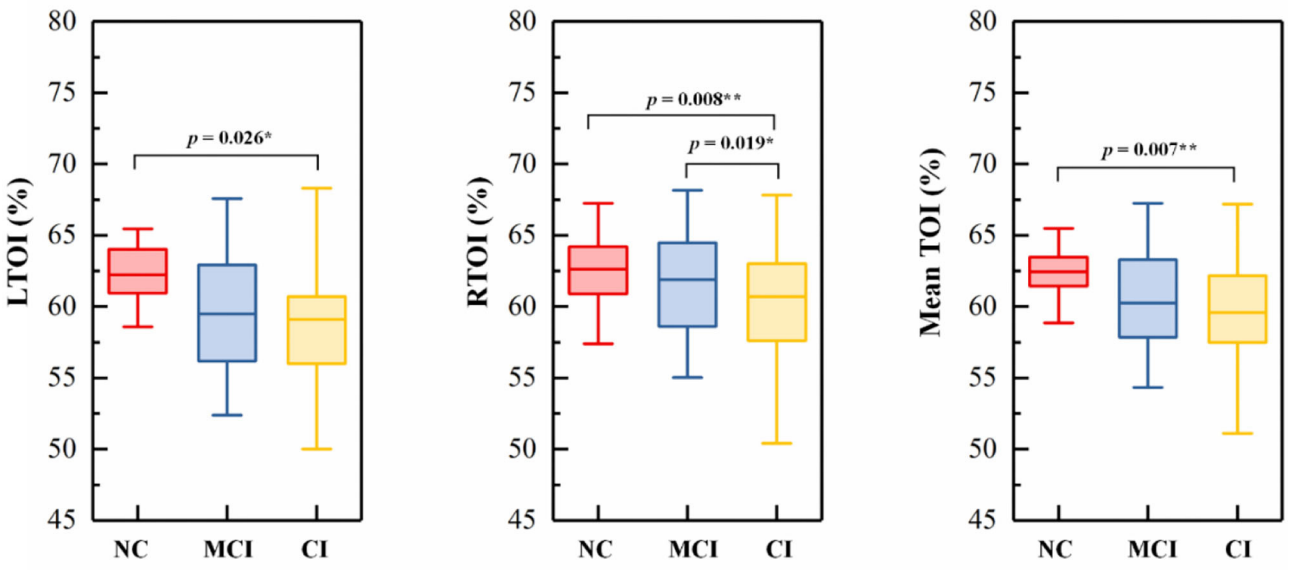

FIGURE 3 | Box-plots picturing the distributions of **(A)** LTOI, **(B)** RTOI, and **(C)** mean TOI. In each boxplot, the NC group (NC) is indicated by red rectangles, the MCI group (MCI) by blue rectangles, and the CI group (CI) by yellow rectangles. The line connectors on the tops of individual panels indicate cases where the difference between two boxplot distributions was statistically significant. $p < 0.05$ are marked with *, $p < 0.0167$ are marked with **.

TABLE 1 | Basic information of the participants.

Characteristic	Group NC	Group MCI	Group CI	*p*-value (NC vs. MCI)	*p*-value (NC vs. CI)	*p*-value (MCI vs. CI)
Age (year)	70.72 ± 7.66	72.02 ± 9.90	72.50 ± 10.93	0.535	0.484	0.829
Gender (male/female)	9/23	31/32	13/13	0.051	0.096	0.945
Body mass index	22.00 ± 1.92	22.89 ± 3.00	22.52 ± 2.33	0.123	0.456	0.550
MoCA score	26.94 ± 1.54	20.60 ± 3.42	10.62 ± 3.16	<0.001*	<0.001*	<0.001*
Systolic blood pressure (mm Hg)	120.55 ±17.18	124.60 ± 19.92	129.14 ± 24.60	0.361	0.113	0.341
Diastolic blood pressure (mm Hg)	70.41 ± 8.60	69.93 ± 12.70	68.20 ± 15.79	0.860	0.506	0.555

*Values are presented as means and standard deviations. *Indicates p < 0.05.*

RTOI and mean TOI values were significantly lower in Group CI than in Group NC.

Coupling Strength

The phase-to-phase coupling functions between ABP and Δ [O_2Hb] were reconstructed, quantified, and compared. **Figure 4** presents the specific-frequency coupling function between ABP and Δ [O_2Hb] in each group and the corresponding coupling strength. In the VLF interval, Group CI exhibited significantly higher coupling from ABP to Δ [O_2Hb] ($CS_{A,O}$) in the LPFC and RPFC than Group NC. In the VLF interval, the $CS_{A,O}$ was significantly higher in Group MCI than in Group NC in LPFC. In the LF interval, the $CS_{A,O}$ in the LPFC was significantly higher in Group CI than in Group NC.

Correlation Analysis

Scatterplots of TOI vs. MoCA scores and coupling strength vs. MoCA scores are presented in **Figure 5**. The correction between the MoCA scores, TOI, and $CS_{A,O}$ in the bilateral PFC is presented in **Table 2**. MoCA scores show a statistically significant positive correlation with LTOI and RTOI. MoCA scores are significantly negatively correlated with $CS_{A,O}$ in the LPFC and RPFC interval VLF and LF.

Receiver–Operator Characteristic Analysis

A receiver–operator characteristic analysis with the corresponding area under the curve was performed on TOI and coupling strength values to determine the optimal threshold value for distinguishing subjects with mild cognitive impairment from those with normal cognition. The discriminant validity for the detection of mild cognitive disorder of the mean TOI

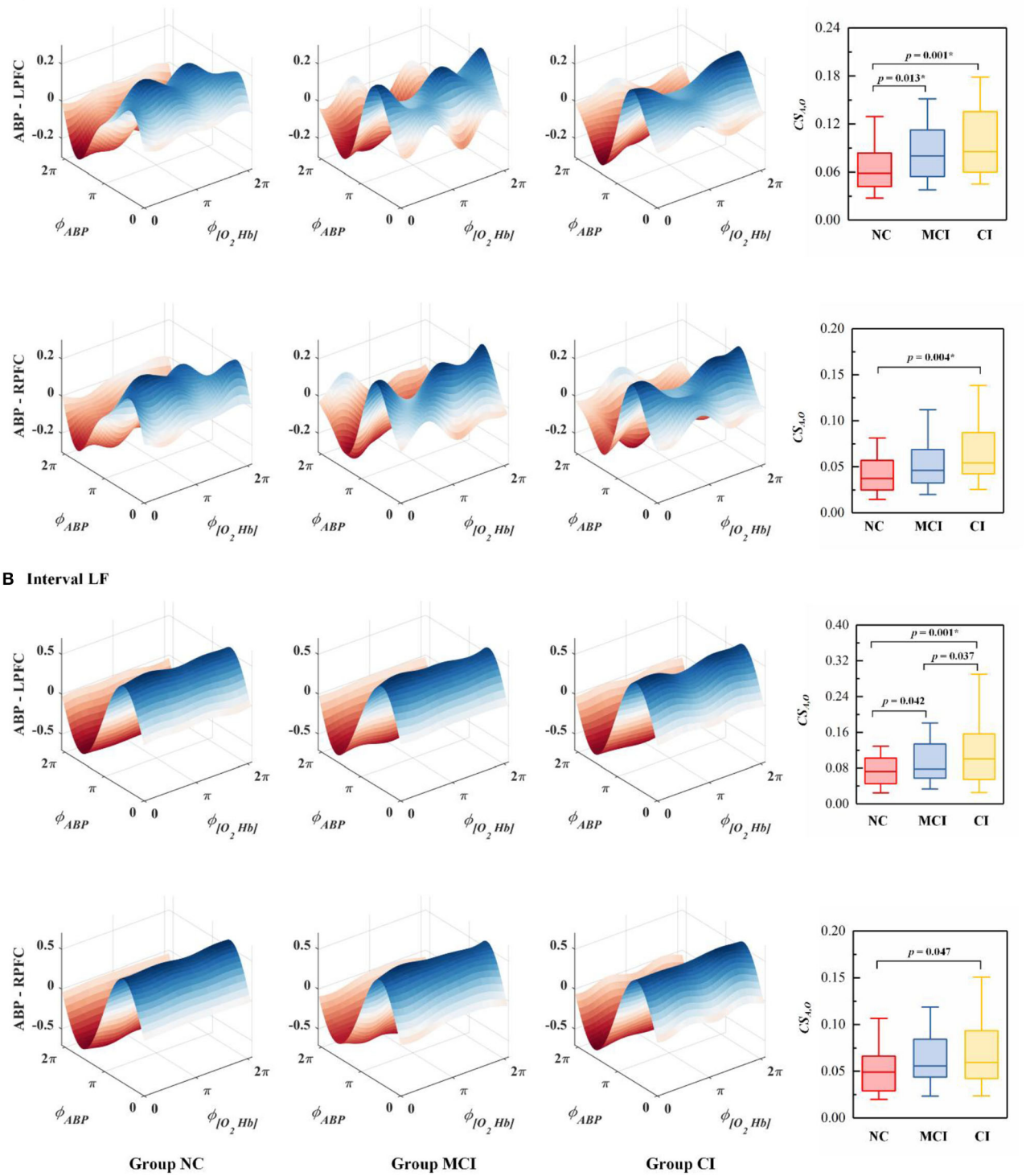

FIGURE 4 | The average coupling functions from all subjects within the group between ABP and Δ [O_2Hb] in the **(A)** VLF interval and **(B)** LF interval. The coupling between ABP and Δ [O_2Hb] in the bilateral PFC is denoted as ABP - LPFC and ABP - RPFC. ϕ_{ABP} and $\phi_{[O2Hb]}$ represent the dynamical phase information of ABP and Δ [O_2Hb] signal, respectively. Each boxplot shows the coupling strength distribution of a specific coupling relationship in the resting state indicated by the NC (Group NC), MCI (Group MCI), or CI (Group CI). The NC group is indicated by red rectangles, the MCI group by blue rectangles, and the CI group by yellow rectangles. The line connectors on the tops of individual panels indicate cases where the difference between two boxplot distributions was statistically significant. $p < 0.0167$ are marked with *.

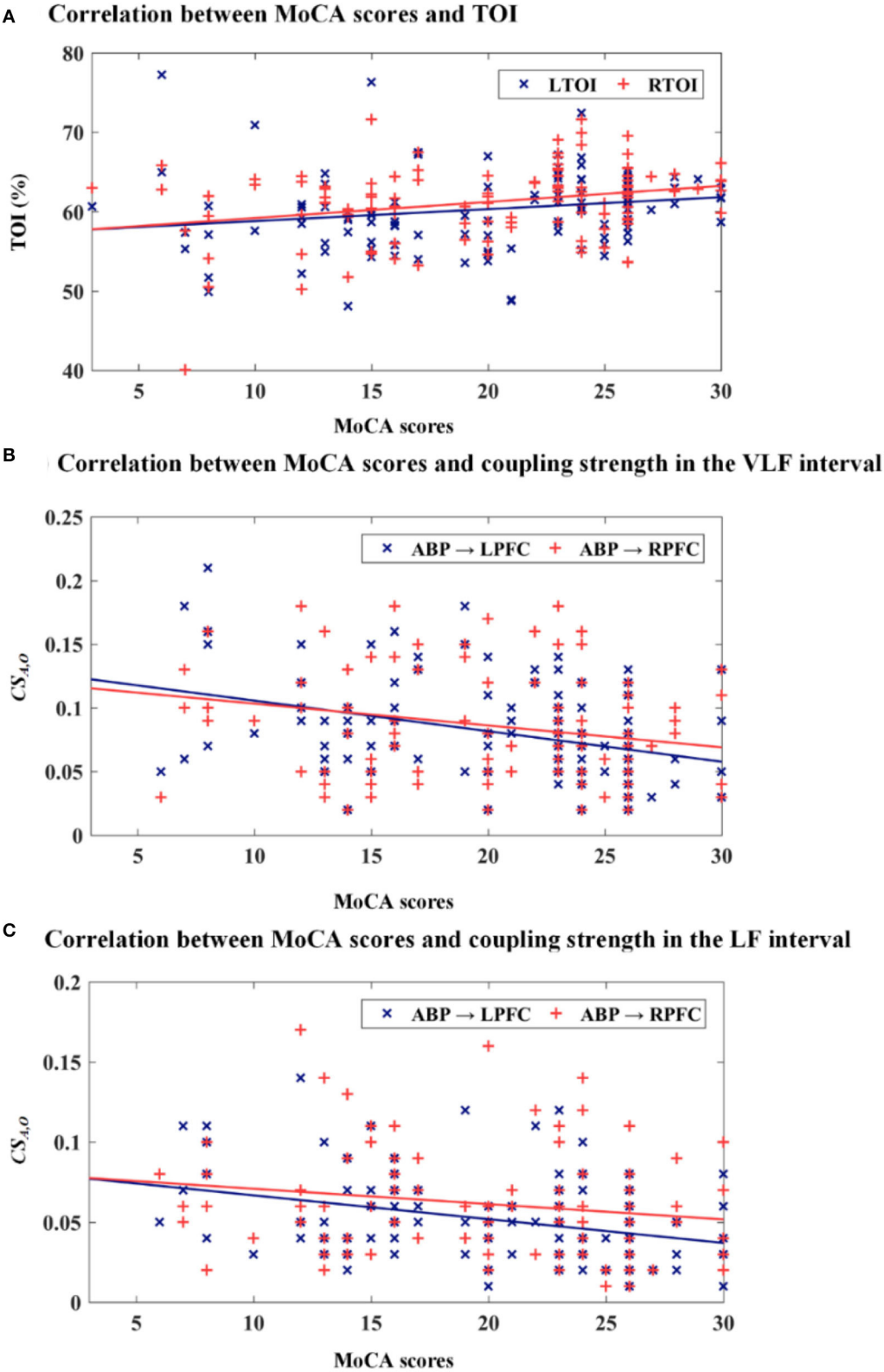

FIGURE 5 | Scatterplots of TOI vs. MoCA scores and coupling strength vs. MoCA scores. **(A)** MoCA scores vs. TOI, **(B)** MoCA scores vs. $CS_{A,O}$ in the VLF interval, and **(C)** MoCA scores vs. $CS_{A,O}$ in the LF interval.

TABLE 2 | Correlations between the MoCA scores, TOI, and $CS_{A,O}$ at rest in the bilateral PFC.

Variables		MoCA	
		r	*p*
TOI	LTOI	0.199	0.029*
	RTOI	0.282	0.002*
$CS_{A,O}$	ABP → LPFC (VLF)	−0.390	<0.001*
	ABP → LPFC (LF)	−0.378	<0.001*
	ABP → RPFC (VLF)	−0.328	<0.001*
	ABP → RPFC (LF)	−0.226	0.014*

*$p < 0.05$ are marked with **.

and coupling strength between the bilateral PFC [receiver–operator characteristic area under the curve (95% confidence interval)]: TOI [0.65 (0.54, 0.74)] and coupling strength [0.64 (0.53, 0.74)]. The optimal threshold value of the mean TOI in the bilateral PFC was 60% (sensitivity = 54.0%, specificity = 84.4%), and the optimal threshold value of the averaged $CS_{A,O}$ in the bilateral PFC was 0.05 (sensitivity = 77.4%, specificity = 41.9%). TOI values below the optimal threshold value and coupling strength values above the optimal threshold value appeared to be closely associated with the diagnosis of mild cognitive impairment.

DISCUSSION

In this research, cerebral autoregulation function was assessed by a coupling function based on dynamical Bayesian inference. The main findings of this study were as follows: (1) TOI was significantly reduced on both sides of the PFC in subjects with cognitive dysfunction; (2) cerebral autoregulation function was impaired in subjects with cognitive dysfunction. The main strengths of this study include the application of a coupling function, which provides insights into the mechanisms underlying the pathophysiology of cognitive impairment. The present study describes the potential mechanism and clinical implications of our findings.

Good oxygenation status is a guarantee for nerve cells to maintain structural integrity and normal function of the brain. A large body of evidence indicates that cerebral hypoperfusion is one of the earliest pathological signs in the development of cognitive failure (de la Torre, 2004). Meta-analyses demonstrated clear abnormalities in cerebral hemodynamic and oxygenation parameters in patients with mild cognitive dysfunction, even at an early stage of cognitive decline (Beishon et al., 2017). It is clinically important to monitor the oxygenation status of cerebral tissue in real-time, detect abnormalities, and initiate timely intervention measures. TOI is an indicator of the oxygen saturation in regional tissues, and variations in TOI can reflect changes in cerebral blood flow to some extent (Jin et al., 2021). Tarumi et al. (2014) found that TOI was reduced at rest in subjects with mild cognitive dysfunction compared with healthy controls. Consistent with the literature, reduced TOI on both sides of the PFC was observed in participants in Group MCI and Group CI compared to controls. This result suggested that cerebral perfusion was reduced in subjects with cognitive dysfunction compared to healthy elderly adults. A possible explanation for this result may be the reduction in brain metabolic demand that parallels cognitive decline. Another possible explanation for this is that chronic brain hypoperfusion in elderly individuals leads to neuronal damage and eventually to neurodegenerative tissue atrophy (de la Torre, 2004, 2008).

A previous study has suggested that sustained mild hypoxia reduces steady-state cerebral blood flow, and continuously impairs cerebral autoregulation (Nishimura et al., 2010). Cerebral autoregulation allows the maintenance of relatively stable cerebral perfusion and brain tissue oxygenation against changes in blood pressure through complex myogenic, neurogenic, and metabolic mechanisms (Addison, 2015). The VLF and LF bands are in the frequency range where cerebral autoregulation is considered operative. The current interpretation of the coupling function metric assumes that pressure fluctuations are more liable to induce linear and pressure-synchronized cerebral blood flow fluctuations with greater magnitude in the condition of disturbed cerebral autoregulation. Therefore, higher values of $CS_{A,O}$ are considered to reflect greater oscillations of Δ [O_2Hb] in response to changes in ABP, that is, poorer damping of the effectiveness of cerebral autoregulation, which represents poorer cerebral autoregulation function, and vice versa. A significantly higher $CS_{A,O}$ was observed in Groups CI and MCI than in healthy controls in the VLF and LF intervals. These results indicated that cerebral autoregulation function was impaired in subjects with mild-to-severe cognitive dysfunction.

In the present study, the significantly increased $CS_{A,O}$ in the VLF interval suggested that one of the mechanisms for impaired cerebral autoregulation in cognitive dysfunction patients might involve alterations in autonomic nervous activities. The continuous activity of the autonomous nervous system serves to maintain the basal level of vessel contraction. The nerves release substances that affect the activities of smooth muscles, leading to changes in the vessel radii and resistance (Shiogai et al., 2010). To maintain flow in the autoregulated range of blood pressure, cerebral resistance vessels undergo vasoconstriction during hypertension and vasodilatation during hypotension. Therefore, failure of vasoconstriction and/or vasodilatation may result in cerebral autoregulation disruption rendering the brain more susceptible to fluctuations in blood pressure (Gommer et al., 2012). In the LF interval, $CS_{A,O}$ was significantly increased in Group CI. This appears to suggest that there is impaired myogenic activity regulation in the PFC in subjects with cognitive dysfunction. This may be related to parasympathetic depression and sympathetic exacerbation in participants with cognitive dysfunction (Toledo and Junqueira, 2008). The significant difference in coupling function was mainly distributed in the LPFC, which may be related to age-related neurodegeneration preferentially affecting the left hemisphere (Thompson et al., 2003).

In the present study, the significant correlation between TOI and MoCA scores indicates the sensitivity of cognitive function to brain oxygenation in elderly individuals. Pearson correlation

analysis showed significant negative correlations between MoCA scores and $CS_{A,O}$ in the VLF and LF intervals. This result suggests that $CS_{A,O}$ could characterize cerebral autoregulation function changes. The results of receiver–operator characteristic analysis support the finding that value of TOI and $CS_{A,O}$ can be used as objective indicators for screening cognitive impairment in the elderly population.

LIMITATIONS

This study assesses brain oxygenation status and cerebral autoregulation function in subjects with cognitive dysfunction. However, the different types of cognitive dysfunction were not further classified in our study due to the relatively small sample size. In future research, more indicators and a larger sample size could be adopted to investigate the relationships between NIRS-related parameters and different types of cognitive impairment.

CONCLUSION

In this pilot study, the effects of cognitive dysfunction on cerebral autoregulation function were investigated by a coupling function based on dynamic Bayesian inference. In the VLF and LF intervals, increased $CS_{A,O}$ in Group CI and MCI indicated that cerebral autoregulation function was impaired in subjects with cognitive dysfunction. The Pearson correlation results suggested that indicators of cerebral oxygenation status and cerebral autoregulation function can reflect cognitive function. This study provides insights into the mechanisms underlying the pathophysiology of cognitive impairment. Although the method is not yet ready for large-scale application, this study provides an objective indicator for the screening of cognitive impairment in the elderly population, and with the development of NIRS and ABP techniques, the method is expected to enable large-scale community screening and routine clinical monitoring in the future.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Rehabilitation Hospital, National Research Center for Rehabilitation Technical Aids. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WL: conceptualization, methodology, and writing—original draft. GQ and HL: data curation. CH: data curation and writing—reviewing and editing. GX: writing—reviewing and editing. XH: investigation and project administration. JZ: project administration. ZL: supervision, funding acquisition, and writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.904108/full#supplementary-material

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Multiple step saccades in simply reactive saccades could serve as a complementary biomarker for the early diagnosis of Parkinson's disease

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Objective: It has been argued that the incidence of multiple step saccades (MSS) in voluntary saccades could serve as a complementary biomarker for diagnosing Parkinson's disease (PD). However, voluntary saccadic tasks are usually difficult for elderly subjects to complete. Therefore, task difficulties restrict the application of MSS measurements for the diagnosis of PD. The primary objective of the present study is to assess whether the incidence of MSS in simply reactive saccades could serve as a complementary biomarker for the early diagnosis of PD.

Materials and methods: There were four groups of human subjects: PD patients, mild cognitive impairment (MCI) patients, elderly healthy controls (EHCs), and young healthy controls (YHCs). There were four monkeys with subclinical hemi-PD induced by injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) through the unilateral internal carotid artery and three healthy control monkeys. The behavioral task was a visually guided reactive saccade.

Results: In a human study, the incidence of MSS was significantly higher in PD than in YHC, EHC, and MCI groups. In addition, receiver operating characteristic (ROC) analysis could discriminate PD from the EHC and MCI groups, with areas under the ROC curve (AUCs) of 0.76 and 0.69, respectively. In a monkey study, while typical PD symptoms were absent, subclinical hemi-PD monkeys showed a significantly higher incidence of MSS than control monkeys when the dose of MPTP was greater than 0.4 mg/kg.

Conclusion: The incidence of MSS in simply reactive saccades could be a complementary biomarker for the early diagnosis of PD.

KEYWORDS

saccade, corrective saccades, MPTP, Parkinson's disease, diagnosis

Introduction

Saccades are rapid eye movements that redirect the fovea from one object of interest to another. A typical saccade consists of a primary saccade that covers all or most of the distance between the fixation point and the target location, which might be followed shortly by a small amplitude saccade (corrective/secondary saccade) if required. Corrective saccades (CS) have been frequently observed in children and young and elderly healthy subjects (Cohen and Ross, 1978; White et al., 1983). Thus, it has been well accepted that CS is a physiological behavior (Troost et al., 1974).

However, eyes do not always jump with the typical form and sometimes they engage in a series of at least two smaller amplitude (hypometric) saccades–namely, multiple step saccades (MSS) (Troost et al., 1974). Although MSS is occasionally observed in healthy subjects (Kimmig et al., 2002; Van Donkelaar et al., 2007), it is clearly more pronounced in Parkinson's disease (PD) patients (Jones and DeJong, 1971; Corin et al., 1972; Troost et al., 1974; Teräväinen and Calne, 1980; White et al., 1983; Hotson et al., 1986; Lueck et al., 1990, 1992; Van Gisbergen et al., 1992; Kimmig et al., 2002) and non-human primates with dopamine depletion (PD monkeys) in the basal ganglia (Brooks et al., 1986; Kato et al., 1995). Thus, MSS is assumed to be a non-physiological behavior (Troost et al., 1974).

A consistent finding among previous studies is that the incidence of MSS in PD patients is significantly higher than that in elderly healthy controls (EHCs) during voluntary saccades such as memory guided saccades (Teräväinen and Calne, 1980; Crawford et al., 1989; Lueck et al., 1992; Van Gisbergen et al., 1992; Kimmig et al., 2002; Ying et al., 2008; Blekher et al., 2009). Furthermore, it has been argued that the incidence of MSS in memory guided sequential saccades could serve as a biomarker for the diagnosis of PD (Blekher et al., 2009). However, practically, memory guided sequential saccade tasks are usually difficult for elderly subjects to perform, particularly for neurodegenerative patients, because participants need to inhibit the reactive saccades to the onset of visual stimulus and then generate saccades based on their memory (Gaymard et al., 1998). Previous studies have found that PD patients made significantly more errors in memory guided saccade tasks (Crawford et al., 1989; Van Gisbergen et al., 1992). Such task difficulties restrict the clinical application of measuring MSS in the diagnosis of PD. Thus, a critical question is whether the incidence of MSS in simple saccade tasks, such as reactive saccades, could provide useful information for the diagnosis of PD.

While the reported incidences of MSS are consistent for memory guided saccades among previous studies, the results are inconsistent for reactive saccades. Some studies reported that, compared with elderly healthy subjects, the incidences of MSS are significantly higher in both PD patients (Jones and DeJong, 1971; Corin et al., 1972; White et al., 1983) and PD monkeys (Brooks et al., 1986; Tereshchenko et al., 2015), but others reported no significant difference (Crawford et al., 1989; Lueck et al., 1990, 1992; Van Gisbergen et al., 1992; Kimmig et al., 2002; Blekher et al., 2009). We think that a possible reason for such inconsistency might be the different definitions of MSS. Some previous studies excluded CS from the analysis of MSS (Troost et al., 1974; Bötzel et al., 1993; Van Donkelaar et al., 2007), while others considered CS as a part of MSS (Becker and Fuchs, 1969; Oliva, 2001). To make a comparison with previous studies, we firstly analyzed the incidence of mixed MSS and CS, and then dissociate CS from MSS for data analysis.

It has been noticed that the impairments of vertical saccades are more severe than horizontal saccades in PD patients (Lemos et al., 2016; Jung and Kim, 2019). One study argued that the characteristics of vertical saccades could serve as a complementary biomarker for the diagnosis of PD (Waldthaler et al., 2019). To the best of our knowledge, no study has compared the incidence of MSS between vertical and horizontal saccades. Therefore, one of the objectives of the present study was to address whether the incidence difference of MSS between vertical and horizontal saccades could also serve as a complementary biomarker for PD diagnosis.

To assess the usefulness of MSS in reactive saccades for the diagnosis of early PD, we studied the incidence of MSS in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced subclinical hemi-PD monkeys, because it is rare to see early PD patients in the hospital where we collected data of PD patients. In addition, since MPTP selectively damages the dopaminergic neurons in the substantia nigra pars compacta (Langston, 2017), the function of dopaminergic circuit in basal ganglia is impaired after MPTP injection (Israel and Bergman, 2008). Moreover, the substantia nigra pars reticulata directly sent output to the intermedia and deep layers of superior colliculus–a saccadic center in brain (Hikosaka et al., 2014), the superior colliculus is dysfunctional in PD monkeys (Rolland et al., 2013). Therefore, this study could help to understand the role of basal ganglia-superior colliculus circuit in the development of MSS.

Furthermore, to the best of our knowledge, previous studies have only compared the incidence of MSS between PD patients and EHC, but no one has investigated whether there is a difference in MSS between PD and mild cognitive impairment (MCI) patients. Thus, the specificity of MSS for the diagnosis of PD is unclear. To address this knowledge gap, we compared the incidence of MSS between PD and MCI patients. We set MCI patients as a control in the present study for the following consideration. PD and MCI are two common neurodegenerative diseases and share certain pathological changes (Xu et al., 2012; Tosto et al., 2015).

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Materials and methods

Participants in the human study

We recruited four groups of participants in the present study. These four groups included PD patients (n = 37), MCI patients (n = 37), age-matched EHCs (n = 37), and young healthy controls (YHCs) (n = 37). The demographic data and clinical scores were shown in Table 1. All participants had normal or corrected-to-normal vision. All participants have written informed consents to take part in the study. The experimental protocols were approved by the Ethics Committee of Beijing Normal University and the Chinese PLA General Hospital (Medical School of Chinese PLA).

All participants except the EHC completed the Folstein mini-mental state examination (MMSE) and Montreal Cognitive Assessment (MoCA) for cognitive function evaluation. Considering the fact that it is rare to see the real healthy participants in hospital, we recruited young and elderly healthy participants in the college community and the residential community, respectively. To make the present study more practical, data collection was carried out by different experimenters in different places. For PD and MCI patients, their cognitive function was evaluated by neurologists in hospital. For YHCs, their cognitive function was evaluated by using MMSE and MoCA tests in university. For EHCs, their cognitive function was evaluated by using MMSE in residential community. For PD patients, MMSE, and MoCA were performed after medication on-state (approximately 1 h after taking levodopa and/or amantadine); the Part 3 of the Movement Disorders Society-revised Unified Parkinson's Disease Rating Scale (MDS-UPDRS-motor scores), Hoehn and Yahr scale (H&Y stage) were administered during the medication off-state (approximately 4 h after taking the medicine). The UPDRS was administered only to PD patients. The data of saccadic eye movements were collected from PD patients during the medication off-state.

TABLE 1 Demographic and clinical characteristics of the human subjects.

	YHC	EHC	MCI	PD
N (male/female)	37 (20/17)	37 (27/10)	37 (28/9)	37 (24/13)
Age in years[a]	22.68 ± 2.92	62.76 ± 7.13	75.24 ± 8.88	62.49 ± 6.97
MMSE[a]	29.49 ± 0.64	25.77 ± 4.31	23.38 ± 3.94	25.95 ± 3.73
MoCA[a]	29.52 ± 0.61	–	23.38 ± 3.94	21.95 ± 4.83
MDS-UPDRS[a]	–	–	–	56.81 ± 13.41[b]
H-Y scale[a]	–	–	–	2.62 ± 0.39[b]

[a]Mean ± SD.
[b]MDS-UPDRS III and H-Y scale were evaluated only for PD patients. The table shows the scores in medicine off state.

Mild cognitive impairment patients meet the diagnosis criteria of MCI according to the National Institute on Aging Alzheimer's Association workgroups in 2011 (Albert et al., 2011). The main diagnostic criteria were as follows: (1) Concern regarding a change in cognition; (2) Impairment in one or more cognitive domains by cognitive assessment (MMSE and MoCA tests); (3) Preservation of independence in functional abilities; (4) Not demented. MCI patients continued their regular medication routine.

Participants in the non-human primate study

Seven male rhesus monkeys were recruited in the present study, including four subclinical hemi-PD (9–12 kg, 12–14 years old) and three healthy control monkeys (10–12 kg, 12–14 years old). Four subclinical (prodromal) hemi-PD monkeys were modeled by injection of MPTP (dissolved in saline with concentration of 0.12 mg/ml) through the unilateral internal carotid artery (Bankiewicz et al., 1986) by a peristaltic pump (RWD Life Science Co., Ltd., Shenzhen, China) with flow rate 1.54 ml/min. The doses of MPTP injection were referred to the previous study (Ovadia et al., 1995) with 0.38, 0.40, 0.41, and 0.43 mg/kg for the four hemi-PD monkeys, respectively. All monkeys were housed in separate cages with a 12 h light/dark cycle. Before training, each monkey was surgically implanted with a head post and two eye coils. The experimental protocols and surgical procedures were approved by the Ethics Committee of Beijing Normal University.

Experimental task

We used a visually guided reactive saccade task to study MSS. We collected eye movement data from one block of trials for each participant.

The task in the human experiment consisted of 40 or 60 trials in a block according to individual participant's affordability. Since some PD and MCI patients had difficulty performing 60 trials in a block we reduced the number of trials to 40 for PD and MCI patients, while for YHCs and EHCs the number of trials in a block was 60. To balance the trial numbers among different groups, we randomly picked up 40 trials from each block of healthy controls for further data analysis.

As for monkey studies, the trial number was varied from 100 trials to 800 trials among blocks. To balance the trial numbers among blocks, we randomly picked up 100 trials form blocks with trial numbers larger than 100. It cost about 5 min for monkeys to perform 100 trials in a block.

Visually guided reactive saccade task (Figure 1A). Each trial began with a white cross (fixation point) appearing at the center of the screen for 800 ms. Simultaneously, with the disappearance

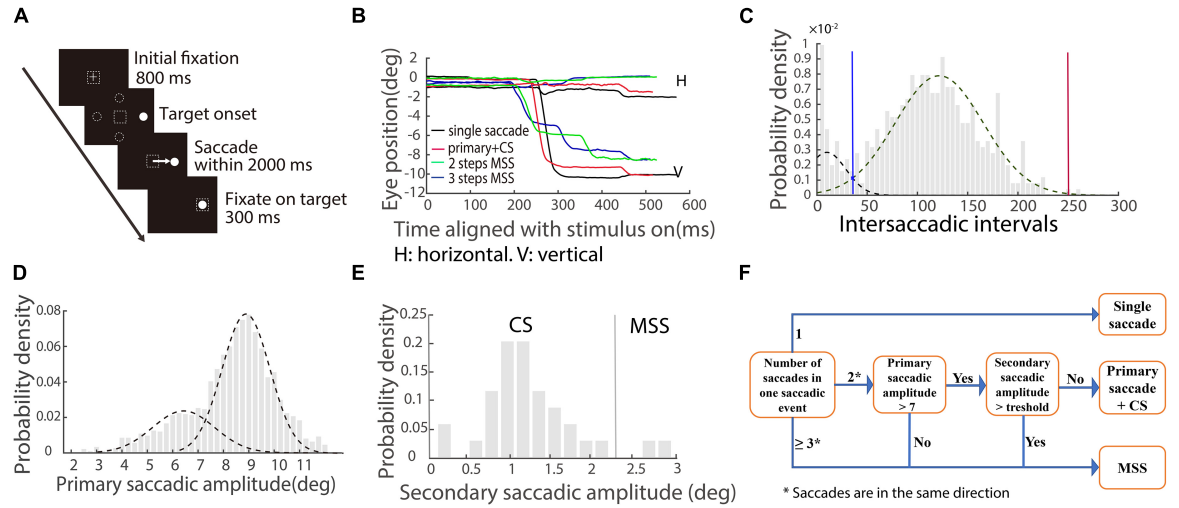

FIGURE 1
Schematic illustration of saccadic tasks and methods of quantifying saccades. **(A)** The paradigm of the visually guided reactive saccade task: White cross and circle represent fixation point and target, respectively. Dashed squares indicate the location of fixation. Dashed circles indicate other potential locations of the target. The white arrow represents the required saccade. **(B)** Exemplified eye trace of a PD patient. The *X*-axis represents the time aligned with the saccadic target onset. The *Y*-axis represents the eye position. Different colors denote different types of saccadic events. **(C)** The distribution of intersaccadic intervals. Dotted lines represent two unimodal distributions. The blue line is the cross point of the two unimodal fitting curves. The red line is the right zero point of the right unimodal fitting curve. **(D)** The distribution of primary/largest saccadic amplitudes. Two dotted lines indicate two unimodal distributions. **(E)** The distribution of secondary saccadic amplitude in which the first responsive saccadic amplitude is ≥7°. The vertical line indicates the mean + 2 × STD of the distribution. **(F)** Illustration of classifying different types of saccadic events.

of the fixation point, a white dot (target) appeared in one of four peripheral locations randomly (right, left, up, and down, with eccentricity of 10°). The size of the fixation points and target were 1° in length or diameter, respectively. Participants were instructed to fixate at the central cross (check window 4° in radius) and then make a saccade toward the target as accurately and fast as possible. The target disappeared after the eye entered and was maintained in the check window (4° in radius) for 300 ms. A blank screen was interposed between trials with an interval of 800 ms. It only took 3–4 s per trial, so each participant in the human study spent approximately 4 min on this test.

Data acquisition

In human experiments, eye movements were monitored at 1 kHz with a head-restrained infrared video-based eye tracker (Eye Link 1000 desktop mount, SR Research Ltd., Kanata, ON, Canada; EM-2000R, Jasmine Science and Technology Ltd., Beijing, China). Participants were seated in a dark room 57 cm away from the monitor (XL2720-B; resolution: 1920 × 1080; 27-inch; refresh rate: 100 Hz). The system was calibrated prior to the experiment by having the participants make saccades to nine targets forming a rectangle (3 × 3 targets). The online check window (maximum calibration error) of the eye tracker is 2° in radius. The average calibration error was 1.65°. The background luminance of the monitor was 0.08 cd/m^2, and the luminance of visual stimuli was 23.9 cd/m^2. Stimuli presentation and behavioral data collection were controlled by MATLAB (R2009b; MathWorks, Natick, MA, United States) with Psychtoolbox (PTB-3) running on a Windows system PC (HP).

For the monkey experiment, eye position signals were recorded using the scleral eye coil technique (Crist Instrument Company, Hagerstown, MD, United States), and data were sampled at 1 kHz. Visual stimuli were displayed on a 27-inch screen (XL2720-B, resolution: 1920 × 1080, refresh rate: 144 Hz) that was placed 57 cm in front of the monkeys' eyes. The background luminance of the monitor was 0.25 cd/m^2, and the luminance of the visual stimuli was 341 cd/m^2. We used a Windows PC system (DELL) to control the visual display and to run the real-time data acquisition system (Monkey Logic; NIH, Bethesda, MD, United States). The calibration procedure of the monkeys was similar to that in the human experiments. The recorded eye movement data were analyzed offline in MATLAB (R2017b; MathWorks, Natick, MA, United States).

Trials in which participants blinked after the target onset were excluded from further analysis. The average number of analyzable trials per participant group were 37, 36, 34, and 32 for YHC, EHC, MCI, and PD groups, respectively. Error trials, including fixation breaks and incorrect directions, were also excluded from further analysis. Overall, the excluded percentage of trials in the human study was 3.1, 6.7, 8.4, and 14.7% in the YHC, EHC, MCI, and PD groups, respectively. The excluded

percentage of trials in the monkey studies was 3, 12, 17, and 26% for four hemi-PD monkeys and 1% for three control monkeys.

Quantitative measures of saccades

A velocity threshold was set to find all responsive saccades from target onset to the end of the trial. The velocity threshold is the mean velocity ± 2.58 × STD (99% confidence interval) during a time interval of 200 ms prior to the target onset. The first responsive saccade was defined as a saccade with a minimum amplitude of 2° and a minimum latency of 30 ms, and its direction was toward the target location. While we plotted the eye traces, we found that there were different types of saccades with varied spatiotemporal properties (**Figure 1B**). To well classify the different types of saccades, we combined all responsive saccades within a trial together as one saccadic event if the intersaccadic intervals between adjacent saccades were within the two boundaries (blue and red vertical lines) of the distribution, as shown in **Figure 1C** (an example of the EHC group). We made the two unimodal fitting curves by employing maximum likelihood estimation. The blue line is the cross point of the two unimodal fitting curves. The red line is the right zero point of the right unimodal fitting curve. Since the distributions of intersaccadic intervals are different among the four groups of participants, the boundaries are varied.

From the exemplified eye traces shown in **Figure 1B**, it is obvious that there are different types of saccadic events, including typical saccades (a single saccade and a single saccade followed by a CS, black and red traces) and MSS with 2 or 3 steps (green and blue traces). The amplitude distribution of primary/largest saccades contains two separated unimodal distributions (**Figure 1D**, data of EHC), which supports at the population level that there are two different types of saccades, i.e., typical saccades and MSS. We will classify CS (a possible component of typical saccades) and MSS by the following criteria for further data analysis.

We first classified CS based on previous findings (Cohen and Ross, 1978). It has been reported that for a 10° required saccade, if the amplitude of the primary responsive saccade is ≥7°, the probability of generating CS is high (Cohen and Ross, 1978). Thus, we analyzed our data about the distribution of the amplitudes of secondary saccades for each group of participants (**Figure 1E**, EHC data), while the amplitudes of the first responsive saccades were ≥7°. CS is defined as the amplitude of secondary saccade in a saccadic event being less than a threshold, i.e., the mean amplitude + 2 × STD of secondary saccades and the direction is toward the target location.

We then classified MSS if a saccadic event met any one of the following criteria: (1) The number of saccades within a saccadic event is ≥3; (2) The number of saccades within a saccadic event is two, and the amplitude of the first responsive saccade is <7°; (3) The number of saccades within a saccadic event is two, the amplitude of the first responsive saccade is ≥7°, and the amplitude of the secondary saccade is ≥ the threshold. The directions of all mentioned saccades are the same. To help understand the logic and process of classification of the saccadic events, we schematically summarized the aforementioned definitions in **Figure 1F**.

To ensure that there was a sufficient number of correct trials for data analysis, the incidence of MSS was calculated when the correct rate of a session was ≥70%. In addition, the incidence of MSS in horizontal and vertical saccades was calculated when the correct rates of the two directions were ≥ the mean − 1.5 × STD (minimal trial number was 10) of each group of participants.

Statistical analysis

The Kruskal–Wallis test (a non-parametric approach to one-way ANOVA) was applied to determine the significant difference among four independent groups of participants based on the incidence of MSS and CS. This was corrected by the Bonferroni correction. If there were significant differences among the four groups of participants, a *post-hoc* test was performed to determine the significance between each pair of participants either by the Wilcoxon rank-sum for unpaired tests or by the Wilcoxon signed-rank for the paired test. The alpha level was set to 0.05.

Furthermore, we employed a curve fitting tool (MATLAB, cftool function) to examine the relationship between MSS/CS and scores of UPDRS motor, MMSE and MoCA in PD group. We justified the goodness of fit curves based on the statistical results of the fitting function, including the sum of squares due to error (SSE), the root mean squared error (RMSE), the coefficient of determination (R-square), and the degrees-of-freedom adjusted coefficient of determination (adjusted R-square).

According to our results, we considered two useful parameters, i.e., the incidence of MSS and the incidence difference of MSS between vertical and horizontal saccades, that might help discriminate PD from the EHC or MCI group. We first applied a logistic regression model to predict the probability of PD by using the two parameters. The formula of this model is $\text{logit}(P_{PD}) = a_0 + a_1x_1 + a_2x_2 + \varepsilon$, where x_1 is the incidence of MSS, x_2 is the incidence difference of MSS between the vertical and horizontal saccades, a_0 is a constant, a_1 is the coefficient of x_1, a_2 is the coefficient of x_2, ε is the random error, and P_{PD} is the probability of being diagnosed with PD. a_0, a_1, a_2, and ε were determined by maximum likelihood estimation. We set the alpha level of the regression model to be 0.05. We next obtained the distribution of P_{PD} for each group of participants. Finally, we used P_{PD} to plot the receiver operating characteristic (ROC) curve and calculated the area under the curve (AUC), which indicates the ability to discriminate PD from the EHC and MCI groups by using the logistic regression model. We justify

the predictors by the AUC which is the output of the logistic regression model.

Results

Results of the human experiment

Incidence of multiple step saccades and corrective saccades

We first compared the incidence of MSS among four groups of human participants. If the CS was not excluded from MSS analysis, the incidence of multiple saccades in PD was significantly higher than that in both the EHC and YHC groups ($p < 0.001$, Wilcoxon rank-sum test, **Figure 2A**), which is consistent with some previous findings (Jones and DeJong, 1971; Corin et al., 1972; White et al., 1983). However, while MCI patients also showed significant differences comparing to EHC and YHC ($p < 0.001$, Wilcoxon rank-sum test), there was no significant difference between the PD and MCI patients ($p > 0.05$, Wilcoxon rank-sum test). Considering that MSS and CS might reflect non-physiological and physiological behaviors (Troost et al., 1974), we dissociated CS from MSS and compared their incidence among the four groups of participants, respectively (**Figure 2B**). First, the incidence of MSS in PD was significantly higher than that in YHC, EHC, and MCI groups ($p < 0.05$ for all comparisons); the incidences of MSS in the MCI and EHC groups were not significantly different ($p > 0.05$), while the incidences of MSS in the MCI and EHC groups were higher than those in the YHC group ($p < 0.001$ for all comparisons). Second, regarding the incidence of CS, only the MCI group showed a higher incidence of CS than the EHC and YHC groups ($p < 0.05$), whereas there was no significant difference among the EHC, YHC, and PD groups ($p > 0.05$, Kruskal–Wallis test, Bonferroni correction, $alpha = 0.05/6$).

Correlation between incidence of multiple step saccades/corrective saccades and scores of unified Parkinson's disease rating scale motor, mini-mental state examination, and Montreal Cognitive Assessment, respectively

We have shown that the incidence of MSS in the PD group was significantly higher than that in YHC, EHC, and MCI groups, whereas the incidence of CS was not significantly different between the PD group and YHC, EHC, and MCI groups. Here, we hypothesized that the incidence of MSS rather than CS might increase as the motor deficits of PD patients become more severe. To test this hypothesis, we analyzed the correlation between UPDRS motor scores and the incidence of MSS and CS. After data selection by the correct rate >70%, the number of PD patients in this analysis was 32. While the incidence of MSS and UPDRS motor scores showed a modestly positive correlation (R-square: 0.087) (**Figure 3A**), the incidence of CS and UPDRS motor scores showed no significant correlation (R-square: 0.023) (**Figure 3B**). Moreover, to test the relationship between MSS/CS and the scores of MMSE and MoCA, we did the same correlation analysis. The results showed that the incidence of MSS was not significantly correlated with the scores of MMSE and MoCA (R-squares: 0.0019 and 0.012) (**Figures 3C,E**), whereas the incidence of CS showed a modestly positive correlation with the scores of MMSE and MoCA, respectively (R-squares: 0.11 and 0.041) (**Figures 3D,F**). Such results support the argument that MSS might be a pathological behavior whereas CS be physiological behavior.

Incidence of multiple step saccades in horizontal and vertical saccades

While CS was not excluded from MSS analysis, the incidences of MSS were not significantly different between vertical and horizontal saccades in YHC, EHC and PD groups ($p > 0.05$, **Figure 4A**). In MCI group, the incidence of MSS

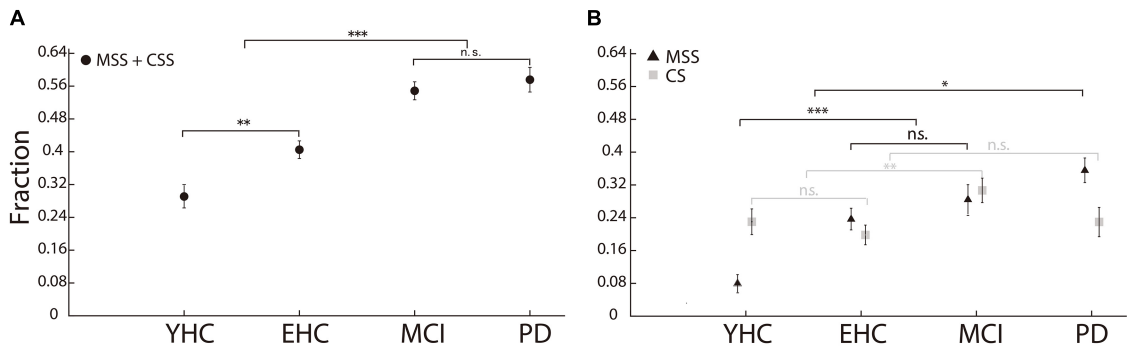

FIGURE 2
Incidence of MSS and CS in the human study. **(A)** The incidence of MSS including CS. There was no significant difference in MSS incidence between PD and MCI. **(B)** The incidence of MSS and CS, respectively. The incidence of MSS in PD was significantly higher than that in YHC, EHC, and MCI participants. Error bars show the standard error of the mean; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n.s., no significant difference (Wilcoxon rank-sum test).

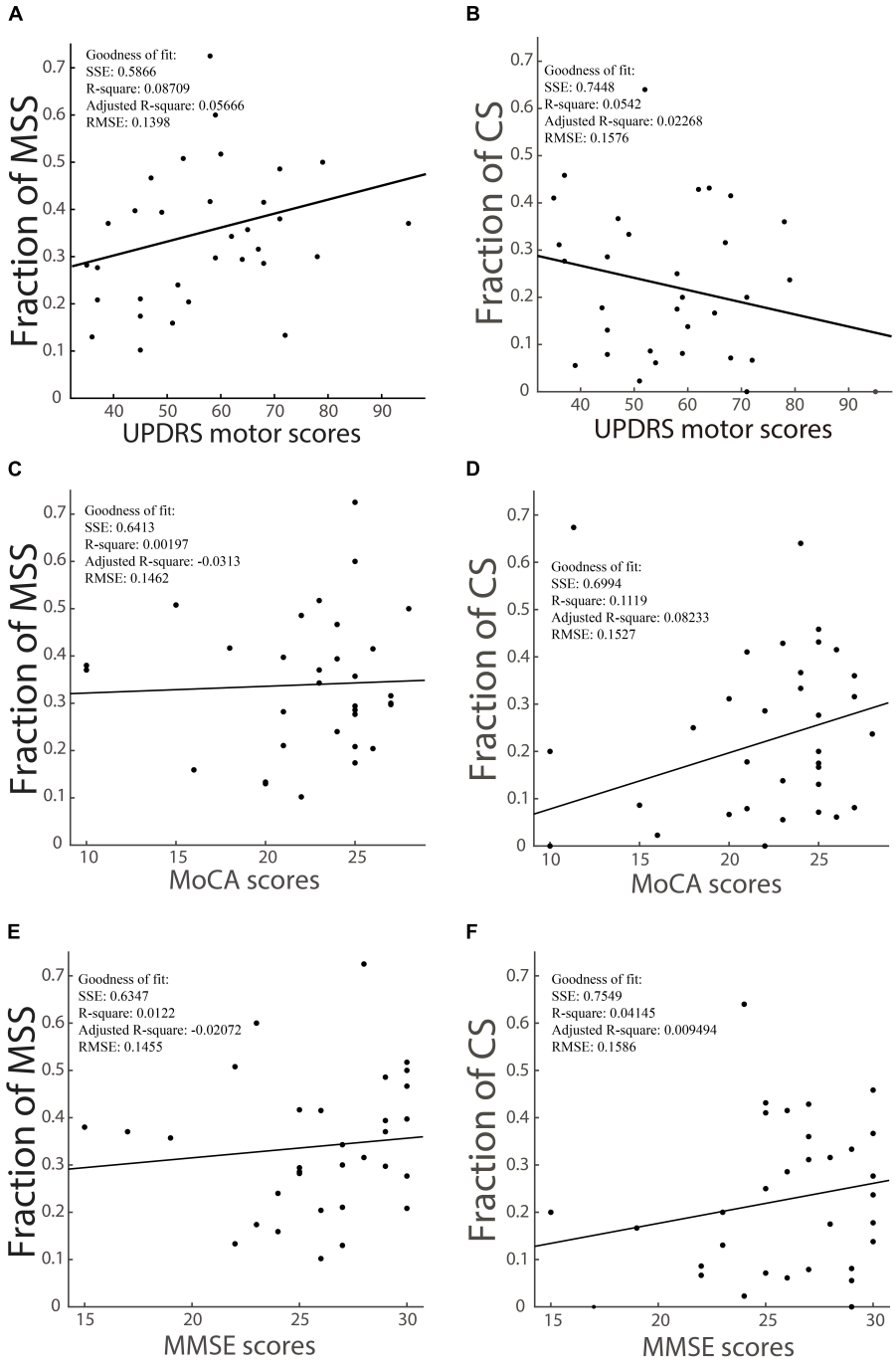

FIGURE 3
The correlation between the incidence of MSS, the incidence of CS and the UPDRS motor scores, scores of cognitive tests of PD patients. The incidence of MSS is modestly and positively correlated with UPDRS motor scores, as shown in panel **(A)**, while the incidence of CS has a slightly negative correlation with UPDRS motor scores, as shown in panel **(B)**. The incidence of MSS is not correlated with MoCA **(C)** and MMSE **(E)** scores, while the incidence of CS has a modestly positive correlation with MoCA **(D)** and MMSE **(F)** scores. SSE, the sum of squares due to error; RMSE, the root mean squared error; R-square, the coefficient of determination; Adjusted R-square, the degrees-of-freedom adjusted coefficient of determination.

in horizontal saccades was higher than in vertical saccades ($p < 0.01$, Figure 4A). However, while CS was excluded from MSS analysis, the incidence of MSS was significantly higher in the vertical saccade group than in the horizontal saccade group in the PD and MCI groups ($p < 0.01$, $p < 0.05$ for the PD and MCI groups, respectively, Figure 4B). Moreover, while the incidence of MSS in vertical saccades was the highest in the PD group ($p < 0.05$ for the comparisons of PD vs. EHC and

PD vs. YHC, respectively Figure 4C), the incidence of MSS in horizontal saccades was not significantly different both between the PD and EHC groups and between the PD and MCI groups ($p > 0.05$, Figure 4C). Such results indicate that the incidence of MSS in vertical saccades provides more reliable information than in horizontal saccades for the diagnosis of PD.

Logistic regression model and receiver operating characteristic analysis for discriminating Parkinson's disease from elderly healthy control and mild cognitive impairment groups

Thus far, our results have shown that the incidence of MSS in the PD group is significantly higher than that in YHC, EHC, and MCI groups, and it occurs more frequently in vertical saccades than in horizontal saccades. To test the likelihood of discriminating PD from the EHC and MCI groups, we first performed logistic regression analysis (detailed information given in Section "Statistical analysis"). The distributions of the probability of being PD are shown in Figures 5A,B for PD vs. EHC and PD vs. MCI, respectively. It is obvious that the distributions between the PD group and EHC, MCI groups were different. To further measure the ability to discriminate PD from EHC and MCI by employing the probability of PD, we then plotted the ROC curve and obtained AUCs of 0.76 and 0.69 for the PD vs. EHC and PD vs. MCI groups, respectively (Figure 5C). Such results indicate that the incidence of MSS in reactive saccades could be a complementary biomarker for the diagnosis of PD.

Results of the monkey experiment

To investigate whether the incidence of MSS could serve as a complementary biomarker for the diagnosis of PD in the early stage, we compared the incidence of MSS between four subclinical hemi-PD monkeys and three healthy monkeys. Despite the fact that these four monkeys did not show any typical PD motor symptoms, we are able to confirm the effect of MPTP injection by the following observations. Firstly, immediately after MPTP injection, the pupil size of ipsilateral injection side became significantly smaller comparing to the contralateral side which is consistent to the previous report (Metzger and Emborg, 2019). However, about 30 min later, the pupil size reversed between ipsilateral and contralateral sides. Secondly, as soon as the start of MPTP injection, the rate of heartbeat increased about 10–20%. Thirdly, after MPTP injection, monkeys lost appetite and reduced weight about 10–20%. Since the stage of subclinical PD is prior to the early stage of PD regarding the natural progress of PD and considering the fact that the severity of behavioral impairments (symptoms) increases following the development of PD, thus, the phenomenon of increased MSS in subclinical PD will also be observed in the early stage of PD. Before MPTP injection, two hemi-PD monkeys were trained to do some saccadic tasks, whereas other two monkeys were naïve to the saccadic tasks.

Incidence of multiple step saccades in the monkeys

As shown in Figure 6A, when the injection dose of MPTP was ≥0.4 mg/kg, three subclinical hemi-PD monkeys showed a significantly higher incidence of MSS than three control monkeys and one hemi-PD monkey SG ($p < 0.001$, Wilcoxon signed-rank test). We next compared the incidence of MSS before and after the MPTP injection in monkey PK. It is clear that the incidence of MSS significantly increased after MPTP injection (Figure 6B).

Since our PD monkeys were induced by unilateral injection of MPTP, it is interesting to see whether the incidence of MSS

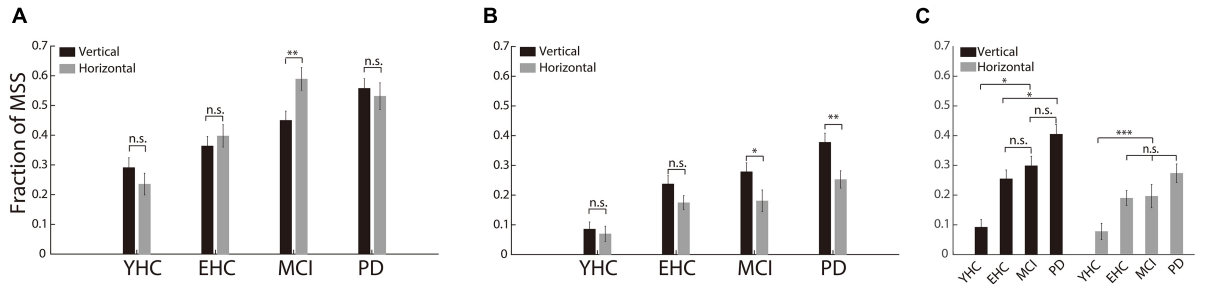

FIGURE 4
The incidence of MSS in horizontal and vertical saccades. **(A)** The incidence of MSS combined with CS between horizontal and vertical saccades. There was no significant difference in MSS incidence between vertical and horizontal saccades in any of the four groups of participants. **(B)** The incidence of MSS between the horizontal and vertical saccades. There was a significant difference between the vertical and horizontal saccades in PD and MCI patients. **(C)** The comparison of the MSS incidence among the four groups of participants. The incidence of MSS in vertical saccades was significantly higher in PD than in YHC, EHC, and MCI participants. Error bars show the standard error of the mean; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n.s., no significant difference (Wilcoxon rank-sum and sign-rank tests).

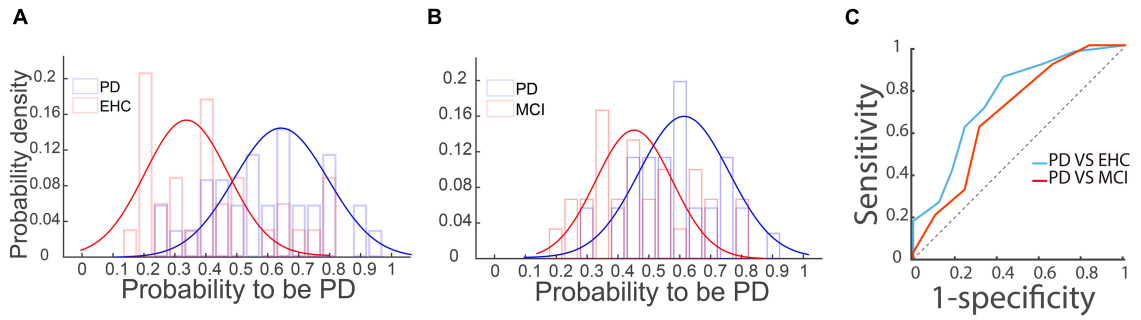

FIGURE 5
Logistic regression and ROC analysis of the PD vs. EHC and PD vs. MCI groups. **(A,B)** The distributions of the probability of PD based on the logistic regression analysis are shown in panels **(A,B)** for the PD vs. EHC and PD vs. MCI groups, respectively. Blue and red curves represent the two unimodal distributions of the PD and EHC/MCI groups, respectively. **(C)** ROC curve of the probability of PD between the PD and EHC and MCI groups. The *X*-axis represents 1-specificity, and the *Y*-axis represents sensitivity. The AUCs of PD vs. EHC (blue curve) and PD vs. MCI (red curve) were 0.76 and 0.69, respectively. The dotted line is the diagonal line.

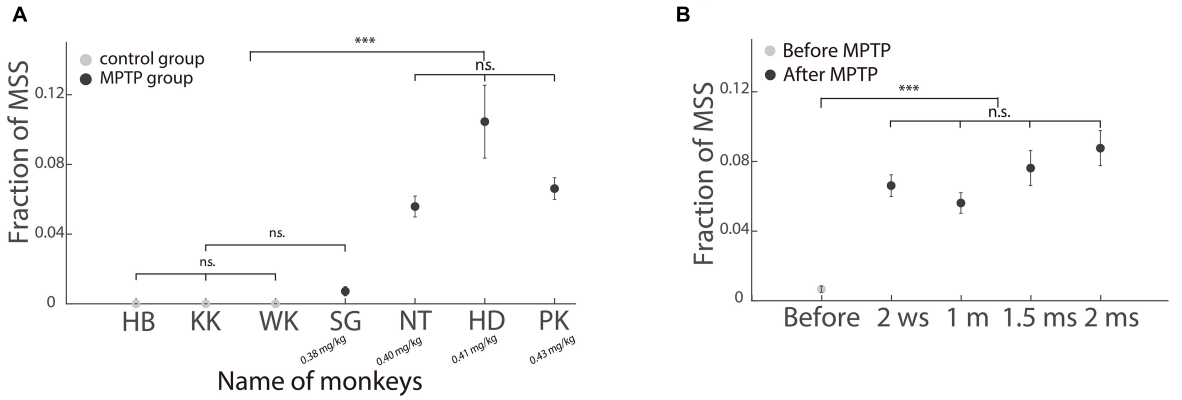

FIGURE 6
Incidence of MSS in healthy and subclinical hemi-PD monkeys. **(A)** Comparing the incidence of MSS between healthy and subclinical hemi-PD monkeys. The incidence of MSS was significantly higher in the three hemi-PD monkeys with MPTP dose larger than 0.4 mg/kg. Please be aware that the dosage of MPTP increases from left to right. **(B)** The incidence of MSS before and after MPTP injection in one hemi-PD monkey (PK). The *X*-axis is the time (w: week, m: month). The incidence of MSS increased significantly after MPTP injection. Error bars show the standard error of the mean; ***$p < 0.0001$, n.s., no significant difference (Wilcoxon rank-sum test).

is different between ipsilesional and contralesional saccades. To make it visible, we randomly picked up 10 trials from left and right saccades, respectively, in two exemplified sessions one before and one after MPTP injection. The horizontal eye positions were plotted in **Figures 7A,B**. It clearly showed that the number of trials with MSS (gray traces) increased after MPTP injection. Moreover, the incidence of MSS in ipsilesional saccades was higher than that in contralesional saccades (**Figure 7B**). The population results showed that the mean incidences of MSS in four PD monkeys were higher in ipsilesional saccades than in contralesional saccades, in which the difference was statistically significant ($p < 0.001$, **Figure 7C** Wilcoxon signed-rank test) in monkey PK and HD who received bigger dose of MPTP injection. Such results are opposite to our expectation, i.e., the impairment of saccades should be more serious in contralesional direction than in ipsilesional direction. One possible explanation is that the dopaminergic system in basal ganglia over compensates with its function after being damaged in certain level, with the similar mechanisms after damaging of cerebral cortices (Dennis et al., 2010). Such results indicate that the incidence of MSS could serve as a complementary biomarker for the diagnosis of early PD even though typical motor symptoms are absent.

Discussion

Although PD has been detected and studied for more than 200 years (Dorsey et al., 2018), objective and reliable biomarkers for the diagnosis of PD, particularly for its early diagnosis, are still lacking (Waninger et al., 2020). Saccadic eye movement might be a valuable behavioral biomarker because it reflects

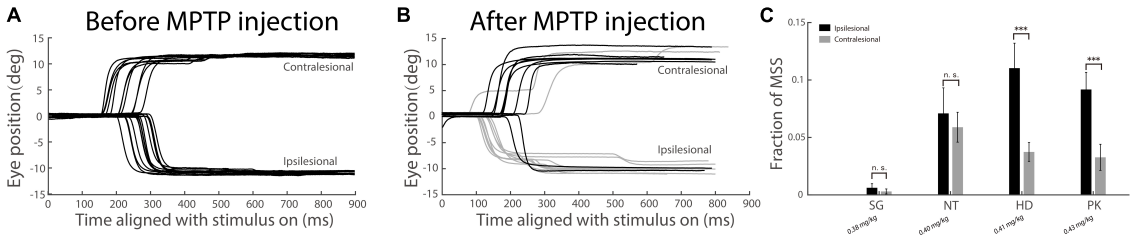

FIGURE 7
Comparison of the incidence of MSS between ipsilesional and contralesional saccades in subclinical hemi-PD monkeys. **(A,B)** Exemplified horizontal eye traces of monkey PK before and after MPTP injection. The *X*-axis represents the time aligned with the saccadic target onset. The *Y*-axis represents the eye position. Black traces denote one step saccades, or one primary saccade followed by a corrective saccade. Gray traces denote MSSs. The incidence of MSS is greater in ipsilesional saccades than that in contralesional saccades. **(C)** The incidence of MSS in population level between the ipsilesional and contralesional saccades. There is a significant difference between the ipsilesional and contralesional saccades in monkey HD and PK who received bigger dose of MPTP injection. Error bars show the standard error of the mean; ***$p < 0.001$, n.s., no significant difference (Wilcoxon rank-sum and sign-rank tests).

the physiological and cognitive functions of the brain and shows high test–retest constancy (Bargary et al., 2017). Indeed, previous studies have found that PD patients exhibit significant changes in certain saccadic parameters, such as the multiple-step pattern (Blekher et al., 2009) and vertical saccadic gain and error rate (Chehrehnegar et al., 2019; Irving and Lillakas, 2019; Waldthaler et al., 2019) in various oculomotor tasks. Researchers have argued that some changes in saccadic parameters could serve as complementary biomarkers for the diagnosis of PD (Chan et al., 2005; Armstrong, 2015; Patel et al., 2019). However, since most previous studies employed voluntary saccadic tasks, such as antisaccade and memory-guided saccade were usually difficult for elderly subjects to complete, particularly for patients with neurodegenerative diseases (Walker et al., 2000), the task difficulty highly restricted the application of measuring saccades in the diagnosis of PD. If the incidence of MSS in simply reactive saccades shows a significant difference between PD and control subjects, it might extend the application of MSS in the diagnosis of PD.

The incidence of multiple step saccades in reactive saccades could serve as a complementary biomarker for the diagnosis of early Parkinson's disease

In the present study, we applied a visually guided reactive saccade task, which is the easiest oculomotor task and only takes approximately 4 min to complete. The majority of participants completed the reactive saccadic task well (with average correct rates of 96.9, 93.3, 91.6, and 85.3% for the YHC, EHC, MCI, and PD groups, respectively). PD patients had significantly more MSS than YHC, EHC, and MCI participants (**Figure 2**). In addition, the incidence difference of MSS between vertical and horizontal saccades was significantly greater in PD patients than in YHC, EHC, and MCI participants (**Figure 4**). Such results indicate that the incidence of MSS in reactive saccades could serve as a complementary biomarker for the diagnosis of PD, with AUCs of 0.76 and 0.69 for discriminating PD from EHC and MCI, respectively.

Although the number of male and female participants is not perfectly balanced in the present study, it has been reported that there is no significant difference between male and female participants in performing visually guided saccade task (Bonnet et al., 2013). Thus, the unbalanced gender does not alter our results. Moreover, the age of MCI group was significantly older than PD group. Our results have shown that the incidence of MSS is significantly higher in EHC than in YHC (**Figure 2**), which indicates that aging would increase the occurrence of MSS. Nevertheless, the incidence of MSS in PD group is significantly higher than MCI group, which supports our argument that MSS could serve as a behavioral biomarker for the diagnosis of PD.

In addition, the correct rates of all monkeys but one (monkey PK: 74%) were >83%. The results of the monkey study show that the incidence of MSS was significantly higher in the three subclinical hemi-PD monkeys with MPTP injection doses ≥0.4 mg/kg than in healthy control monkeys (**Figure 6**). Such results indicate that the incidence of MSS could serve as a complementary biomarker for the diagnosis of early PD even though typical motor symptoms are absent.

Discrimination between Parkinson's disease and mild cognitive impairment indicating acceptable specificity by employing multiple step saccades to diagnose Parkinson's disease

Since previous studies only compared the incidence of MSS between PD patients and EHC participants, the question

regarding the specificity of MSS for the diagnosis of PD among other neurodegenerative diseases remains. It is important to evaluate the specificity of MSS for the diagnosis of PD because other neurodegenerative diseases also show abnormal saccadic behavior, e.g., MCI patients exhibit decreased saccadic latency, accuracy and velocity in reactive saccades (Chehrehnegar et al., 2021). Theoretically speaking, it is possible that the incidence of MSS might increase in MCI patients. Our results show that PD patients had significantly more MSS than MCI patients (**Figure 2B**), and we could discriminate PD from MCI with an AUC of 0.69 in the ROC analysis (**Figure 5**).

Possible reasons for the significantly higher multiple step saccades incidence in vertical saccades than in horizontal saccades in Parkinson's disease and mild cognitive impairment patients

For the first time, we showed that the incidence of MSS in vertical saccades was significantly higher than that in horizontal saccades in PD and MCI patients (**Figure 4B**). For PD patients, such results share certain commonalities with previous studies that reported a more severe impairment of saccades in the vertical direction (Rottach et al., 1996; Antoniades and Kennard, 2015; Lemos et al., 2016; Jung and Kim, 2019). An elongated saccadic latency (Lemos et al., 2016) and shortened saccadic amplitude (hypometria) in the vertical direction have been reported in PD patients (Jung and Kim, 2019). Here, we provide additional evidence to support the argument that the impairment of vertical saccades is more severe than that of horizontal saccades in PD. Since vertical and horizontal saccades are controlled by different brain regions and neural networks (Lemos et al., 2016; Takahashi and Shinoda, 2018; Irving and Lillakas, 2019), an intuitive thinking is that such directional differences in saccades might be due to the asymmetric impairment between these brain structures. Such an assumption is supported by a functional magnetic resonance imaging (fMRI) study, which shows that in PD patients, vertical reactive saccades cause higher activity in the right frontal eye field, cerebellar posterior lobe, and superior temporal gyrus than horizontal saccades (Lemos et al., 2016).

We are also, for the first time, able to report a higher incidence of MSS in vertical saccades than in horizontal saccades in MCI patients. The possible reason for this behavioral phenomenon might be the similar pathological alterations in MCI and PD patients, both of which are neurodegenerative diseases. Although there is no direct evidence to support this assumption, it is well known that MCI is the early stage of Alzheimer's disease (AD) (Morris et al., 2015). According to the findings of previous studies, approximately 80% MCI patients develop to dementia in 6 years (Petersen et al., 2001). Moreover, PD and MCI are two common neurodegenerative diseases that share certain pathological changes such as alteration of neurotransmitter receptors and accumulation of misfolded proteins (Xu et al., 2012; Tosto et al., 2015).

The possible neural mechanisms underlying the generation of multiple step saccades

Despite the advanced knowledge about the neural control of saccades, (Gaymard et al., 1998) there are few studies of the neuronal mechanisms underlying the generation of MSS. Thus, it is not clear how the brain develops MSS. Nonetheless, previous studies have found that some brain regions, i.e., the frontal cortex, basal ganglia and cerebellum, are involved in the generation of MSS (Avanzini et al., 1979; Kimmig et al., 2002; van Donkelaar et al., 2009). Stimulating the frontal eye field and supplementary eye field with transcranial magnetic stimulation (TMS) increased the incidence of MSS (van Donkelaar et al., 2009). Loss of dopaminergic neurons in the basal ganglia increased the incidence of MSS, as observed in PD patients (Kimmig et al., 2002; Blekher et al., 2009) and PD monkeys (Tereshchenko et al., 2015). Lesions in the cerebellum caused an increase in MSS incidence in human and non-human primates (Avanzini et al., 1979). Considering that the abovementioned brain regions have either direct or indirect (via the superior colliculus) anatomical connections with premotor circuits (comprised of omnipause and burst neurons) in the brainstem, (Munoz and Wurtz, 1995) the function of these regions in MSS generation is very likely to be a modulator rather than a generator. It is well known that the interinhibition between omnipause and burst neurons in premotor circuits causes pulse (saccade) and step (fixation) patterns of eye movements (Munoz and Wurtz, 1995). Moreover, a previous study found that electronically stimulating omnipause neurons during execution of a saccade immediately stopped the movement of the eyes and froze the eyes midway (Bergeron and Guitton, 2002). After releasing the electronic stimulation in omnipause neurons, the saccade resumed. Such a pattern of eye movement highly resembles MSS. Therefore, we assume that the lower oculomotor structures in premotor circuits of the brainstem are the generator of MSS, whereas the higher oculomotor structures in cortical and subcortical regions are the modulators of MSS.

Limitations and future study

First, since the incidence of MSS has been studied only in one type of reactive saccade, i.e., the visually guided step saccade task (**Figure 1A**), it is interesting to study the incidence of MSS in other types of reactive saccades, such as the visually guided

gap saccade task. Second, although the specificity of using MSS to diagnose PD is evaluated by comparing the incidence of MSS between PD and EHC and MCI, more studies are required to compare the incidence of MSS between PD and other neurodegenerative diseases, particularly movement disorder diseases such as essential tremor and Progressive Supranuclear Palsy. Third, PD patients were not in a full off-state in the present study and they were also combined with some cognitive deficits which might affect the comparison between PD and MCI patients. Fourth, although studies of subclinical hemi-PD monkeys directly explore the role of the basal ganglia in the generation of MSS, more studies are needed to systematically explore the neural mechanisms of MSS, such as single neuron recordings from oculomotor structures in the brain.

Conclusion

Multiple step saccades in visually guided reactive saccades could be the biomarker for the early diagnosis of PD. In addition, the results from hemi-Parkinson monkeys indicate that the dopaminergic system in basal ganglia plays an important role in the development of MSS.

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 Ethics Committee of Beijing Normal University and the Chinese PLA General Hospital (Medical School of Chinese PLA). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Ethics Committee of Beijing Normal University.

Author contributions

MZ and XX designed the experimental paradigm. WM, JW, ML, ZZ, and FJ performed the experiments. WM, JW, ZZ, HB, and MZ completed the MPTP injection operation of the four monkeys. WM analyzed the data. WM and MZ wrote and edited the manuscript. XX, ZL, and XL supervised the data collection and discussed the results. 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.

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Detecting apathy in patients with cerebral small vessel disease

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Background: Apathy is attracting more and more attention in clinical practice. As one of the most common features of cerebral small vessel disease (CSVD), the assessment of apathy still mainly relies on observers. With the development of Information and Communication Technologies (ICTs), new objective tools take part in the early detection of apathy.

Objectives: To detect apathy in patients with CSVD and find out the relationship between apathy and actigraphic data sampled from the diurnal and nocturnal periods.

Methods: A total of 56 patients with CSVD were recruited for a cross-sectional observational study. Apathy was diagnosed by the diagnostic criteria for apathy in neurocognitive disorders. The presence of lacunes, white matter hyperintensities, cerebral microbleeds (CMBs), and perivascular spaces (PVS) in magnetic resonance imaging (MRI) images were rated independently. Actigraph devices were worn in the non-dominant hands of each subject for 7 consecutive days to collect samples of raw data, and diurnal vector magnitude (VM) and a series of sleep quality variables were obtained.

Results: We found that the frequency of apathy in Chinese patients with CSVD reached 37.50%. Patients in the Apathy+ group showed more lacunes and CMBs, and higher Fazekas scores in comparison to apathy-group individuals. Diurnal VM, instead of other sleep quality variables, was lower in CSVD patients with apathy relative to those without apathy. Lastly, we discovered that diurnal VM and total time in bed (TTB) correlated negatively with apathy severity in patients with CSVD.

Conclusion: Actigraphy is a promising choice to evaluate apathy in patients with CSVD.

KEYWORDS

actigraphy, apathy, small vessel disease, sleep, neuropsychiatric disorders

Introduction

Apathy, defined as a disorder of goal-directed behavior, has attracted more and more attention in aging neuroscience (Hachinski et al., 2022). It has been evidenced that apathy is be found in many neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (Zhao et al., 2012; Niino et al., 2014; Brown et al., 2019), and apathy has been confirmed to be correlated with cognitive impairments in cross-sectional studies (Niino et al., 2014; Yu et al., 2020). Several longitudinal studies demonstrated that an obvious decline in cognitive function and daily living ability was accompanied by apathy (Robert et al., 2006; Lechowski et al., 2009). For example, a 4-year prospective longitudinal study recently discovered that a higher level of apathy at baseline enables the prediction of a progressive cognitive decline at 4-year follow-up in patients with MS (Raimo et al., 2022). In most previous research, apathy was diagnosed and assessed by scales such as the Neuropsychiatric Inventory (NPI), the Apathy Evaluation Scale (AES), the Apathy Inventory (AI), and the Geriatric Depression Scale (GDS) (David et al., 2012; Kuhlmei et al., 2013; Zhao et al., 2014; Tay et al., 2019). In Robert et al. (2018), Miller et al. (2021), the diagnostic criteria for apathy in neurocognitive disorders were discussed actively by experts from academia, industry, and regulatory bodies all around the world. In line with the evolvement of the diagnostic criteria of apathy, unreliable self-reporting has been proposed to be substituted and improved by informant-based observable traits and objective tools.

Cerebral small vessel disease (CSVD) refers to a group of pathological processes that affect the small arteries, arterioles, venules, and capillaries of the brain (Pantoni, 2010). CSVD is quite common in the older population and is featured as cognitive deficits, gait abnormalities, and affective disorders (Zhào et al., 2020). A recent systematic review revealed that CSVD affects all major domains of cognitive ability (Hamilton et al., 2021). Our previous study showed that the prevalence of apathy reached 37.3% in older Chinese patients with CSVD, with a lack of goal-directed behavior and diminished goal-directed activities in the dimension of behavior/cognition according to the diagnostic criteria for apathy revised in 2018 (Zhào et al., 2021).

Until now, various assessment scales for apathy have been developed, whereas, the detection of apathy is still debated. This phenomenon can be explained by the fact that the application of scales is rather limited, owing to its dependency on the human observer. Based on the development of novel technologies, apathy experts recommended objective ways such as Information and Communication Technologies (ICTs) on evaluating apathy in coordination with subjective inventories (Robert et al., 2013). For example, the ECOCAPTURE@HOME protocol was reported to assess apathy remotely in patients with AD and Frontotemporal Dementia (FTD) recently (Batrancourt et al., 2019; Godefroy et al., 2021).

Actigraph devices, progressing from piezoelectric to captive sensors, from epoch-based count data to raw data sampled at high frequency, are a potential candidate for ICTs of that use (König et al., 2014). Actigraphy was confirmed to measure sleep quality and daytime activity in our recently published paper (Zhao et al., 2022). Thus, we sought to detect apathy in patients with CSVD and found out the relationship between apathy and actigraphic data sampled from the diurnal and nocturnal periods.

Methods

Participants

We launched a cross-sectional observational study from 1 November 2021 to 20 April 2022 and recruited 56 elderly patients with CSVD consecutively from the Department of Neurology at the Seventh Medical Center of PLA General Hospital (Beijing, China). Our study was approved by the Academic Ethics Committee of the Biological Sciences Division of PLA General Hospital in Beijing, China.

The exclusion criteria were as follows: patients with major stroke or cerebral bleeding episodes or tremor; other causes of leukoencephalopathy (e.g., immune, demyelination, genetic); use of psychotropic medications; multisystem diseases, such as polyarteritis nodosa, nervous system vasculitis associated with connective tissue disorders, and vasculitis secondary to infectious; arthritis; MRI contraindications; and other neurodegenerative disorders, such as AD, PD, and FTD.

Magnetic resonance imaging measurements

A 3.0T MRI brain (Discovery MR750; GE Healthcare, United States) scan displayed white matter lesions reflecting the degree of CSVD. A brain MRI (slice and interslice thicknesses of 5 and 1.5 mm, respectively) was carried out as follows: longitdinal relaxation time (T1) fluid-attenuated inversion recovery [transverse relaxation time (TR), 1750 ms; time of echo (TE), 23 ms; T1, 780 ms; field of view (FOV), 24 cm] and T2-weighted imaging (TR, 7498 ms; TE, 105 ms; FOV, 24 cm) sequences. The assessors were blinded to imaging findings.

Total cerebral small vessel disease burden score

The total CSVD burden score was calculated according to our previous procedure (Zhao et al., 2022). One point was

allocated to each of the following MRI parameters: moderate to severe white matter hyperintensities (WMH) (Fazekas score: 2–3), presence of lacunes, cerebral Microbleeds (CMBs), and moderate to severe basal ganglia-perivascular spaces (PVS) (semi-quantitative rating > 1), with total scores ranging from 0 to 4 points. To elucidate whether actigraphic data differed according to CSVD burden score, subjects were also divided by total CSVD burden score.

Diagnosis and assessment of apathy

Each patient was diagnosed according to the diagnostic criteria for apathy in neurocognitive disorders (Miller et al., 2021). It is different from the diagnostic criteria proposed in 2018 (Robert et al). In fact, in criteria B (symptoms and duration), Dimensions B1, B2, and B3 were listed as diminished initiative, interest, and emotional expression/responsiveness, respectively. Dimension B4 (social interaction) was removed. The patient exhibits at least one symptom in at least two of the three dimensions. Details were listed in **Supplementary material 1**.

Neuropsychiatric inventor and global cognitive function test

All participants completed both NPI and mini-mental State Evaluation (MMSE). The NPI (12 subscales) is a structured interview with a caregiver who is familiar with the subject. The overall frequency (1–4) and severity (1–3) are then rated. Scores on each NPI subscale range from 0 to 12, with higher scores indicating more severe symptoms (Zhao et al., 2014). MMSE is a widely used 30-point test that reflects the global cognitive function (Zhào et al., 2019).

Wrist actigraphy

According to the procedure, each participant was instructed to wear an ActiGraph GT3X+ device (ActiGraph, Pensacola, United States) on their non-dominant wrist for 24 h per day (except when bathing or swimming) for 7 days.

At the end of the wear period, data were downloaded using ActiLife software (ActiGraph, Pensacola, FL, United States). All data files were visually screened for sufficient wear time and then processed for analysis.

Objective actigraphic variables detection

Data from the Actigraphic data were downloaded and analyzed using the software with a 60 s epoch. As the ActiGraph GT3X+ device is a triaxial accelerometer, the diurnal vector magnitude (VM) was sampled based on the equation listed as follows: VM = $\sqrt{X^2 + Y^2 + Z^2}$ (X, Y, and Z are the VM counts at X-axis, Y-axis, and Z-axis, respectively) (Details given in **Figure 1**). Bedtime and wake time from the sleep diary was used to define sleep-wake variables. The sleep quality variables consisted of sleep efficiency (SE), total time in bed (TTB), total sleep time (TST), wake after sleep onset (WASO), times of awakenings (TA), and average duration of awakenings (ADA) (Zhao et al., 2022).

Statistical analysis

Differences between the groups' clinical and demographic data were analyzed by using Student-*t* analysis or one-way analysis of variance. Correlations were determined using the Pearson correlation coefficient to assess the relationship between apathy severity and actigraphic data on the total sample. The significance threshold was set at $p < 0.05$ in all statistical tests. The analysis was carried out using SPSS 22.0 software.

Results

The frequency of apathy among aged patients with CSVD was 37.50% in this study. **Table 1** showed the demographic characteristics of all patients. The gender (66.67% men *vs.* 48.57% men; $p = 0.268$), age (69.24 ± 8.56 years *vs.* 66.11 ± 10.24 years; $p = 0.246$), height (167.14 ± 8.56 cm *vs.* 165.63 ± 6.83cm; $p = 0.453$), weight (72.52 ± 12.47 kg *vs.* 66.31 ± 11.98 kg; $p = 0.074$) and MMSE score (20.95 ± 4.26 *vs.* 23.40 ± 4.72; $p = 0.057$) did not reach significance. Individuals of Apathy+ group and Apathy- group did not show remarked differences in most NPI-subscale scores, except for the NPI-apathy subscale (6.00 ± 1.92 *vs.* 2.06 ± 1.02; $p < 0.001$) and total score (24.80 ± 9.14 *vs.* 16.69 ± 8.57; $p = 0.002$), details were shown in **Supplementary material 2**. Apathy+ group patients showed more lacunes (mean rank: 23.31 *vs.* 35.06; $p = 0.004$) and CMBs (mean rank: 34.12 *vs.* 24.33; $p = 0.017$), higher Fazekas score (2.09 ± 0.83 *vs.* 1.59 ± 0.74; $p = 0.023$), and higher CSVD burden score (3.24 ± 0.89 *vs.* 2.21 ± 1.27; $p = 0.0001$) compared with Apathy- group individuals. For actigraphic data, diurnal VM were lower in CSVD patients with apathy relative to those without apathy. On the contrary, all the sleep quality variables did not differ statistically between Apathy+ and Apathy- groups. Details are shown in **Table 1**.

Patients with a high CSVD burden (≥ 2 points) exhibited lower diurnal VM relative to those with a low CSVD burden (1 point). All the sleep quality variables, including SE, TTB, TST, WASO, TA, and ADA, were not statistically different between groups. Details are shown in **Figure 2**.

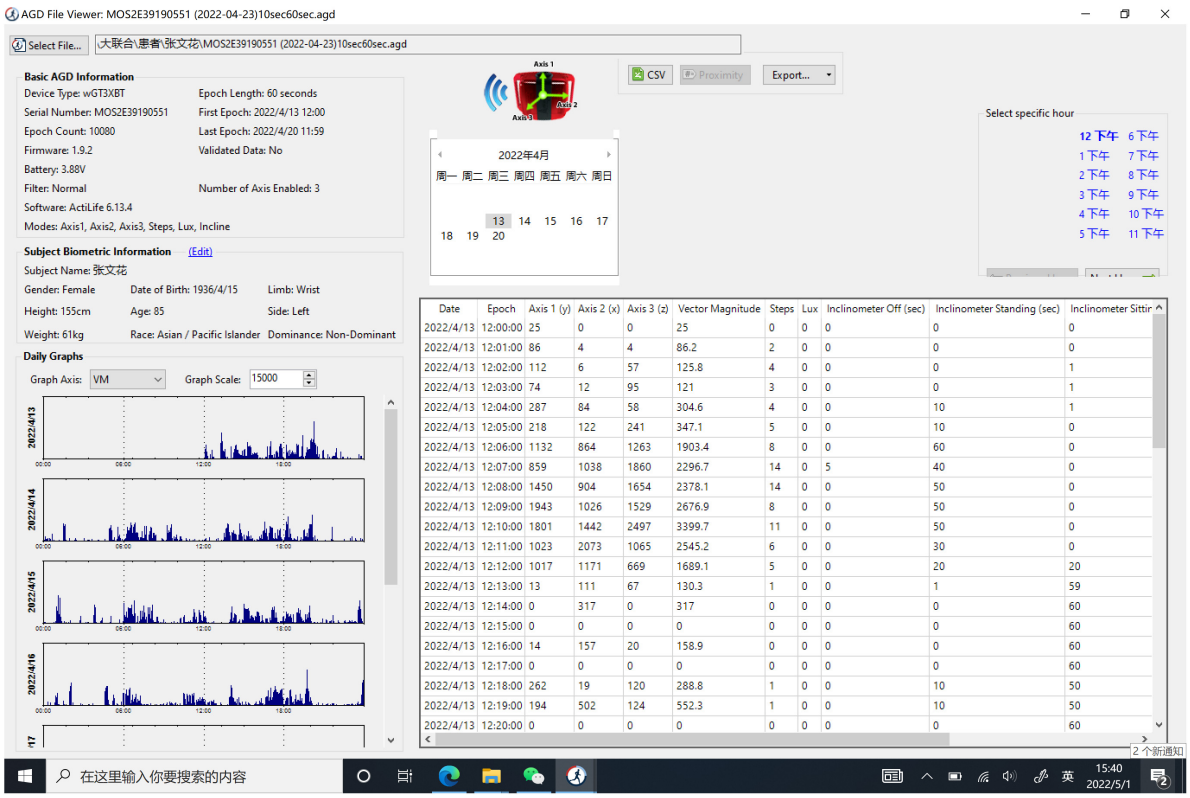

FIGURE 1
An example of a participant's actogram, that is, raw actigraphy data of vector magnitude.

TABLE 1 Clinical and demographic characteristics of the subjects with apathy and without apathy.

Characteristics	Apathy + (N = 21)	Apathy- (N = 35)	Overall (N = 56)	P value
Men,%	14(66.67%)	17(48.57%)	31(55.36%)	0.268
Age, years	69.24(8.56)	66.11(10.24)	68.07(10.08)	0.246
Height, cm	167.14(7.95)	165.63(6.83)	166.07(7.48)	0.453
Weight, Kg	72.52(12.47)	66.31(11.98)	68.29(12.42)	0.074
MMSE, score	20.95(4.26)	23.40(4.72)	22.49(4.64)	0.057
SE,%	83.94(8.07)	79.31(7.00)	81.19(7.49)	0.066
TTB, minutes	377.11(109.77)	406.02(110.92)	392.62(114.16)	0.432
TST, minutes	314.54(88.83)	321.90(81.83)	317.63(87.54)	0.793
WASO, minutes	59.47(39.97)	78.40(40.81)	70.42(40.58)	0.164
TA, times	15.16(8.92)	17.12(6.26)	16.18(7.34)	0.425
ADA, minutes	3.73(0.93)	4.84(2.31)	4.42(1.92)	0.054
Diurnal VM, counts	602.39(253.50)	1281.08(348.92)	1026.57(456.67)	0.000***
Lacunes	36.80	22.97	/	0.010**
PVS	28.50	27.71	/	0.450
CMBs	23.94	35.10	/	0.007**
Fazekas score,	2.15(0.81)	1.57(0.74)	1.81(0.80)	0.009**
CSVD burden score	3.35(0.75)	2.17(1.27)	2.65(1.23)	0.001***

Mean (Standard Deviation) for age, height, weight, Fazekas score, and CSVD burden score. Number (Percentage) for gender. Mean rank for LI, PVS, and CMBs. **$p < 0.01$ Apathy+ relative to Apathy-, ***$p < 0.001$ Apathy+ relative to Apathy-. PVS, enlarged perivascular spaces; CMBs, cerebral microbleeds; CSVD, cerebral small vessel disease; SE, sleep efficiency; TTB, total time in bed; TST, total sleep time; WASO, wake after sleep onset; TA, times of awakenings; ADA, average duration of awakening; VM, vector magnitude.

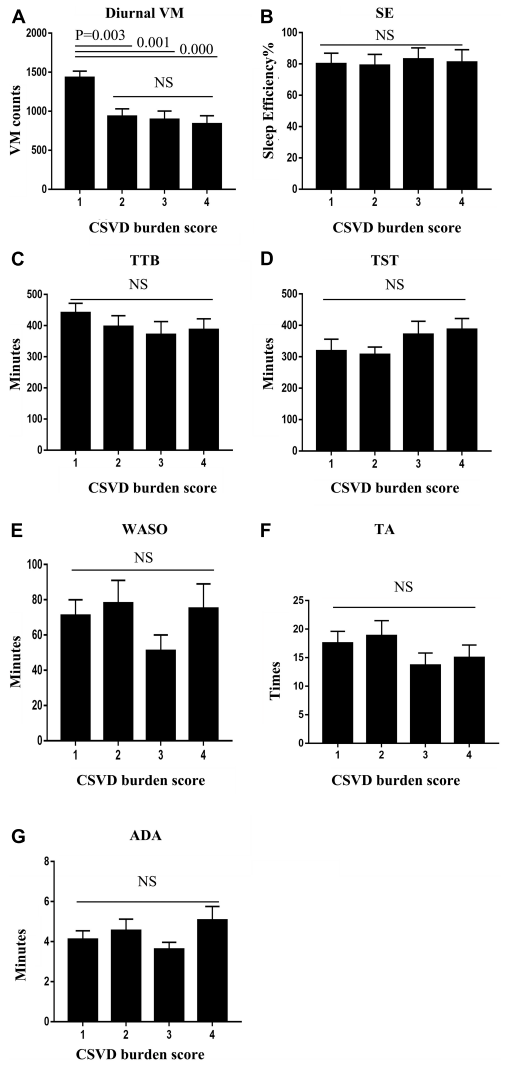

FIGURE 2
Patients with high cerebral small vessel disease (CSVD) burden (≥ 2 points) exhibited higher diurnal VM relative to those with a low CSVD burden (1 point) **(A)**. Sleep quality variables, including sleep efficiency (SE), total time in bed (TTB), total sleep time (TST), wake after sleep onset (WASO), times of awakenings (TA), and average duration of awakenings (ADA), were not statistically different between groups **(B–G)**.

Furthermore, correlation was adopted to analyze the relationship between the NPI–apathy score and actigraphic variables; we found that apathy severity score was negatively associated with diurnal VM score ($r = -0.811$, $p = 0.000$) and TTB ($r = -0.349$, $p = 0.030$). Details are shown in **Figure 3**.

Discussion

Among patients with CSVD, the current study demonstrated that apathy showed more lacunes, CMBs, and higher Fazekas scores relative to individuals without apathy. Furthermore, diurnal VM rather than sleep quality variables were statistically lower in subjects with apathy in comparison with those without apathy. Meanwhile, patients with a high CSVD burden exhibited lower diurnal VM relative to individuals with a low CSVD burden. Lastly, diurnal VM and total time in bed (TTB) correlated negatively with apathy severity in the total sample.

Although the prevalence of apathy in kinds of neuropsychiatric disorders differs, it is no doubt that apathy is one of the most commonly encountered symptoms in the aged population with high frequency (Yao et al., 2015). In patients with CSVD, together with our previous reports, apathy can be found in more than one-third of hospitalized aged adults (Zhào et al., 2021). Systematic review and meta-analysis confirmed that apathy was associated independently with worse CSVD severity (Clancy et al., 2021), and a hypothesis of "vascular apathy" has been proposed on the ground of the empirical findings that supported the close relationship between apathy and CSVD (Wouts et al., 2020). The hypothesis was based on the inference that apathy symptoms and CSVD share the same functional brain area. In detail, the brain circuits such as the frontal regions with their projections to the prefrontal regions, the basal ganglia, the parietal regions, and the anterior cingulate, which play key roles in planning, motivation, and autoactivation, could be injured in CSVD (Wouts et al., 2020). More recently, apathy, combined with gait impairment and executive dysfunction, was conveyed as a new vascular triad in patients with CSVD by Hachinski et al. (2022). Hypertension, cerebral hypoperfusion, white matter tract disconnection, and other CSVD etiological factors were reported to cause apathy (Moretti et al., 2015; Tay et al., 2019; Hachinski et al., 2022).

Given that individuals with apathy often manifested in those with a lack of goal-directed behavior, there exists several studies that tried to elucidate the definite relationship between apathy and actigraphic data in patients with different neurological disorders. Müller et al. (2006) observed that patients with high apathy exhibited significantly reduced locomotor activity and more episodes of inactivity (naps) during the daytime in patients with traumatic brain injury. David et al. (2012) found that individuals with AD who had symptoms of apathy had significantly lower daytime mean motor activity than AD patients without apathy. In patients with mild cognitive impairment (MCI) and dementia, AES scores correlated negatively with actigraphic daytime activity (Kuhlmei et al., 2013). The present study also concluded that apathy severity was negatively associated with diurnal VM, which implied that lower daytime activity sampled by actigraph devices could be a representation of apathy in patients with CSVD. The difference observed with the other studies using actigraphy was mainly due to the population selection. In addition, the algorithm we used was different from other research, which might be another reason.

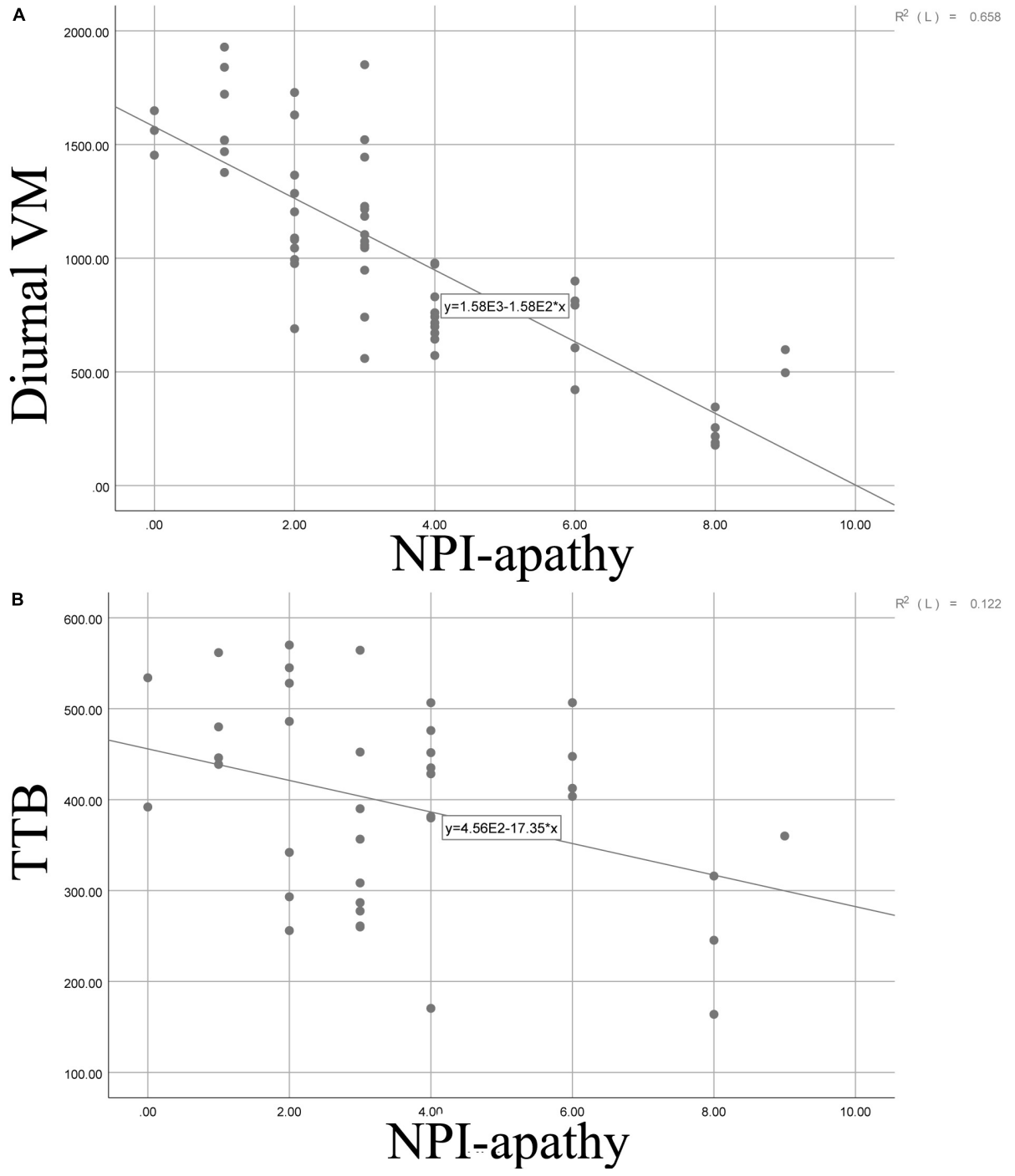

FIGURE 3
Scatter graphs demonstrating the relationship between apathy and diurnal VM **(A)** as well as between apathy and TTB **(B)**. VM, vector magnitude; SE, sleep efficiency; TTB, total time in bed; TST, total sleep time; WASO, wake after sleep onset; TA, times of awakenings; ADA, average duration of awakenings.

Contrary to the findings which revealed that high CSVD burden individuals showed less diurnal VM, all the sleep quality variables did not differ between groups of patients with distinct CSVD burden. This phenomenon is not similar to the findings of Zhào et al. (2021). We considered that these dissimilarities resulted from the sleep evaluation methods we selected. Actigraphy is an objective tool to measure sleep quality, which is different from the subjective questionnaire chosen

by Zhao. Pearson correlation demonstrated that apathy was negatively associated with TTB, which is in accordance with the results from patients with AD (Mulin et al., 2011). Taken together, the close relationship between apathy and diurnal activity, as well as TTB, might imply a potential alteration of the sleep/wake circadian rhythm in CSVD patients with apathy. A previous study indicated that abnormalities in sleep/wake circadian rhythm detected by actigraph devices were associated with a high risk of post-stroke apathy (Cosin et al., 2015). It is quite attractive in recent years that patients with CSVD were found to show disrupted 24-h activity rhythm (Zuurbier et al., 2015; Sommer et al., 2021).

Several limitations of this study need to be mentioned. First, apathy was assessed using NPI-apathy, instead of diagnostic criteria of apathy, due to a lack of consensus criteria for apathy diagnosis in CSVD these years. Second, the sample size was not large. In future studies, we need to collect more patients with CSVD.

In conclusion, patients with CSVD have a high incidence of apathy. Actigraphy is a promising choice to evaluate apathy in patients with CSVD.

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 Academic Ethics Committee of the Biological Sciences Division of PLA General Hospital in Beijing, China. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XC was responsible for statistical analysis. HZ was responsible for manuscript writing. ZL and YD were responsible for data collection. YH was responsible for studying concepts and design. 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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.933958/full#supplementary-material

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The discriminant validity of single-question assessments of subjective cognitive complaints in an Asian older adult population

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Objective: To compare the discriminant validity of three different single-question assessments of subjective cognitive complaints (SCC) for dementia in a community-based older adult population in Singapore.

Methods: Eligible older adults aged ≥60 were recruited into phase I for identifying those who require further assessment using the Abbreviated Mental Test (AMT) and progressive forgetfulness question (PFQ). Participants who failed either tests entered phase II and were administered various single-question assessments of SCC, such as the 8th question on the patient Ascertain Dementia 8 (AD8-8_{pt}), informant AD8 (AD8-8_{info}), and the 10th item on the Geriatric Depression Scale (GDS-10), followed by the Montreal Cognitive Assessment (MoCA) and a formal neuropsychological battery to identify the participant's cognitive status by a research diagnosis and DSM-IV criteria. Differences in characteristics among diagnostic groups were compared. All discriminatory indices (sensitivity, specificity, positive, and negative predictive values, overall accuracy) for these single-question assessments and their combinations with the MoCA were calculated and reported to confirm their discriminant validity in identifying the existence of subjective complaints and objective impairment.

Results: A total of 3,780 participants were assessed at phase I, of which 957 entered and completed phase II. Of whom, 911 were dementia-free and 46 had dementia. The MoCA (13/14) displayed good sensitivity (95.6%), specificity (81.5%), and overall accuracy (82.1%) for dementia detection. The GDS-10 and AD8-8_{pt} showed poor discriminant validity, while the AD8-8_{info} had the highest specificity (83.2%) and the greatest overall accuracy (82.5%) for dementia. Compensatory combination of the AD8-8_{info} with MoCA, the sensitivity and positive predictive values were optimized (100%), while the conjunctive combination of two tools achieved excellent specificity (96.3%) and overall accuracy (94.8%) in discriminating dementia patients.

Conclusion and implications: Combining a reliable single-question SCC assessment with an objective tool can efficiently discriminate dementia patients from healthy older adults in the community.

KEYWORDS

single-question assessment, subjective cognitive complaints, dementia, cognitive screening, discriminant validity

Introduction

Dementia is one of the top causes of death among all diseases. Currently, more than 55 million people live with dementia worldwide and there are nearly 10 million new cases every year (World Health Organization, 2021). The increase in dementia cases has caused a serious challenge for the health system and society. Although it mainly affects older people, it is not an inevitable consequence of aging. Studies have shown that early detection of cognitive impairment prior to the occurrence of dementia could benefit early management and intervention for at-risk older adults, to delay the process of cognitive decline and prevent dementia onset (Livingston et al., 2020). Hence, early screening for dementia in the community is particularly essential.

Subjective cognitive complaints (SCC), also known as subjective cognitive decline, or subjective memory complaints (Rabin et al., 2017), a key sign of preclinical Alzheimer's Disease dementia, refers to a persistent decline in memory and/or other cognitive abilities reported by individuals or informants in the absence of objective neuropsychological evidence (Jessen, 2014). SCC is common among the older adults, and its prevalence increases with advancing age. Subjects with SCC are at a high-risk conversion to mild cognitive impairment and dementia, especially those aged over 75 (Jessen et al., 2020; Slachevsky et al., 2020). While objective cognitive assessments remain the gold standard for assessing cognitive function, which assesses the cognitive performance at a single point in time, such as the Mini-mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), and comprehensive cognitive batteries, SCC assessments can be used to determine the existence of subjective complaints from participants and capture longitudinal cognitive changes (Jessen, 2014). Hence, using a self-reported SCC assessment as an additional tool in a large-scale cognitive screening may be an easy and more cost-beneficial way to identify those at-risk for cognitive decline and dementia (Wasef et al., 2021).

Among all SCC assessments, the use of a single-question format of SCC assessment has been introduced and popularized. There is growing evidence to confirm that SCC, as assessed by single-question tools as well as more comprehensive tools, was associated with an increased risk of cognitive decline and dementia (Jungwirth et al., 2008; Rönnlund et al., 2015; Rabin et al., 2017; Peters et al., 2019). The progressive forgetfulness question (PFQ) was reported a simple but effective in screening for dementia in a primary care setting in Singapore, by ruling out people at lower risk of dementia (Chong et al., 2006). Similarly, the Hypertension in the Very Elderly (HYVET) Trial examined the role of the 10th item on the geriatric depression scale (GDS) "do you feel have more problems with memory than most?" in predicting incident dementia in a hypertensive older population and found that baseline SCC was associated with an increased risk of developing any dementia (Peters et al., 2019). The England and Wales Departments of Health Commissioning for Quality and Innovation (CQUIN) strategy initiative have recommended and implemented the use of a single-question as the first step in the assessment pathway in large-scale dementia screening (Kmietowicz, 2012; Hendry et al., 2014).

Although these studies have highlighted the applicability of such SCC assessments for large-scale use, they did not compare such SCC assessments with gold standard neurocognitive evaluation, hence could not ascertain the discriminant validity of such brief tools for dementia screening purposes. Thus, there is a need for further validation of the single-question assessments of SCC in a large population of community-dwelling older adults.

Hence, the present study aimed to (1) explore the discriminant validity of single-question assessments of SCC for dementia detection in an Asian older adult population; (2) examine whether the combination of single-question SCC assessments with a structured cognitive tool (MoCA) could improve the discriminant indices for dementia detection. We hypothesized that a single-question SCC performed by the participants, or their caregivers can quickly identify those at higher risk of developing dementia and who would benefit more from a detailed cognitive assessment. Secondly, the single-question SCC can improve discriminant indices when used with in combination with the MoCA.

Materials and methods

Study design

The Singapore Epidemiology of Eye Diseases study (SEED) was conducted in multi-ethnic subjects aged 60 years or older living in the community in Singapore. Community residents

of three ethnic groups (Chinese, Malays, and Indians) were recruited from the baseline participant pool by telephone or home visits between 2011 and 2017. The details of the SEED study have been previously reported (Majithia et al., 2021). The SEED study had two phases, with the phase I consisting of a questionnaire administered by trained investigators on the participants' demographic information and relevant risk factors, along with a primary screening of participants' cognitive function using the Abbreviated Mental Test (AMT) and PFQ (Sahadevan et al., 1997). The optimal cut-off of AMT adjusted for education is 6/8 (screen positives were defined as AMT score $\leq$6 among those with $\leq$6 years of formal education, or AMT score $\leq$8 among those with >6 years of formal education), which has been previously validated in Singapore (Sahadevan et al., 2000). The PFQ is a single format question by asking participants or their informants ("Do you/he/she have progressive forgetfulness"), and those who answer YES is considered positive (Chong et al., 2006). Participants who failed on either the AMT or/and PFQ tests were tested positive and hence invited to the second phase of the study. In the phase II, participants underwent a set of single-question SCC assessments, such as GDS-10 and AD8-8, followed by the MoCA and a comprehensive neuropsychological evaluation (Xu et al., 2016a). Details on the SEED study procedures can be found elsewhere (Hilal et al., 2013, 2017; Wong et al., 2019).

Study participants

At phase I, a total of 3,780 individuals completing both the AMT and/or the PFQ assessments, of whom 1,593 were screened positive and 957 underwent comprehensive cognitive and clinical investigations in phase II.

Single-question subjective cognitive complaints assessments

Three single-questions for assessing SCC were used:

Single-question SCC	Respondent	Question
GDS-10[a]	Participants	Do you feel have more problems with memory than most?
AD8-8_{pt}[b]	Participants	Do you have daily problems with thinking and/or memory?
AD8-8_{info}[c]	Main caregivers	Does the participant/patient have daily problems with thinking and/or memory?

[a]GDS-10, the 10th item of Geriatric Depression Scale.
[b]AD8-8_{pt}, the 8th item of patient AD8.
[c]AD8-8_{info}, the 8th item of informant AD8.

Cognitive assessments and dementia diagnosis

Brief and comprehensive cognitive assessments were administered to all participants in phase II. The MoCA was performed, followed by a formal neuropsychological battery (the vascular dementia battery, VDB) (Narasimhalu et al., 2011; Xu et al., 2016b), which was locally validated for Singaporean older adults (Hilal et al., 2013). Dementia was diagnosed according to the DSM-IV criteria (American Psychiatric Association, 1994), by consensus at formal meetings of the research team.

Statistical analyses

Demographic characteristics and cognitive outcomes were presented as mean $\pm$ SD, number with/without number of cases (%) as appropriate. One-way ANOVA and chi-square tests were used to compare differences of sample characteristics by the cognitive outcome. Furthermore, Bonferroni correction was applied for multiple comparisons between groups, and that a p-value of <0.016 was considered statistically significant.

In addition to sensitivity and specificity, discriminant validity of the tools was estimated by positive predictive value (PPV) and negative predictive value (NPV). PPV and NPV are defined as a proportion of people with a positive/negative result who actually have/do not have the disease. A higher PPV in the observed population signifies less false positives and a higher NPV will have small number of false negatives (Chu, 1999; Stojanovic et al., 2014).

$$\text{Sensitivity} = \text{True Positive}/(\text{True Positive} + \text{False Negative})$$

$$\text{Specificity} = \text{True Negative}/(\text{True Negative} + \text{False Positive})$$

$$\text{PPV} = \text{True Positive}/(\text{True Positive} + \text{False Positive})$$

$$\text{NPV} = \text{True Negative}/(\text{True Negative} + \text{False Negative})$$

$$\text{Accuracy} = (\text{True Positive} + \text{True Negative})/(\text{True Positive} + \text{False Positive} + \text{True Negative} + \text{False Negative})$$

The discriminant indices of dementia were calculated for GDS-10, AD8-8_{pt}, AD8-8_{info}, and MoCA (using the optimal cut-off points) were calculated using the above formula separately. The Pearson correlation coefficient and Cohen's kappa coefficient were calculated for different single-question SCC tools. Compensatory and conjunctive combinations of the single-question SCC with MoCA were employed to determine if combination approaches would enhance the discriminatory values over MoCA alone. Compensatory combination requires either test to be positive, whereas conjunctive combination requires both tests to be positive. Compensatory combination

generally improves sensitivity whereas conjunctive combination generally improves specificity (Kan et al., 2019).

All analyses were done on IBM SPSS.26.0, and a p-value < 0.001 was considered statistically significant.

Results

Demographic data

Figure 1 shows the study recruitment flow chart. A total of 957 participants were included in the final analysis, 46 (4.8%) were diagnosed with dementia. Compared with those who were diagnosed as dementia-free subjects, those who were diagnosed as dementia were older (mean age 78.8 year vs. 69.8 year), more often female (78.3% vs. 50.4%), have lower education levels (91.3% vs. 61.0%), and that these differences were statistically significant. We also find a notable difference in ethnicity between the two groups. Sample characteristics at phase II are shown in Table 1.

Discriminant validity of single-question subjective cognitive complaints

Table 2 summarizes all discriminant indices of the different single-question SCC tools and MoCA for detecting dementia. Results showed that the GDS-10 and AD8-8_{pt} had low sensitivity and moderate specificity, while the AD8-8_{info} had the highest specificity (83.2%) and the greatest overall accuracy (82.5%), although all SCC questions showed a high NPV (>95%) and low PPV (<20%). At an optimal cut-off of 13/14, MoCA displayed good sensitivity (95.6%) and specificity (81.5%). All three single-question SCC tools have poor agreement among each other (Supplementary Tables 1, 2). There was a statistically significant difference in the proportion of endorsement on the three SCC questions, whereas the AD8_{pt} has more endorsement than the other tools (Supplementary Figure 1).

Combined utility of the Montreal cognitive assessment and single-question subjective cognitive complaints tools for detecting dementia

Subsequently, we explored whether the combination of the MoCA with another single-question SCC tool can improve the discriminant indices. The compensatory combination of MoCA and AD8-8_{info} reached an optimal sensitivity and PPV. The specificity of MoCA can be increased to 96.3% and 96.2% by combining with the AD8-8_{info} and GDS-10 in a conjunctive manner, respectively. Also, the overall accuracy of this conjunctive combination was improved to 94.8% and 93.6% (Table 3).

Discussion

In this study, we found that the discriminant indices of all single-question SCC assessments, such as the GDS-10, AD8-8_{pt}, and AD8-8_{info}, were inferior to the MoCA for dementia detection. However, combining an SCC assessment (AD8-8_{info}) with an objective tool (MoCA) maximized the discriminant validity of dementia detection.

Our study showed that, although feasible, using a single-question of SCC itself does not yield optimal discriminant validity for dementia screening, which is consistent with other studies (Eichler et al., 2015). The finding from our present study also reaffirms the importance of objective cognitive assessments in dementia screening, as most older people are unable to make an accurate assessment of their cognitive performance, even though SCC has some predictive value. Moreover, some participants with depressive symptoms may exaggerate their subjective memory complaints but not have objective cognitive decline, which can lead to false positive results (Brailean et al., 2019). Therefore, using such a single-question SCC assessment alone may be difficult to achieve case detection in large-scale screening, especially in community populations.

We found that using a combination of objective tools with a single-question SCC can maximize sensitivity (compensatory) and specificity (conjunctive). The optimal cut-off of the full version of MoCA (13/14) for dementia detection in our study is indeed lower than other reports, but was consistent with the previous studies in Singapore, possibly due to the generally low level of education in the Asian population (62.5% with 0–6 years of education) (Chan et al., 2015; Phua et al., 2018; Kan et al., 2019). When combined in a compensatory manner, the AD8-8_{info} with MoCA, the sensitivity, and PPV is optimized (100% in our study), which allows the inclusion of as many patients as possible in a large-scale dementia screening. In addition, the conjunctive approach of the AD8-8_{info} with MoCA showed improvement in specificity (96.3%) and overall accuracy (94.8%), which helps to narrow the screening pool and exclude as many healthy people as possible, while also saving time and human resources. From the findings of the present study, the use of a single-question SCC tool should be used in combination with an objective cognitive assessment test in large-scale dementia screening in the community.

Evidence showed that a combination approach can improve the utility of cognitive tests in dementia screening. According to that the priority of the two combination strategies, a compensatory combination is capable to enhance the overall sensitivity, while a conjunctive combination may improve

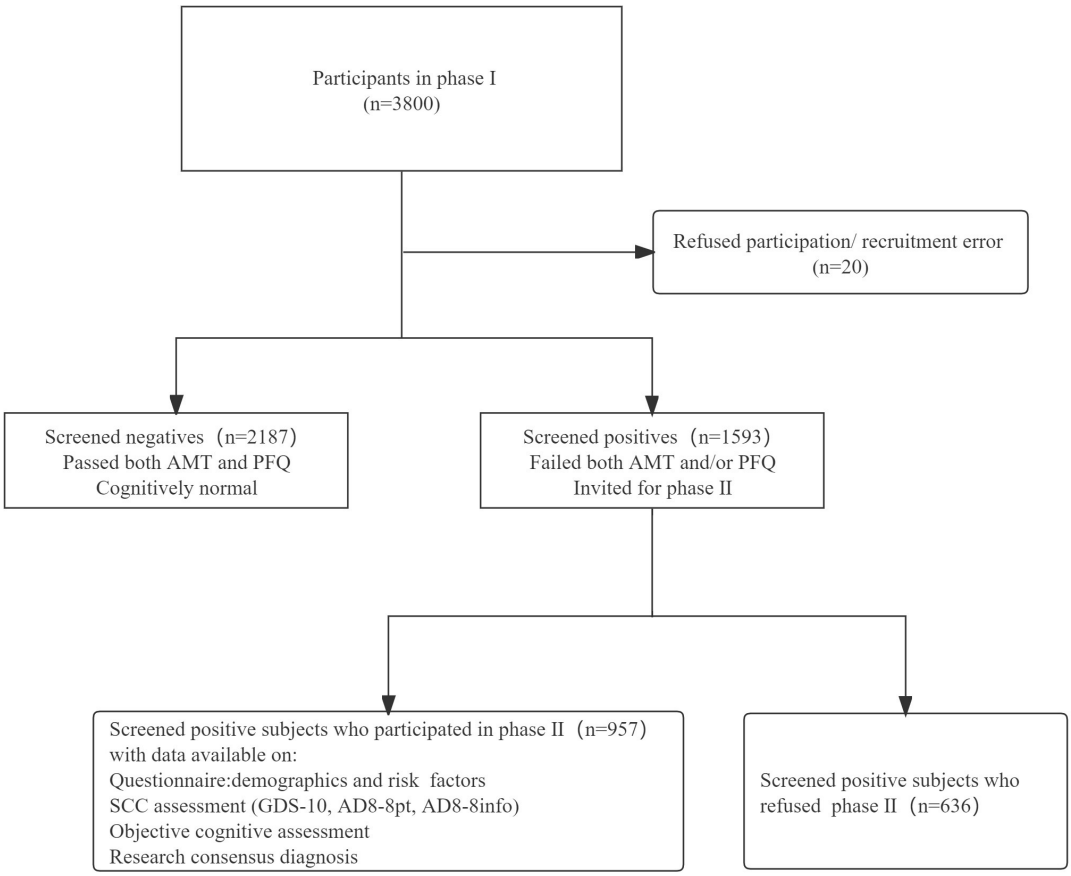

FIGURE 1
Study recruitment.

PPV (Chan et al., 2016). Combination strategies were usually based on specific research settings and objectives. In clinical settings, where patients were referred from somewhere else due to memory complaints may benefit from a compensatory combination to optimize screening sensitivity. However, in the community setting, the conjunctive combination approach may be preferred to achieve better PPV and reduce false positives. This approach will also help facilitate and reserve resources in community healthcare systems, where screening infrastructure and resource is scarce (Iliffe et al., 2009). Meanwhile, before the structured objective cognitive assessment, adding a single-question SCC assessment can establish a good relationship with the participants and relieve their tension.

We found that the discriminant indices of AD8-8_{info} were superior to other SCC assessments, including AD8-8_{pt}. Moreover, we can see that using the 8th item of AD8, 67.5% of informants reported memory problems with their study partners among dementia participants, while only 39.5% of patients self-reported subjective memory problems. Similarly, in the dementia-free group, the proportion of informants who correctly reported no memory decline was slightly higher than that of participants (83.3% vs. 76.6%). This result is consistent with the previous studies which showed asking informants are more reliable than subjects, particularly noticeable among patients with dementia (Yim et al., 2017; Kan et al., 2019). It could be that dementia is a progressive neurodegenerative disease; many old people do not have an accurate assessment of their cognitive abilities, especially those who have already shown symptoms of cognitive decline. In contrast, informants can capture such progressive changes because of regular interaction with the subject. Meanwhile, the informant confirmation is a key feature of clinical cognitive decline and might be a better predictor of objective performance as disease severity progresses (Morrison et al., 2022). Although such informant-based tools may be affected by individual differences of caregivers, such as familiarity between caregivers and subjects, reliability of answers, etc. These problems can be well solved by combining them with objective cognitive tools.

It should also be mentioned that the cognitive changes observed by various SCC tools are different. In terms of the implications of the SCC questions, the GDS-10 asks about

TABLE 1 Sample characteristics at Phase II.

Characteristics	Dementia ($n = 46$)	Dementia-free ($n = 911$)	Total ($n = 957$)	*P*-value
Age (mean, SD)	78.8 ± 5.6	69.8 ± 6.4	70.2 ± 6.6	<0.001
Gender, female (*n*%)	36 (78.3)	459 (50.4)	495 (51.7)	<0.001
Education, 0–6 years (*n*%)	42 (91.3)	556 (61.0)	598 (62.5)	–
Ethnicity	–	–	–	0.001*
Chinese (*n*%)	7 (15.2)	293 (32.2)	300 (31.3)	–
Malay (*n*%)	27 (58.7)	296 (32.5)	323 (33.8)	–
Indian (*n*%)	12 (26.1)	322 (35.3)	334 (34.9)	–
GDS-10[a] (yes, *n*%)	18 (40.9)	155 (17.0)	173 (18.6)	<0.001
AD8-8_{pt}[b] (yes, *n*%)	15 (39.5)	194 (23.4)	209 (24.1)	<0.001
AD8-8_{info}[c] (yes, *n*%)	27 (67.5)	123 (16.7)	150 (19.3)	<0.001
MoCA[d] (mean, SD)	8.3 ± 4.1	19.4 ± 5.1	18.8 ± 5.6	<0.001

[a]GDS-10, the 10th item of Geriatric Depression Scale.
[b]AD8-8_{pt}, the 8th item of patient AD8.
[c]AD8-8_{info}, the 8th item of informant AD8.
[d]MoCA, the Montreal Cognitive Assessment.
*The differences are significant after Bonferroni correction.

TABLE 2 Discriminant indices of the different tools for detecting dementia.

Brief tools	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	No of cases correctly identified	No of healthy subjects correctly identified	Overall Accuracy (%)
GDS-10[a]	40.9	82.9	10.4	96.7	18/44	756/911	80.0
AD8-8_{pt}[b]	41.7	76.5	7.2	96.8	15/36	631/825	75.0
AD8-8_{info}[c]	69.2	83.2	18.0	98.1	27/39	609/732	82.5
MoCA[d] (cut-off: 13/14)	95.6	81.5	20.3	99.7	43/45	742/911	82.1

[a]GDS-10, the 10th item of Geriatric Depression Scale.
[b]AD8-8_{pt}, the 8th item of patient AD8.
[c]AD8-8_{info}, the 8th item of informant AD8.
[d]MoCA, the Montreal Cognitive Assessment.

TABLE 3 Discriminant indices of the combination of single-question subjective cognitive complaints (SCC) with Montreal cognitive assessment (MoCA) for detecting dementia.

Combination of brief tools	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	No of cases correctly identified	No of healthy subjects correctly identified	Overall accuracy (%)
MoCA[a] or GDS-10[b]	95.6	68.3	13.0	99.7	43/45	622/911	69.6
MoCA and GDS-10	40.9	96.2	34.0	97.1	18/44	876/911	93.6
MoCA or AD8-8_{pt}[c]	97.8	63.4	12.4	99.8	44/45	536/846	65.1
MoCA and AD8-8_{pt}	36.8	94.1	20.9	97.2	14/38	840/893	91.7
MoCA or AD8-8_{info}[d]	100	66.2	14.8	100	45/45	508/767	68.1
MoCA and AD8-8_{info}	62.5	96.3	43.1	98.3	25/40	849/882	94.8

[a]MoCA, the Montreal Cognitive Assessment.
[b]GDS-10, the 10th item of Geriatric Depression Scale.
[c]AD8-8_{pt}, the 8th item of patient AD8.
[d]AD8-8_{info}, the 8th item of informant AD8.

one's memory problems compared to most people, which is a "cross-sectional" comparison; while the AD8-8 asks about changes in memory over recent years, which is a longitudinal comparison. Hence, during implementation phase, the SCC assessments need to be carefully selected according to the type of interviewees (Diaz-Galvan et al., 2021; Morrison et al., 2022).

The strength of this study is that it is a community-based research design and has a large sample size covering

different ethnic groups in Singapore. The second strength was the inclusion of multiple single-question SCC assessments in the present study which enabled the comparison among varying tools. The third strength was the use of a comprehensive objective cognitive assessment which provided the diagnosis of a spectrum of cognitive function in the present study.

Several limitations require acknowledgment. First, the gold standard diagnosis of dementia was only administered in a subset of individuals who were screened positive in phase I, which may have result an underestimation of the prevalence of cognitive impairment. Also, due to the small number of dementia cases, we did not perform further studies on the subtypes of dementia. Future studies could target at preclinical stages of dementia, as well as different dementia subtypes. Secondly, our study was conducted in a community-based population in Singapore, the proportion of people who refused to participate in a comprehensive cognitive assessment was high (39.9%), hence more prone to selection bias. Besides, as the present study was conducted in the community, although the prevalence of dementia was consistent with other literature, the generally lower prevalence of dementia may have affected the estimation of PPV and NPV of cognitive screening tools (Chu, 1999). Future studies could further adjust the actual predictive values of these SCC tools according to the census data.

Conclusion

This study demonstrated that using a combination of objective tools with the 8th question on the informant AD8 as a single-question SCC measure can maximize discriminant capacity for dementia detection in the community. Future studies are warranted to examine if single-question SCC measures can predict pathology-related cognitive changes among older adults at-risk of dementia.

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 the Singapore Epidemiology of Eye Diseases study (SEED) was approved by the National Healthcare Group Domain Specific Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XX and CC designed the study, developed the protocol, and obtained the ethics of this study. TP and XZ performed the data analysis and wrote the manuscript. XH, CK, NV, CC, and CY revised the manuscript. All authors approved of the final version of this 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.2022.901592/full#supplementary-material

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Targeting autonomic nervous system as a biomarker of well-ageing in the prevention of stroke

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Stroke prediction is a key health issue for preventive medicine. Atrial fibrillation (AF) detection is well established and the importance of obstructive sleep apneas (OSA) has emerged in recent years. Although autonomic nervous system (ANS) appears strongly implicated in stroke occurrence, this factor is more rarely considered. However, the consequences of decreased parasympathetic activity explored in large cohort studies through measurement of ANS activity indicate that an ability to improve its activity level and equilibrium may prevent stroke. In support of these observations, a compensatory neurostimulation has already proved beneficial on endothelium function. The available data on stroke predictions from ANS is based on many long-term stroke cohorts. These data underline the need of repeated ANS evaluation for the general population, in a medical environment, and remotely by emerging telemedicine digital tools. This would help uncovering the reasons behind the ANS imbalance that would need to be medically adjusted to decrease the risk of stroke. This ANS unbalance help to draw attention on clinical or non-clinical evidence, disclosing the vascular risk, as ANS activity integrates the cumulated risk from many factors of which most are modifiable, such as metabolic inadaptation in diabetes and obesity, sleep ventilatory disorders, hypertension, inflammation, and lack of physical activity. Treating these factors may determine ANS recovery through the appropriate management of these conditions. Natural aging also decreases ANS activity. ANS recovery will decrease global circulating inflammation, which will reinforce endothelial function and thus protect the vessels and the associated organs. ANS is the whistle-blower of vascular risk and the actor of vascular health. Such as, ANS should be regularly checked to help

draw attention on vascular risk and help follow the improvements in response to our interventions. While today prediction of stroke relies on classical cardiovascular risk factors, adding autonomic biomarkers as HRV parameters may significantly increase the prediction of stroke.

KEYWORDS

aging, longevity, parasympathetic activity, stroke, ANS activity, epidemiology, neuroendothelial disease, inflammation

Epidemiology of stroke and autonomic nervous system

The World Health Organization (WHO) describes stroke as a pandemic. From 1990 to 2010 the world burden of stroke increased significantly with regard to the recent increase in the numbers of patients, of deaths (20% increase), and of disability-adjusted life years (DALY; 16% increase) (Krishnamurthi et al., 2013). According to the WHO, the estimated incidence of stroke events in Europe is likely to increase from 1.1 million per year in 2000 to more than 1.5 million per year in 2025 in relation to demographic changes (Truelsen et al., 2006). Worldwide, the number of deaths due to stroke was 4.4 million in 1990 (Murray and Lopez, 1997). This prevalence is underestimated as many strokes do not exhibit clinical evidence and remain unnoticed; indeed, systematic autopsies of large populations disclosed a 12.9% pathological evidence of cerebral infarction, although any clinical sign of stroke had ever been experienced by these patients who died from other diseases (Shinkawa et al., 1995). Many studies also reported previous silent strokes in those patients investigated for acute stroke as in the Framingham (Kase et al., 1989) and the Copenhagen (Jorgensen et al., 1994) cohorts. In the Veterans Affairs Cooperative Study, 14.7% of cerebral scans showed previous silent brain infarctions (Hénon et al., 1995). This percentage reached 38.3% in the SEPIVAC survey (Ricci et al., 1993).

Death from stroke is not the only societal drama as 40% of survivors demonstrate severe sequelae, and 30% fall in depression in the year following a stroke. Dementia is a frequent evolution. In developed countries, each case from stroke occurrence to death costs almost $80,000 US or €70,000, with an annual amount of more than $100 billion US for the United States only (Feigin et al., 2014); The incidence may be largely under evaluated as there is approximately 15 silent stroke for one clinically patent stroke (Leary and Saver, 2003).

Autonomic nervous system activity and traditional risk factors are strongly related as ANS imbalance contributes to the creation of a pre-pathological milieu of composite risk factors, such as hypertension, diabetes or atrial fibrillation and alterations of endothelial homeostasis in favor of pro-thrombotic/proinflammatory state, eventually leading to increased risk of stroke (Saeed et al., 2005; Bäck et al., 2019; Carandina et al., 2021). Thus ANS monitoring is of importance in stroke prevention focusing on the imbalance characterized by a decrease in parasympathetic activity and a concurrent increase in sympathetic activity.

As ANS monitoring is most often based on mathematical analysis of successive ECG sinusal intervals (RR intervals), those which are neuro-controlled, subjects suffering from arrhythmias are often excluded from the studies. As a matter of fact, antiarrhythmic drugs alter significantly the HRV measurements, according to their class (Zuanetti et al., 1991).

Autonomic nervous system and longevity

Autonomic nervous system parasympathetic outflow is a recognized predictor of longevity in centenarians, ANS decrease with aging being considered as a natural decrease of allostatic systems (Hernández-Vicente et al., 2020). In the general population, three longitudinal studies underlined the predictive value of decreased ANS activity for death, mainly cardiovascular, namely the ARIC (Atherosclerosis Risk in Communities), the ZUTPHEN and the FRAMINGHAM studies (Tsuji et al., 1996; Dekker et al., 1997, 2000). The same predictive value was attributed to a decrease in ANS after stroke of myocardial infarction (Kleiger et al., 1987). The ultracentenarians over 100 years of age have significantly higher parasympathetic activity than the elderly subjects from 81 to 100 years of age and the parasympathetic predominance may be the neuroautonomic feature that helps to protect ultra-centenarians against cardiovascular disease (Piccirillo et al., 1998). In animal models, increased sympathetic activity and decreased parasympathetic activity (Vanoli et al., 1991) are associated with a higher risk of sudden cardiac death (Kleiger et al., 1987; Bigger et al., 1993; Tsuji et al., 1996; Chen and Tan, 2007). Specifically, reduced 24-h HRV is independently associated with increased risk of myocardial infarction, congestive heart failure, death from cardiovascular

disease, and total mortality (Kleiger et al., 1987; Bigger et al., 1993; Tsuji et al., 1996). Experimental evidence also suggests that the autonomic nervous system is implicated in the development of vascular atheroma and occlusion (Hamaad et al., 2004). On the other side, cholinergic stimulation protects endothelium by blocking endothelium cells activation and leukocyte recruitment during inflammation (Saeed et al., 2005). This opens a promising area for future research in the effects of common non-cardioactive drugs (Nicolini et al., 2012). In the cardiovascular health study (CHS), greater total leisure-time activity, walking distance, and walking pace were each associated with more favorable HRV indices, supporting cardiovascular benefits (Soares-Miranda et al., 2014).

Autonomic nervous system, an easy tool

Stress is the response of the organism to the interoceptive and exteroceptive changes, i.e., to any biological, psychological, or environmental context change in daily life. The stress is most often beneficial but may become deleterious if solicited intensively or for a too long period of time. The regulation from ANS is immediate and even adapts to the perceived risk as well as to the perspective of future risks. This may even alter transgenerational epigenetical modulation of stress (Babenko et al., 2015).

Among the many adaptative changes, the stress mechanism activates the sympathetic nervous activity (ANS), with a mirrored decreased in parasympathetic nervous activity (PNS). This resultant prevailing sympathetic activity sets a multitude of negative side effects, particularly at the vascular level. There is also a biological cost to activate the biological regeneration through mechanisms including the sleep, vagal tone and tissue regeneration (Canini, 2019).

Stress response is traditionally considered to represent the *fly or fight* response to a significant external variation condition. Today, stress frequently deals with more common changes, such as hunger, thirst, temperature changes, walking, sleeping, speaking, eating, meeting people, listening noise or music, getting up or lying. All these actions require an adaptative answer of ANS to keep the biological equilibrium in spite of an unusual context. In that view, the stress response is present at many occasions and accompanies also many classical cardiovascular risk factors.

Several methods are available to measure ANS activity. Invasive methods include biological catecholamine measurements or direct nerve sympathetic activity measurement using microneurography (Wallin and Sundlöf, 1979). Although providing precise measures, these techniques do not measure parasympathetic activity and cannot be applied to large populations.

Fortunately, ANS activity can be also non-invasively investigated giving access to both the sympathetic and parasympathetic arms of ANS activity. The importance of heart rate behavior in response to exercise is illustrated by a predictive value for sudden death (Jouven et al., 2005). Even the notion of a relationship between longevity and heart rate as a simple measurement of ANS activity, has been raised (Jensen, 2019). Quantification of heart rate variability (HRV) provides more precise measurements than simple heart rate values. REF any change in ANS activity is reflected by HRV due to the very rich ANS innervation of the heart. In that view, the heart is an open window on the neurological regulatory ANS activity. The lack of variability of RR intervals means that there is no ANS activity to regulate heart rate, and often, no ANS activity at all. It means neuronal inactivity. This total lack of variability can be observed when a cerebral death has occurred in ICU, where heart rate becomes absolutely regular (Rapenne et al., 2000).

Analysis of heart rate changes allows quantifying ANS activity. Rapid vanishing changes in RR interval length are induced by the parasympathetic drive, due to the rapid elimination of its neurohormone, acetylcholine. These variations are called high frequency (HF) variations and thus represent the parasympathetic drive. Slower changes of RR intervals length are due to slower changes in catecholamine concentrations and are thus called low frequency (LF), they roughly represent the sympathetic drive which is while this LF frequency band also represents some parasympathetic activity (Brown et al., 1993; Lorenzi-Filho et al., 1999; Pichot et al., 1999).

Only the normal RR intervals are taken into account in HRV measurements, as their length is modulated by the autonomic nervous system, which is not the case for intervals linked to ectopic beats which are due to local reentry or local enhanced automaticity. The HRV evaluation implies first a reading of Holter recordings in order to label beats as normal (N), ventricular ectopic beats (V or VE), or supraventricular ectopic beats (S or SVE). This reading also gives access to the length of each normal to normal (NN) beat. HRV is then based on mathematical calculations on these consecutive NN intervals. Once the set of RR (NN) intervals is established, two classical mathematical approach to quantify the autonomic nervous system activity which modulate the NN length variations, namely the temporal and the frequential approaches (Task Force, 1996).

The temporal approach is based on simple standard deviations (SD) of these NN intervals, usually calculated on 24-h periods. The way the SD will be calculated will give several results. The first result is global autonomic activity calculating the SD of all RR (NN) intervals of the set, and it is called SDNN. Then, to take into account fast RR length variations from one RR interval from the previous, it is calculated the SD of the root mean square of the mean of the successive differences of consecutive RR (NN) intervals squared, giving a representation of the parasympathetic activity. It is called RMSSD. Referring to

the sympathetic activity, the SD of RR (NN) intervals are first calculated on consecutive 5 min intervals, the mean of these SD is thus appropriate to reflect sympathetic HRV changes which are observed on longer periods than parasympathetic ones. It is called SDNNIDX. One last measurement which differs slightly from these SD and means, is the percentage of RR intervals differing for more than 50 ms from the previous one. It is a parasympathetic parameter. It is called PNN50.

The second set of mathematical approach is based on the analysis of cyclical variations of RR (NN) as a sinusoidal signal. This approach is most often performed through a Fourier Transform (FT), which identifies regular repetitive patterns that can be described as sinusoids. This is a general law that is applied to many periodic signals including physiological signals. It is a new mathematical space where signal modulations can be characterized by a frequency and amplitude modulation. Entering the Fourier space allows thus to quantify repetitive fast changes by fast sinusoids and repetitive slow changes by slow sinusoids. In RR length modulation physiology, the slow and fast changes are included in bounds calculated using pharmacological blocking of sympathetic and parasympathetic activities (Pomeranz et al., 1985). These bounds are 0.04 – 0.15 Hz for the low frequencies (LF) representing the sympathetic activity and 0.15 – 0.40 Hz for the high frequencies (HF) representing the parasympathetic activity. This gives also the possibility to calculate a ratio between the sympathetic activity and the parasympathetic activity, the ratio LF/HF, which represents the sympathetic predominance.

Indeed, while the representativity of parasympathetic activity by the HF band of Fourier transform is widely accepted, the representativity of sympathetic activity by the LF component is slightly more questionable since the LF band represents both parasympathetic and sympathetic activities, and does not correlate well with the MSNA activity, this latter being the reference value for sympathetic activity (Saul et al., 1990). The values should also be assessed against total spectral power (Ptot). HF and LF values are thus better assessed when compared to each other, and their ratio LF/HF is a good indicator of ANS imbalance (Pagani et al., 1984), when used in normalized units as well, although this last index remains questioned (Billman, 2013). There is also significant respiratory influences on HRV that affect not only the HF component, but could affect LF as well, depending on the breathing pattern (Brown et al., 1993; Lorenzi-Filho et al., 1999).

These temporal and frequential methods share a limitation in averaging ANS activity on the period analyzed, thus missing transitions in ANS modulation. A first answer is to analyze separately diurnal and nocturnal values. The nocturnal values are of particular interest as they are independent of most environmental stimuli and thus better represent the ability of ANS to activate the parasympathetic drive.

It is also possible to pick up transitional values of ANS activity using a specific frequential measurement based on wavelets analysis (Pichot et al., 1999, 2016). This allows to go beyond the limitation of stationarity needed for temporal and frequential methods. Stationarity is rather difficult to obtain but this status benefits from a general tolerance. Wavelet analysis is of particular interest in experimental conditions such as drug assessment, autonomic answer to physical exercise, or stress evaluation under any environmental changes. The wavelet transform (WT) presents other advantages over the FT. First, the shape of the analyzing wavelet can be freely chosen, and thus is not limited to the sinusoid shape of the Fourier Transform, owing to more accurate measurements. Specific analyzing shapes of interest for EKG were published by Daubechies (1992). The second advantage of WT is the localization of the fitting between the analyzing shape and the analyzed signal, allowed by displacing the analyzing signal along the analyzed signal and thus identifying precisely the time where the change occurs (**Figure 1**).

Beyond these so-called linear approach of HRV, representing its complexity, non-linear approach gained a great interest (Acharya et al., 2004; Stein et al., 2005; Maestri et al., 2007). In fact, RR interval series demonstrating identical statistical linear properties (mean and SD) and power spectra can differ profoundly in terms of the "fine texture" of the rhythm (Nicolini et al., 2012).

These variables are the Poincaré plot (Kamen et al., 1996), the fractal analysis (Peng et al., 1995), the entropy, heart rate turbulence (Bauer et al., 2008), deceleration and acceleration capacities (Bauer et al., 2006), empirical mode decomposition (Balocchi et al., 2004), largest Lyapunov exponent (Wolf et al., 1985), symbolic dynamics (Porta et al., 2001), and empirical mode decomposition (Balocchi et al., 2004), among others. The readers interested may find some details in a dedicated paper from Pichot (Pichot et al., 2016). Unfortunately, these promising variables were seldom chosen due to the lack of available software before the publication of Pichot's software, HRVanalysis (Pichot et al., 2016). Fortunately, we hope that past data may be reanalyzed using this software. New biomarkers gain a significant place as predictors of stroke as they did in postmyocardial infarction (Stein et al., 2005). Data about HRV in the elderly may be corrected to take into account ECG fragmentation, which may artificially increase variability in that population (Costa et al., 2017).

Spontaneous baroreflex (BRS) measurement is another non-invasive approach to ANS measurement. Smooth muscle in arteries, arterioles, and veins and pericytes in capillaries receive a rich autonomic innervation. The baroreflex measures the parasympathetic response to variations in blood pressure. The test is performed by analyzing the increase or decrease in blood pressure and the corresponding lengthening or shortening response of the following interval RR. This measurement is made possible without pharmaceutical administration due the spontaneous permanent change in systolic blood pressure (SBP) from one beat to the following. To do that, the ECG and blood

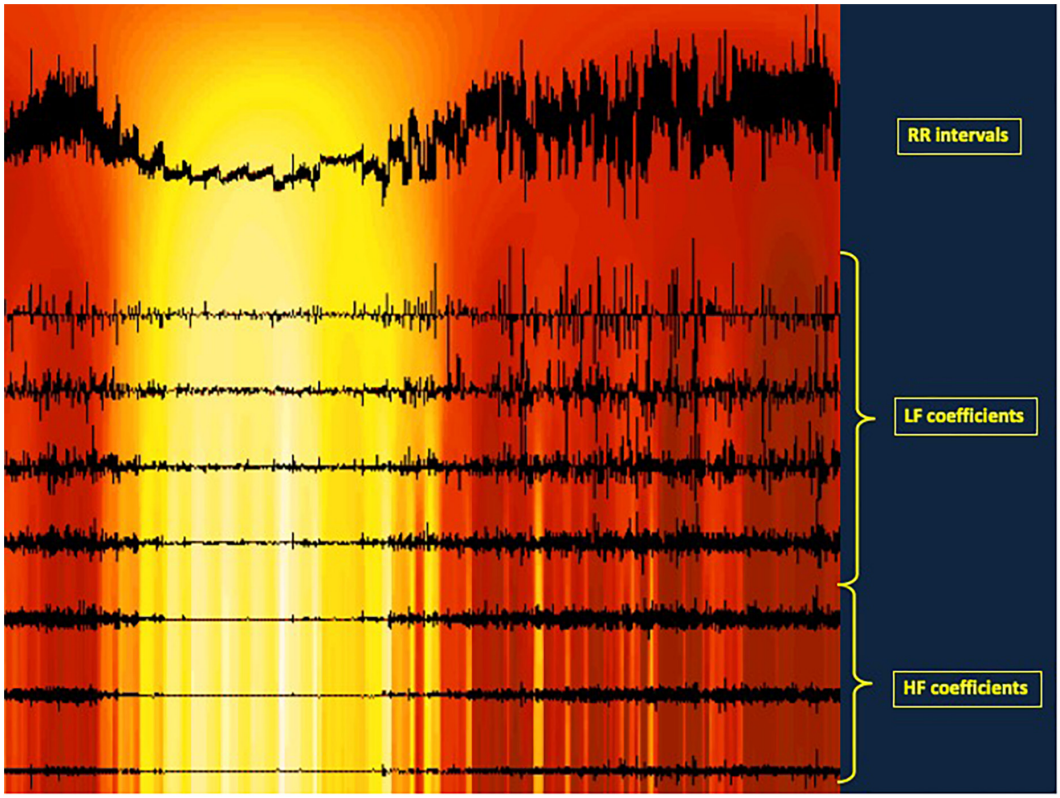

FIGURE 1
Illustration of a wavelet analysis for HRV. The upper trace represents the RR intervals length. The seven horirontal lines illustrates the autonomic nervous system activity, the three lowest lines representing he parasympathetic activity, the four upper lines the sympathetic activity. On each horizontal line, a vertical line is traced each time a parasympathetic or a sympathetic activity is detected, i.e., each time the RR length changes at a fast time (HF) or at a slower pace (LF). The additions of these coefficients give the total parasympathetic and sympathetic activity, respectively. In addition, in that experiment, a transient profound artificial aging was induced as a result of intravenous atropine administration at 20-min intervals. That administration shortens progressively th RR intervals. At the same time, the autonomic coefficients representing heart rate variability decrease then disappear. Then they reappear also progressively after the last atropine administration. This illustrates how a wavelet analysis of HRV can measure both the quatitative variations of parasympathetic and sympathetic activity by summing the coefficients along the period analyzed, but can also localize HRV change along the time. The colored background illustrates the ANS activity along the time from high activity in red to low activity in yellow.

pressure have to be measured simultaneously to belong to the same time frame. The non-invasive blood pressure is measured continuously non-invasively via fast digital balloon counter pressure (Peňáz, 1973). The interest in baroreflex measurement comes from its strong representation of parasympathetic activity as the measure checks at the same time the quality of the sensor of blood pressure located in the carotid body, and the ANS neuronal complete circuitry going from the sensor to the target, here from the carotid to the brainstem then to the heart (Seravalle et al., 2015). Arterial baroreceptors in the carotid sinuses and aortic arch sense pressure changes, and in response to an increase blood pressure they inhibit efferent sympathetic neurons leading to vasodilatation, and they influence the heart rate. They also decrease renin by limiting sympathetic renal outflow (Kaufmann et al., 2020).

Another approach is to evaluate blood pressure variability (BPV). As for HRV, high frequency and low frequency are very different. High frequency means fine adaptation, pulse to pulse, while low frequency means late adaptation trying to catch up the physiological target, but so late that an excessive adaptation is the rule. What is not continuously and finely adjusted will need later stronger corrections, hence the difference meaning of high frequency signing a permanent fine adaptation and low frequency signing a late adjustment.

Arterial stiffness is another approach of ANS activity. This is used on short term variations as a marker of sleep apnea by measuring the pulse transit time (Contal et al., 2013). On the long term, parameters of arterial stiffness, pulse pressure and its other components, are strong predictors of mortality and cardiovascular outcomes (Gavish et al., 2022). Arterial stiffness takes into account vascular aging and thus endothelium health which depends on ANS activity (Abboud, 2010).

Normal values for ANS measurements are scarce. Guidelines for measurement have been published, as well

as some normal values (Shaffer and Ginsberg, 2017), of which nocturnal values are given from the Hypnolaus study (Berger et al., 2022). Various aspects of HRV were explored as short term values (Lee et al., 2018), values in women (Kesek et al., 2009), values in periodic leg movements (Sforza et al., 2005), in patients suffering OSA (Qin et al., 2021), and in those presenting with metabolic syndrome (Assoumou et al., 2012a).

Free software are today available, making possible calculation of HRV from almost any recording source with any available method (Pichot et al., 2016). This illustrates an emerging facilitation of the implication of ANS in cardiovascular risk assessment.

Autonomic nervous system as a predictor for stroke occurrence

While today's prediction of stroke risk relies on classical cardiovascular risk factors, adding autonomic biomarkers may significantly increase the prediction of stroke (Bodapati et al., 2017). The association of two HRV parameters significantly increased the predictive power from 0.61 for the CHS-score (Cardiovascular Health Study clinical stroke risk score, Stein et al., 2008) alone to up to 0.68 ($p = 0.02$). The implication of ANS unbalance in stroke occurrence in the general population is supported by the Framingham cohort. Specifically, a reduced 2-h HRV was independently associated with increased risk of myocardial infarction, congestive heart failure, death from cardiac and cerebral diseases, and total mortality (Kleiger et al., 1987; Bigger et al., 1993; Tsuji et al., 1996). Due to its short-term and long-term neurological interaction with vessels, ANS activity presents a strong relationship with cardiovascular diseases, including stroke. Since many risk factors are shared for MI and for stroke occurrences, the prediction brought through ANS imbalance predicts both vascular diseases as established in the Framingham cohort (Kleiger et al., 1987; Bigger et al., 1993; Tsuji et al., 1996). It should be emphasized that the term *cardiovascular* disease may be misleading as it focuses our attention on the heart disease while it downplays the *cerebrovascular* disease. Both terms should be associated to avoid this shadowing effect.

This makes of ANS imbalance a key factor of stroke occurrence. In The Framingham Heart Study, a 1 SD decrement in autonomic activity, as assessed by the biomarker HVR low-frequency power (LF, natural log transformed), was associated with 1.70 times greater hazard for all-cause mortality, including stroke. It is of note that the Holter recordings were obtained from 2-h diurnal recordings during routine examination in a subset of 1082 subjects (Tsuji et al., 1994). In a stepwise analysis including classical cardiovascular risk factors, SNA variable was the first to enter the model. The authors concluded that the estimation of HRV by ambulatory monitoring offers prognostic information beyond that provided by the evaluation of traditional risk factors (Tsuji et al., 1994). Thus HRV appears as a general cardiovascular (CV) risk marker, and that an individual has the age of his/her ANS activity (Tsuji et al., 1994). Recently, the Framingham Offspring cohort third addressed more specifically dementia and stroke prediction. Dementia was predicted by SDNN [HR (Hazard Ratio) per 1 SD, 0.61] and RMSSD (HR per 1 SD, 0.34). High resting heart rate was associated with increased stroke risk (HR per 10 bpm, 1.18).

Normal SDNN values were associated with lower stroke risk in men but not in women (HR per 1 SD, 0.46) (Weinstein et al., 2021). Nighttime HRV parameters are important as they are strong predictors of stroke in the Copenhagen Holter study, where eighty-one percent of the stroke occurred in the subjects with the lower half of nighttime SDNN (less than 38 ms; HR, 4.31) (Binici et al., 2011).

There are also epidemiologic evidences of ANS implication in clinically silent stroke which was found a predictor of patent stroke (AHA/ASA Scientific Statement). This can go from silent brain infarcts (SBI), white matter hyperintensities, or microbleeds (Smith et al., 2017), as established by the historical Hisayama study (Shinkawa et al., 1995). Age was a prominent factor, since in this cohort the percentage of subjects with silent infarcts increased with advancing age, from 4.4% in ages 40–49 to 19.3% in more than 80-year old humans. Other main established factors were hypertension, AF and diabetes mellitus. A recent meta-analysis including 14764 subjects underlined a 2.94 relative risk of clinically patent stroke following SBI, after adjustment for CV risk factors (Gupta et al., 2016).

Antiarrhythmic drugs alter significantly HRV, making HRV measurements different from basal values in these subjects (Zuanetti et al., 1991). However, this determines the exclusion of many subjects in the cohort studies as this may exclude the subjects with the most severe risk, determining some bias.

A study focusing on brain lacunae in elderly hypertensive patients underlined the lacunae to be rather related to ANS activity impairment than specifically to hypertension. As a matter of fact, while nocturnal dippers demonstrated an appropriate autonomic activity, extreme dippers, defined as having more than 20% nocturnal reduction of compared to their daily value, exhibited a markedly suppressed nervous activity during sleep. An extreme dip with a ratio night/day $\leq$0.8 was associated to lacunar events, and there was a correlation between the decrease of the HF nocturnal value as well as of the increase LF/HF ratio and the asleep/awake SBP ratio (Kario et al., 1997). In other words, an excessive sympathetic activity goes along with extreme nocturnal deep BP and brain lacunae. Non-dippers showed more advanced cerebrovascular disease than normal dippers, but less than extreme dippers. The depression of autonomic activity determining extreme dipping is also associated with brain lacunae (Kario et al., 1997). In a study comparing ANS through HRV, ambulatory blood pressure monitoring (ABPM), brachial artery endothelium-dependent flow-mediated dilation (FMD) and the intima-media thickness

(IMT) of the carotid artery, it was shown an association between FMD and extreme dippers, reflecting an endothelial dysfunction and an increase in sympathetic activity (Hamada et al., 2008). Extreme dippers, with a night blood pressure decrease more than 20 percent of the diurnal values, are more prone to stroke, particularly to hemorrhagic stroke (Metoki et al., 2006). It is always important to consider that subclinical events of stroke on imaging and autopsy are much more frequent than clinically patent strokes, in the elevated proportion of 1–14 (Leary and Saver, 2003). Subclinical events are strong predictors of future clinical events (Gupta et al., 2016).

The way the ECG is recorded may influence the results, particularly if the recording covers less than a full nyctohemeral cycle. Indeed, from a day-time recording with only 2-h duration recording, as in the Framingham study, the data did not take into account the HRV nocturnal values, while they may be of great interest as the prevalence of nocturnal autonomic dysfunction is high in lacunar stroke patients even in the absence of the commonest sleep-related disorders. In this respect a full-day HRV ambulatory measurement may better describe the autonomic balance giving both day and night values (Hamada et al., 2008). As already stated, nighttime HRV parameters are strong predictors of stroke in the Copenhagen Holter study (Binici et al., 2011).

An abnormal HRV not only predicts a first-ever stroke but also contributes to increase the risk of stroke recurrence (Buratti et al., 2020). The prediction of stroke occurrence by low HRV parameters is also significant after a hip surgery (Ernst et al., 2021).

Whatever the recording duration, HRV remains a powerful approach for aging evaluation. Even HRV calculated on 12-s ECG recordings, as performed from standard 12-lead recording at bedside, made possible to establish the order in which people of the Zutphen cohort died within a 30-year time frame for any cause of death including cancer. Again, our age is that of our ANS activity (Dekker et al., 1997).

At the other end, in the Copenhagen Heart Study involving longer ECG recording durations up to 48 h, global HRV value represented by night-time SDNN global ANS activity was significantly associated with death prediction, even after additional adjustment for heart rate and other relevant biomarkers including serum triglycerides, hs-CRP, and NT-pro BNP. The hazard ratio reached 4.31, p 0.003, for those in the lower half of nighttime SDNN. In the same study, 24-h HRV variables were predictive of all-cause mortality, including stroke (Binici et al., 2011).

There is not one ANS parameter recognized as the best marker for stroke prediction. This comes from the lack of systematization of HRV measurements parameters, due to choices of duration of recordings, parameters analyzed, and the mathematical choice to assess the RR intervals dispersion. Global parameters as SDNN, are the most often introduced in the predictive algorithms since they are easier to understand and to perform. Within the choice of temporal measurements, RMSSD is also often chosen to assess the parasympathetic drive. Frequential analysis are also often used as they open the possibility to get the LF/HF ratio, which represents well the ANS imbalance. Diurnal recordings enhance the LF components as the daily life is much challenging than the nocturnal sleeping period which in turn better assesses the parasympathetic activity (Berger et al., 2022). Even the ANS evaluation in the Framingham study was conducted on short diurnal recordings. Free ANS software will benefit future, and possibly passed, studies in giving access to every ANS parameters (Pichot et al., 2015).

For many studies, a decreased sympathetic activity was the most prominent predictor of cardiovascular events. This can be explained as this is a worsening of ANS imbalance. Parasympathetic activity is not taken into account in the Framingham study as only diurnal measurement were performed, a time where the parasympathetic activity is spontaneously rather low and so variations in parasympathetic activity cannot be measured easily and if measured will be hardly significant (Tsuji et al., 1994, 1996). Conversely, an increase in parasympathetic activity, as obtained through vagal nerve stimulation (VNS), will decrease the sympathetic drive (Clancy et al., 2014).

Today, prediction of stroke brought through ANS measurement was mostly performed using SDNN, RMSSD, LF, and HF variables. This approach should be improved using more variables available to researchers including baroreflex which considers not only the ANS circuitry but also the quality of the carotid sensors in the baroreflex.

Ischemic stroke and hemorrhagic stroke are both concerned by ANS prediction. There are both related to vascular disease linked to classical risk factors, as hypertension, dyslipidemias, metabolic syndrome, inflammation, endothelial disease, and lack of physical activity, each being strongly associated to ANS imbalance (Jiang et al., 2022). As a matter of fact, prevention of both stroke types relies on the same guidelines (Caldwell et al., 2019).

Stress allows an efficient answer to aggressions, increasing the chances for survival, but an excessive intensity or duration is deleterious. ANS measurement may thus participate in the prediction of vascular events by identifying the excess of adrenergic activity, offering means of stroke prediction. However, no specific study yet describes predictive differences in ANS parameters for predicting ischemic stroke versus haemorrhagic stroke.

In this context, there is nothing surprising that the diseases including the most severe altered ANS values are also those determining the shortest life duration and the more cerebrovascular complications. This is true in many chronic diseases and we will review some of them (**Figure 2**). These diseases often present multiple factors.

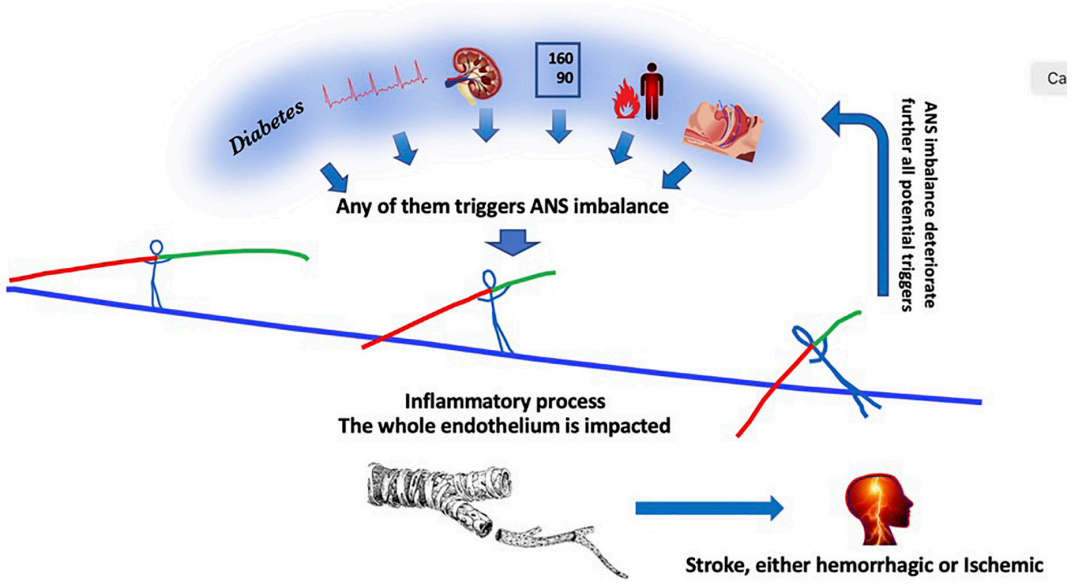

FIGURE 2
From left to right, diabetes, atrial fibrillation, chronic kidney disease, hypertension, inflammation, sleep apnea disorders, any of them contribute independantly to a global decrease in ANS activity with creates a predominance in the sympathetic activity of the remaining ANS activity. The autonomic nervous system activity imbalance determines in turn a global excess in circulating inflammatory products which in turn impacts the whole 300 square meter endothelium monolayer tissue, ending finally in a stroke. The ANS imbalance crushes further endothelium function which in turn increases multi-organ damages, aggravating the deleterious process.

It is important to consider the role of autonomic system in stroke recurrence as it conveys the same predictive value than after a transient ischemic attack (TIA) (Guan et al., 2018). Measuring ANS in TIA allows to identify high risk sub-populations which may benefit from the warning (Guan et al., 2019). This is in concordance with the risk for a first stroke (Kleiger et al., 1987; Bigger et al., 1993; Tsuji et al., 1996).

Autonomic nervous system as a predictive factor for stroke occurrence in the context of diabetes mellitus

The ANS circuits in diabetes are considered an integrative network centered on brainstem modified by and affecting diabetes-induced CVD (Espinoza and Boychuk, 2020). Even short periods of provoked hyperglycemia stimulate sympathetic activity with attenuation of parasympathetic activity (Majeed and Yar, 2020). At 60 years of age, the subjects of the PROOF study show a strong correlation between ANS dysregulation and their dysmetabolism. Furthermore, their ANS deactivation measured through baroreflex was proportional to the metabolic disorder (Assoumou et al., 2012a).

Autonomic nervous system activity is a stronger predictor of stroke in subjects with type 2 diabetes mellitus compared with the general population. Night-time HRV identified diabetics with cardiovascular events, including stroke; at an average follow-up of 14.4 years with a better fitting of the receiver operating curve (ROC) from 0.704 to 0.765 when adding SNA to conventional risk factors (Hadad et al., 2021). Progressive HRV worsening also predicted ischemic stroke independently of classical cardiovascular risk factors in diabetics (Yun et al., 2018). According to the authors, a one-unit increase of their combined predictor index increased by almost 10% the probability of events (Lai et al., 2021).

The ARIC cohort study also showed HRV to be a powerful predictor of cardiovascular sudden death during a mean follow-up of 13 years, with an increase of up to 27% in sudden death associated with low HRV (Fyfe-Johnson et al., 2016). While in their first publication the authors did not specifically analyze stroke occurrences, it is however well established that stroke is almost as frequent as coronary artery disease. A more recent publication relating a 22-year follow-up demonstrated a predictive HRV value for incident stroke, but only in the diabetic patients of the cohort (Fyfe-Johnson et al., 2016).

Autonomic nervous system as a predictive factor for stroke occurrence in the context of chronic kidney disease

Chronic kidney disease (CKD) is associated with an increased risk of both ischemic and hemorrhagic strokes,

in a range of 10–33 per 1000 patient-years depending on study population and design (El Husseini et al., 2014). In addition to the established high cardiovascular risk, the altered autonomic control in hemodialyzed patients further increases the probability of major adverse cardiac events and stroke (Kao et al., 2019). Lower HRV values, mainly low frequency, significantly predicts higher risk of cardiovascular disease (CVD) (Chandra et al., 2012). There is a relationship between the decline in glomerular filtration rate and autonomic neuropathy (Clyne et al., 2016). Renal failure increases sympathetic activity and overrides the stimulation by baroreceptor deactivation during hemodialysis (Boero et al., 2001).

Therapeutic targets for sympathetic activity decrease were proposed in CKD (Seravalle et al., 2021). Although the results of sympathetic renal nerve ablation were first very encouraging toward hypertension, with a profound decrease of systolic and diastolic blood pressure, there is no study analyzing long-term clinical improvement in inflammatory markers (Seravalle et al., 2021). A pilot study based on 1 month non-invasive VNS in CKD showed changes in tumor necrosis factor (TNF), interleukine-1 and -10 (IL-1 and IL-10) concentrations that did not reach statistical difference (Hilderman and Bruchfeld, 2020). Another VNS pilot study in hemodialysis patients determined a decrease in high-sensitivity C-Reactive protein (hsCRP) but not in TNF, IL-1b, or IL-10 (Hilderman and Bruchfeld, 2020). While this suggests that there may be a potential for cholinergic modulation in CKD patients, further studies are needed adding control of other conditions as sleep quality (Kadoya et al., 2021). However, in renal transplant recipients, a complete denervation of the native diseased kidneys by bilateral nephrectomy decreases cardiovascular risk (Obremska et al., 2016).

Autonomic nervous system as a predictive factor for stroke occurrence in the context of inflammation

Chronic systemic inflammation, which is associated with parasympathetic inactivity (Tracey, 2002), is a well-recognized cardiovascular risk factor independently of hypercholesterolemia (Ridker et al., 1998, 2000). This is in line with contribution of chronic inflammation to the residual risk of myocardial infarction and stroke in the absence of elevated levels, or after attaining low levels of low-density lipoprotein cholesterol. The Framingham study, demonstrated ANS activity to hold a strong predictive power, higher than that of cholesterol (Ridker et al., 2002). Conversely, statins reduce sympathetic activity (Lewandowski et al., 2015).

The relationship between cardiovascular disease and inflammation evokes the notion of a role of low ANS activity as a possible inflammation-regulator extended to cardiovascular disease. This major marker of vascular aging should be of interest in primary prevention (Ridker et al., 1998). This is further reinforced by the increased cardiovascular risk and autonomic dysfunction in systemic chronic inflammatory diseases such as rheumatoid arthritis (Hupin et al., 2021), and obesity. Decreased parasympathetic activity is associated with central adiposity and higher SBP, indicative of increased metabolic risk, already at age 5–6 years (Vrijkotte et al., 2015). When a follow-up is performed, it can be seen that increased sympathetic activity predicts an increase in metabolic abnormalities and hypertension over time (Palatini et al., 2006; Licht et al., 2013). The PROOF (Prognostic Indicators of Cardiovascular and Cerebrovascular Events) study confirms a strong correlation between ANS dysregulation and inflammation in subjects aged 65 years old issued from the general population (Dauphinot et al., 2009, 2012; Assoumou et al., 2011). In this study, classical waist circumference clinical factor is strongly associated to inflammation (Assoumou et al., 2011) and increased inflammation predicts in its turn hypertension (Dauphinot et al., 2009).

In a study including people from the Women's Health Study, adding inflammation as a risk factor improved the prediction of stroke (Ridker et al., 2000). Hs-CRP was the strongest univariate predictor out of the 12 markers measured. This is in relation to poor ANS activity. This relationship is tight enough to be considered as a reflex, the inflammatory reflex (Tracey, 2002). A confirmative cohort meta-analysis showed a linear association between CRP level and stroke (P = 0.940), as well as with CVD (P = 0.429), and CHD (P = 0.931); for each 1-mg/L increase in CRP level, the pooled RRs for stroke, CVD and CHD were 1.07, 1.18, and 1.12, respectively (Yang et al., 2021). Other studies found even higher risk predictive values, with a RR being 1.43 for CVD mortality (95% CI, 1.22–1.68) in the group with high CRP values (Ni et al., 2020). The strong relationship between ANS activity and inflammation allows to link these CVD risks to a defect in parasympathetic activity (Tracey, 2002; Doux and Yun, 2006; Ni et al., 2020; Rupprecht et al., 2020).

Another link for ANS implication in stroke occurrence is the independent association between IL-6 levels and cardiovascular events (Miwa et al., 2013). The chronic cardiovascular inflammation related to atherosclerosis-driven cerebrovascular disease is maintained through a failure in the resolution of inflammation (Bäck et al., 2019). Murine models of a disrupted parasympathetic signaling through vagotomy have demonstrated a reduction in specialized proresolving lipid mediators (Mirakaj et al., 2014), which exert key atheroprotecive effects (Arnardottir et al., 2021). Transcutaneous vagal neurostimulation (tVNS) protects from the increase in inflammation induced by administered lipopolysaccharide in rats (Zhao et al., 2012). However, if tVNS protects against inflammation in animal models, the long term effects have not been set in patients (Tynan et al., 2022).

Microglia activation due to sympathetic activation (Li et al., 2020) has severe consequences as inflammation favors neurodegeneration following stroke (Stuckey et al., 2021). Thus the ANS imbalance not only favors stroke occurrence but may also aggravate the post-stroke neurodegeneration.

Autonomic nervous system as a predictive factor of stroke occurrence in the context of atrial fibrillation

Atrial fibrillation is becoming very frequent with an incidence reaching 1.01 and 2.16 for 100 person-years for the age ranges 65–74 and 75–84, respectively (Charlemagne et al., 2011). The incidence value may triple in 2050 (Miyasaka et al., 2006). Incidence is better measured through automatic long duration recordings than using patient-triggered devices (Roche et al., 1997, 2002). It was described as high as 83% after 85 years of age (Rajala et al., 1984).

Atrial fibrillation is an important predictor of stroke (Wolf et al., 1978, 1991; Harrison and Marshall, 1984; Flegel et al., 1987; Yamanouchi et al., 1989; Cabin et al., 1990; Broderick et al., 1992; Jerntorp and Berglund, 1992; Aronow et al., 1996a,b; Jorgensen et al., 1997). In the Framingham study, the risk ratio was 5.6, but reached 6.9 in the Whitehall Study of London Civil Servants cohort (Flegel et al., 1987). Even when not observed at the time of the stroke, AF is often suspected (Liao et al., 2007). Furthermore, silent strokes are frequently associated with AF (Yamanouchi et al., 1990, 1997a,b; Shinkawa et al., 1995; Barthelemy et al., 2003), the risk ratio reaching 2.5 (Yamanouchi et al., 1997b). The recent StrokeStop (Svennberg et al., 2021) and the Loop (Svendsen et al., 2021) studies were aimed at searching for AF and analyze the benefits of a preventive treatment in a monitored group versus a control group in terms of stroke incidence. They differ as the StrokeStop study recorded the ECG from intermittent patient intervention while the Loop study used an implanted loop recorder (ILR) (Svennberg et al., 2015). The StrokeStop Study determined a significant decrease in ischemic stroke (HR 0.76), while the Loop study did not, the incidence using IRL being equivalent to its control group. The differential results of the studies may reflect (a) the healthy user bias, (b) detection mode and burden of AF, (c) background detection, and (d) choice of endpoints (Svennberg and Braunschweig, 2021). Since hand-held single-lead ambulatory ECG screening twice daily for 2 weeks was able to detect AF for stroke-prevention, heart rate monitoring appears feasible with possible extension to a refined stroke prediction based on ANS activity.

Autonomic nervous system imbalance plays an important role in the initiation and maintenance of AF (Chen et al., 2014; Acampa et al., 2018), which is itself frequently associated with stroke. AF is frequently present in unexplained stroke (Barthelemy et al., 2003). AF accounts for uneven atrial conduction which can be induced by uneven sympathetic or parasympathetic stimulations of the atria. In relation to aging, the excess of sympathetic becomes easily predominant. The effects of NE, and nerve growth factor (NGF) on AF vulnerability have a relationship with the ionic remodeling, while the sympathetic hyperinnervation did not have a strong association with the induction of AF (Yang et al., 2019).

Heterogenous effective refractory periods (ERPs) are the hallmark of electrophysiological remodeling in AF. Although high parasympathetic tone prolongs ventricular ERPs, atrial ERPs are shortened and, importantly, this parasympathetic shortening is not uniform through the atria, rather, it is heterogeneously distributed even in healthy hearts (Linz et al., 2019). Adrenergic stimulation exerts most of its arrhythmogenic influence through increases intracellular Ca^{++}. When this Ca^{++} loading is associated to an impaired CA^{++} reuptake mechanism, it permits favorable conditions for Ca^{++} triggered arrhythmic activity by inducing heterogeneously shortened action potential duration (Pfenniger et al., 2021).

Because of its pharmacologic property of preferentially blocking the N-type calcium channel, the dual (L-/N-) calcium channel blocker cilnidipine primarily blocks release of norepinephrine from sympathetic nerves, with a 20-fold smaller effect on L-type calcium channels. Evaluation in the canine atrial tachypacing model of AF showed that this compound could attenuate norepinephrine release and reduce electrical remodeling (both ERP and conduction velocity) as well as structural remodeling (Tajiri et al., 2019).

On the acetylcholine side, high sequence homology between all isoforms of muscarinic receptors (M1–M5) makes difficult the development of highly selective M2 inhibitors (Aistrup et al., 2009).

Methods that reduce autonomic innervation or outflow have been shown to reduce the incidence of spontaneous or induced atrial arrhythmias, suggesting that neuromodulation may be helpful in controlling AF (Chen et al., 2014).

Furthermore, the autonomic atrial receptors can themselves be unevenly stimulated as a consequence of autoantibodies. At least three types of autoantibodies have been found: anti-myosin, anti-M2 muscarinic receptor, and anti-heat shock protein autoantibodies. The best evidence concerns the M2-autoantibody. The proarrhythmic effect of the patients' purified immunoglobulin G containing anti-M2 autoantibodies was confirmed by the occurrence of atrial premature contraction using IgG from patients with AF either idiopathic or in relation with dilated cardiomyopathy (Baba and Fu, 2008).

Any cause of parasympathetic decreased activity gives its full place to adrenergic activity, which is a strong risk enhancer of AF. An atrial parasympathetic receptor decrease due to autoantibodies eventually leads to AF (Baba et al., 2004; Zou et al., 2013; Gurses et al., 2015). This is also confirmed by prediction of AF recurrence when these antibodies are present in post pulmonary vein isolation (Gurses et al., 2015). In such

conditions, strong sympathetic hyperactivity seems of particular frequency and importance (Linz et al., 2014).

The lack of parasympathetic activity is by itself a major factor of unbalance, since vagal neurostimulation (VNS) either acute or progressive is able to resolve AF (Kulkarni et al., 2021). Indeed, experimental studies and clinical trials that have explored the effects of neurostimulation on cardiac autonomic control have related to HF, ventricular arrhythmia and AF.

An intensity-dependent effect of vagal activation has been demonstrated in AF (Coumel et al., 1978; Bettoni and Zimmermann, 2002; Patterson et al., 2005). Thus high intensity vagal stimulation, i.e., at a level sufficient to slow sinus rhythm or atrioventricular conduction rate, induced AF. On the other hand, low intensity stimulation, below the threshold for slowing down the activity of the nodal tissue, exerted an anti-arrhythmic influence (Li et al., 2009; Shen et al., 2011; Sheng et al., 2011; Yu et al., 2011).

In anesthetized dogs, chronic low intensity stimulation of the tragus has been shown to protect against the development of AF induced by direct mechanical auricular stimulation (Li et al., 2009). In general, a shift in the sympathovagal balance in favor of the parasympathetic is observed among patients responding favorably to tVNS (Popov et al., 2013). Also, in people referred for AF ablation, Stavrakis et al. (2015) demonstrated an antiarrhythmic and anti-inflammatory effect of tVNS. In this study, patients under anesthesia received an auricular stimulation to induce AF. With stimulation, the duration of AF was significantly reduced as well as the blood concentration of inflammatory markers TNFα and CRP (Stavrakis et al., 2015).

Stavrakis et al., 2015, 2017, 2020 previous studies supported that tVNS could greatly facilitate induction and maintenance/resolution of AF according to the intensity of the stimulation. Indeed, (i) a strong vagal stimulation leads to an important sinus rate slowing, which promotes AF inducibility, whereas (ii) a low intensity stimulation produces antiarrhythmic effects. Moreover, Stavrakis et al. (2017) showed that electrical stimulation of the vagus nerve at levels substantially below the bradycardia threshold decreased the incidence of postoperative AF (POAF) and suppressed inflammation induced by cardiac surgery.

These observations suggest that vagal stimulation can exert either proarrhythmic or antiarrhythmic effects on the atrial function based on the intensity of stimulation.

Autonomic nervous system as a predictive factor for stroke occurrence in the context of hypertension and blood pressure variability

Hypertension is a clinical landmark of stroke occurrence (Beckett et al., 2008). The relationship is continuous, and independent of other risk factors. The risk for death from and stroke increases steadily beginning at SBP as low as 115 mm Hg. The mortality of stroke double with each increment of 20 mm Hg SBP (Chobanian et al., 2003; Grysiewicz et al., 2008). The Hemorrhagic Stroke Project reported an adjusted odds ratio of 5.71 for hypertension among hemorrhagic stroke cases compared with age-matched controls (Feldmann et al., 2005). There is a tight relationship between hypertension and excessive sympathetic activity (Mancia and Grassi, 2014). Also, hypertension accelerates vascular ischemic disease, as does sympathetic activity (Mancia and Grassi, 2014). There is also an increase in the density of β-adrenergic receptors (Brodde et al., 1984).

Autonomic regulation of BP is of major importance. Autonomic regulation of blood pressure involves several factors, including heart rate, myocardial contractility, and vascular resistance. Baroreflexes are the major factor of BP modulation (Mancia et al., 1986). This system is rather simple, reporting information relative to distension of carotid and aortic arch and inducing a decrease in BP. The information sent to a central regulator located in the brainstem is updated at each arterial pulse. The neuronal output drives a parasympathetic command which lowers BP and slows heart rate. Unfortunately, this reflex, the baroreflex, which depends on a mechanical sensitivity to artery stretching, decreases with age (Abboud, 2010). The consequences of baroreflex impairment are chronic increased levels of BP, an impaired ability to respond to acute challenges to BP stability, low blood pressure periods, and an associated increased risk of sudden cardiac death.

The combination of CKD and hypertension is a situation in which an excess of sympathetic activity is very deleterious. Renal sympathectomy has demonstrated some advantages.

BP regulation is highly dependent on the ANS. In spontaneously hypertensive rats, there was a relationship in BP with the age-related loss of cardiac vagal preganglionic neurons (Corbett et al., 2007). Baroreflex impairment with age in humans also depends on loss of vagal innervation (Sagawa, 1983; Monahan, 2007). In the subjects aged 65 years old from the PROOF cohort, there are strong correlations between ANS dysregulation and hypertension (Dauphinot et al., 2013). On short term studies, Non-Esterified Fatty Acids (NEFA) can raise blood pressure, heart rate, and α1-adrenoceptor vasoreactivity, while reducing baroreflex sensitivity, endothelium-dependent vasodilatation, and vascular compliance (Egan, 2003).

The control of hypertension protects against stroke at any age (Bjorklund et al., 2004). An intensive approach targeting a BP below 140 mm Hg determined a hazard ratio of 0.75 of fatal and non-fatal cardiovascular events in the general population (Group et al., 2015).

Blood pressure variability adds also a predictive power to CV events. Methods to measure BPV measurements vary from repeated intra-daily measurements, successive daily or monthly measurements as well, the most common representation being standard deviation of 24-h ambulatory blood pressure (ABP)

measurements. Other approaches propose to calculate the coefficient of variation of successive measurements, which is their SD divided by mean blood pressure, as well as many other parameters (Andalib et al., 2020). Indeed, current ambulatory recording devices do not give individuals successive systolic, or diastolic, peaks, and the calculations performed on ambulatory BP measurements are thus performed on already averaged data.

Blood pressure variability is a significant predictor of CV events including stroke as shown from the Uppsala Longitudinal Study of Adult Men (ULSAM) where BPV, reflected by the standard deviation (SD) of daytime and nighttime SBP, significantly predicted stroke (Bjorklund et al., 2004). Twenty-four hour ambulatory pulse pressure (PP) gave a HR reaching 1.29 for one SD increase in daytime ambulatory PP independently of other established CV risk factors (Bjorklund et al., 2004). In the Chinese residents in Pu-Li town and Kinmen county, Taiwan, average real variability (ARV) index recorded on a short period added significant prognostic information (Hsu et al., 2016). BPV is also associated with cerebral white matter hyperintensity and might be one of the pathophysiological phenomena involving in the small vessel disease independent of hypertension (Zhang et al., 2022). The Hisayama study showed that increased day-to-day BPV is, independently of average home blood pressure, a significant risk factor for the development of all-cause dementia (Oishi et al., 2017). BPV predicts also CV events after a first stroke (Dawson et al., 2000; Kakaletsis et al., 2022). Regardless of whether they had hypertension, higher visit-to-visit SBP variability was significantly associated with a higher risk of MACE (Liu et al., 2022). Nighttime BPV was said to contribute to the prediction of CV events (Palatini et al., 2014) while this may be related to OSA. Also, the BPV prediction analysis showed that, in a large population cohort, which provided sufficient statistical power, BPV assessed from 24-h ambulatory recordings did not contribute much to risk stratification over and beyond 24-h BP (Hansen et al., 2010).

Due to its central role in blood pressure regulation, the baroreflex dysfunction may increase BP as well as disrupts BPV, and become a significant marker (Kaufmann et al., 2020).

Autonomic nervous system as a predictive factor for stroke occurrence in the context of vascular stiffness

Arterial stiffness is a recognized risk factor for stroke and patients with acute ischemic stroke show higher arterial stiffness index values (Tuttolomondo et al., 2010). Among stroke patients, lacunar subtype has the highest arterial stiffness indexes, underlining the relationship between vascular aging and endothelial dysfunction (Tuttolomondo et al., 2010, 2017). Abboud underlined the relationship between endothelium health and ANS activity (Abboud, 2010). The combination with OSA is also underlined by the association with arterial stiffness (Saeed et al., 2022). Arterial stiffness is also an independent risk factor for hemorrhagic transformation in ischemic stroke undergoing thrombolysis (Acampa et al., 2017). Increased arterial stiffness is consistently associated with the presence of deep cerebral microbleeds and severe enlarged perivascular spaces burden at the BG and CS (Bae et al., 2021). Direct evidence of the dependence of artery stiffness from sympathetic activity was demonstrated (Holwerda et al., 2019; Tsioufis and Dimitriadis, 2019).

Autonomic nervous system as a predictive factor for stroke occurrence in the context of sleep apnea

The prevalence of sleep apnea varies among studies. In the Wisconsin Sleep Cohort (WSC) study, the prevalence was as high as 24% for men and 9% for women. In Europe, the prevalence is around 10% (Heinzer et al., 2015; Haba-Rubio et al., 2016). In the PROOF cohort, it was set at 56% in people aged 67 years (Assoumou et al., 2012a,b). The Hypnolaus study from Lausanne showed a prevalence reaching 23.4% in women and 49.7% in men, with a median age of 57 years (range 40–85) (Heinzer et al., 2015).

The profile of autonomic function found in OSA includes increased sympathetic activity and reduced parasympathetic activity (Sequeira et al., 2019). Overactivation of the sympathetic nervous system was shown to be positively correlated with hypoxia, negative intrathoracic pressure swings, and recurring cortical arousals (Ucak et al., 2021). In patients suffering from OSA, the arousal index strongly correlated with LF/HF ratio and VLF, markers of ANS imbalance (Sforza et al., 2007), they were also negatively correlated with the vagally mediated HF components (Ucak et al., 2021). The PROOF study shows strong correlations between ANS dysregulation and OSA (Roche et al., 2003, 2009, 2012; Barthelemy et al., 2007). Furthermore, autonomic sympathetic activation during sleep predicts new-onset ambulatory hypertension (Roche et al., 2012). These strong relationships reinforce the interest in using Holter systems to detect OSA from Holter ECG (Pichot et al., 2015; Lao et al., 2021), and ANS activity may also be particularly well suited to follow treatment efficacy (Kim et al., 2020).

Altered autonomic function in OSA was implicated in increased cardiovascular risk (Dissanayake et al., 2021). Autonomic abnormalities in OSA are associated with a 46% greater risk of cardiovascular events (Fang et al., 2020). These vascular risks were well described, but the authors did not discriminate cardiact events from cerebral events (Marin et al., 2005). The development of cardiovascular disease in OSA is multifactorial and induces a cascade of events. The primary contributing factor is sympathetic overactivity (Ucak et al., 2021). Even unrecognized sleep apnea is associated with elevated

ABP (Roche et al., 2012), elevated CRP values (Roche et al., 2009), and cardiac arrhythmias (Roche et al., 2003).

Autonomic nervous system as a predictive factor of stroke occurrence in the context of combined factors

Interactions are most frequently linked to sympathetic hyperactivity brought on by acute and mostly chronic stress. Cerebrovascular risk factors are often associated.

A less clinically patent risk factors is probably sleep apneas are there is no biological markers and no obvious clinical marker as obesity or hypertension while sleep apneas may be at the origin of several established cerebrovascular risk factors (Van Ryswyk et al., 2018).

Sleep apnea accounts for a chronic intermittent stress and strongly leads to increased AF (Braga et al., 2009; Tavares et al., 2021), hypertension (Peppard et al., 2000), and dyslipidemia. Sleep apnea, obesity, diabetes mellitus, hypertension, and more generally the periods before AF, all share a decrease in ANS activity as a common factor of stroke occurrence. Interestingly, ANS activity, specifically the parasympathetic activity, may be enhanced through exercise training which may benefits sleep apnea disorders (Berger et al., 2018, 2019). The chemoreceptor reflex contribution to the pressor response is usually small compared with the baroreflex contribution, but it becomes very significant and predominant with senescence (Abboud, 2010). Furthermore, baroreflex impairment is associated with an enhanced chemoreceptor sensitivity resulting in a major sympathetic stimulation (Abboud, 2010). In patients with cardiac heart failure (CHF), untreated OSA is associated with an increased risk of death independently of confounding factors (Wang et al., 2007). Chronic artificial baroreceptor activation enhances survival in dogs presenting a pacing induced heart failure (Zucker et al., 2007). This stimulation suppresses the increases in plasma norepinephrine (NE) and angiotensin (ANG II).

Controlling one of combined factors can be beneficial, as refers to diabetes, where the control of hypertension divides the occurrence of stroke by a factor three (Bragg et al., 2021).

The PROOF cohort study demonstrated frequent arousals from sleep, with or without hypoxia, to increase BP and put healthy elderly volunteers at an higher risk of hypertension and thus of stroke (Chouchou et al., 2014). The importance of not interrupting sleep cycles, to allow to reach profound sleep states, has been illustrated (Jurysta et al., 2003). Any sleep interruption prevents the sleep deepening and will thus not permit parasympathetic activation. Hypoxia determines a strong supplementary sympathetic stimulation through chemoreceptors activation. This stimulation further decreases the parasympathetic activity through a central counter action, multiplying further the aggressivity of adrenergic hormones.

One risk factor can easily be associated with many others. Interestingly, the PROOF cohort study pointed out a linear relationship between a decreased baroreflex, which is a major factor of parasympathetic activity, and the number of components of metabolic syndrome (Assoumou et al., 2012a).

Even the memory performance was shown to be dependent on ANS. This underlines the need for a permanent high level of parasympathetic activity for a preserved long-term brain microvascular health (Saint Martin et al., 2015).

Some views on a common consequence of autonomic nervous system unbalance: Neuroendothelial disease

Endothelial function is highly dependent on ANS activity which regulates blood flow as well as tissue-blood exchanges. During severe biological unbalance, as hypoxia or acidosis, the endothelium function may be severely compromised. Inflammation is a factor common to many pathologies and dysfunctions. Endothelial dysfunction may be found in each of the chronic diseases just described.

Endothelium disease is associated to AF (Corban et al., 2021). The decrease in parasympathetic activity associated with an increase in sympathetic activity plays a key role in vascular vulnerability (Bäck et al., 2019). This triggers several pathways including increased reactive oxygen species (ROS) and increased inflammation (Harada et al., 2015; Bäck et al., 2019). Beyond their high cellular aggressivity and disruptive effects on regulations, ROS further enhance sympathetic activity by stimulating the hypothalamic subfornical centers (Abboud, 2010). By this way, inflammation triggers a large increase in vascular inflammasome (Bäck et al., 2019). The disease further extends to the content of the vessels by activating platelets and many other factors (Harada et al., 2015).

Increased ROS induce microvascular dysfunction including impaired endothelium-dependent vasodilator and enhanced endothelium-dependent vasoconstrictor responses, along with increased vulnerability to thrombus formation (Yu et al., 2019). Increased ROS thus result in enhanced fluid filtration associated with protein extravasation and activation of leukocytes. All of these pathologic microvascular events involve the increased production of ROS, and consecutive induced ischemia reperfusion, which further activates inflammasomes, provokes severe mitochondrial disorders, and determines release of microvesicles in endothelial cells (Bäck et al., 2019). There is a relationship between HRV and endothelial function evaluated through brachial artery flow mediated dilation (Pinter et al., 2012). This approach underlines the strong dependency of endothelium to ANS parasympathetic activity. Chronic baroreceptor stimulation improves endothelium

function (Chapleau et al., 2016), as does direct acetylcholine administration (Borovikova et al., 2000). Endothelium health is thus strongly dependent on parasympathetic activity. Rebalancing autonomic activity through VNS attenuates this dysfunction and protects from stroke (Carandina et al., 2021).

The effects of autonomic nervous system improvement by neural stimulation

Both VNS and renal denervation are intended to increase parasympathetic activity and decrease sympathetic predominance. Acute, or more often chronic, VNS enhances endothelium health which enhances vagal protective activity (Saeed et al., 2005; Chapleau et al., 2016).

The powerful downregulation of inflammatory process by vagal stimulation is a fundamental key of the protection brought by vagal nerve activity. Transcutaneous auricular vagus nerve stimulation (tVNS) was shown to protect rats from the potent lipopolysaccharide inflammatory stimuli, by decreasing TNFα, IL-6, and IL-1β response to the inflammatory stimuli. Conversely, inhibition of the tVNS effects by α7nAChR antagonist injection confirmed this mechanism (Zhao et al., 2012). Using the Shwartzman reaction, nicotine and the CAP55 cholinergic agent decreased substantially both VCAM-1 mRNA and *E*-selectin mRNA expression by the endothelium, and reduce leukocyte adhesion (Saeed et al., 2005). *In vivo* VNS in the carrageenan air pouch model induced similar effects (Saeed et al., 2005). Vagal stimulation determines a peripheral vascular protection in a rat model of myocardial ischemia/reperfusion through the cholinergic anti-inflammatory pathway which is dependent on a7nAChR (Zhao et al., 2013).

In CHF, a strong negative correlation was shown between the plasmatic increase in NE and survival (Cohn et al., 1984). This underlines the central role of NE as a highly toxic endocrine factor. On the other hand, the strong protective effect of parasympathetic activity was demonstrated in CHF through vagal stimulation (Zucker et al., 2007). Neurovagal stimulation was shown to correct or help reducing AF (Kiuchi et al., 2016; Stavrakis et al., 2020). Low-intensity vagal stimulation inhibits AF in an animal model of OSA (Gao et al., 2015). In another critical clinical field, CKD, complete denervation of the native diseased kidney by bilateral nephrectomy decreases the cardiovascular risk (Obremska et al., 2016).

After an induced ischemic stroke, infarct size was significantly reduced in response to vagal stimulation in animals (Yang et al., 2018); the infarct size decrease was associated with a decrease in blood-brain transfer in the stimulated group, spatially correlated with the attenuation of the infarct size. It was also shown that vascular tight junctions were protected in microvessels with lower serum proteins leakage, which underlines the protection brought to the endothelium. After stroke, the non-invasive VNS reduces blood-brain barrier disruption in a rat model of ischemic stroke (Yang et al., 2018).

Conclusion

Today, since Hilz et al. (2011) proposed to substitute the NIH Stroke Scale (NIHSS) clinical values score to encompass the lack of ANS variables availability and take advantage of that ANS variables have become readily available through accessible devices and software (Pichot et al., 2016). If either low ANS variables values or ANS severe imbalance is detected, a priority should be to identify the disorder explaining the fall in ANS activity and taking the needed corrective decisions to avoid further disease extension. This includes mainly OSA, hypertension, diabetes, CKD, and inflammation. Recording ANS activity in large populations should be encouraged to measure ANS on large populations, the recordings may be validated through cardiologist teams to ensure the needed quality.

Vagal activity is a key factor which, when maintaining its activity through physical exercise is not possible, may be boosted by non-invasive transcutaneous vagal stimulation.

Obtaining individual values only needs analysis of an ECG recording, preferably on a full nyctohemeral period, which is now easy to perform and repeat. Using growth curves for sympathetic and parasympathetic activity would be straightforward using growth curves similar to children's weight and height. An annual recording may be a good preventive target. The recording may be performed at young age when unfavorable clinical conditions are present.

The predictive power of ANS activity for cardiovascular diseases may lead to a large utilization of autonomic modulation in preventing the occurrence of ischemic and hemorrhagic stroke and limiting their severity (Carandina et al., 2021). We may well have the age of our autonomic nervous system and we can enhance its protective activity through physical exercise or, if is difficult to exercise, through tVNS.

Author contributions

J-CB, VP, DH, MBe, SC, LM, MBä, J-RL, and FR contributed to conception and design of the study. J-CB wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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

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A novel predictive strategy for the incidence of postoperative neurocognitive dysfunction in elderly patients with mild cognitive impairment

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Objective: Preoperative levels of cognition-related biomarkers and intraoperative cerebral ischemia and hypoxia might cause postoperative neurocognitive dysfunction (PND). The aim of this study was to evaluate the predictive ability of preoperative plasma biomarkers along with cerebral oxygen saturation ($SctO_2$) for the incidence of PND in elderly patients with mild cognitive impairment (MCI).

Methods: A total of 210 patients aged 65–80 years undergoing spinal surgery were randomly assigned to three groups (n = 70 each): propofol, sevoflurane, and propofol/sevoflurane as anesthesia maintenance protocols. Propofol was administrated target-controlled infusion of 4 μg/ml (group P), the minimum alveolar concentration (MAC) of inhalation anesthetic sevoflurane was 1.3 (group S), and propofol was injected with a target-controlled plasma concentration of 1.2 μg/ml, accompanied by sevoflurane inhalation 0.7 MAC (group PS). Cognitive function was evaluated 1 day preoperatively and on the 7th day postoperatively. Preoperative levels of amyloidβ-40 (Aβ-40), Aβ-42, total tau protein (T-tau), phosphorylated tau protein (P-tau), and triggering receptors on myeloid cells-2 (TREM2) were investigated. $SctO_2$ was monitored intraoperatively.

Abbreviations: PND, postoperative neurocognitive dysfunction; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; Aβ-40, amyloidβ-40; Aβ-42, amyloidβ-42; $SctO_2$, cerebral oxygen saturation; AUC, the area under the curve; AD, Alzheimer's disease; MAC, minimum alveolar concentration; POD, postoperative delirium; PACU, post-anesthesia care unit; MMSE, Mini-Mental State Examination; NFTs, neurofibrillary tangles; TREM2, Triggering receptors on myeloid cell-2; BMI, body mass index; ASA, American Society of Anesthesiologists' physical; CDR, Clinical Dementia Rating; ADL, activities of daily living.

Results: Aβ-42 had the strongest significant correlation with preoperative MoCA score. The value of Aβ-42 associated with a high risk of PND was 28.34 pg/ml, and the area under the curve (AUC) was predicted to be 0.711. When the preoperative level of Aβ-42 was 28.34 pg/ml, $SctO_{2max}$% was 9.92%. The AUC was predicted to be 0.872, and the sensitivity and specificity were 0.833 and 0.841, respectively.

Conclusion: Under the conditions of preoperative Aβ-42 less than 28.34 pg/ml, the intraoperative fluctuation range of cerebral oxygen saturation should be maintained within 9.92% to reduce the occurrence of PND in geriatric patients with MCI.

KEYWORDS

elderly, mild cognitive impairment, postoperative neurocognitive dysfunction, cerebral oxygen saturation, plasma amyloid β-42

Introduction

Postoperative neurocognitive dysfunction (PND) is an objectively measured decline in cognition compared with that of the preoperative state (Evered and Silbert, 2018) and it adversely affects the quality of life and rehabilitation of patients (Lin et al., 2020). The clinical diagnosis of PND is a long process that requires the application of various cognitive tests and postoperative follow-up (Miniksar et al., 2021). Preoperative cognitive function is an important factor that cannot be ignored. PND leads to neurological complications due to multifactorial causes, with a high rate of morbidity, especially in geriatric patients with mild cognitive impairment (MCI; Bekker et al., 2010; Kline et al., 2012; Xin et al., 2021). A retrospective analysis of 2014 patients aged above 65 years who underwent surgery found that the incidence of postoperative delirium in patients with MCI before surgery was significantly higher than in patients of the same age with normal cognitive function before surgery (8.7% vs. 2.6%; Sprung et al., 2017). Preoperative plasma biomarkers are closely correlated with MCI and subsequent cognitive dysfunction. Identification of the most optimal-related biomarkers and their proper concentration could enhance PND prevention and treatment practices.

MCI is defined as the clinical stage between the expected cognitive decline of normal healthy aging and a more serious decline characterizing dementia (Ng et al., 2021). The incidence of MCI is 10%–20% of adults above 65 years of age, and approximately 20%–40% of patients with MCI progress to dementia every year (Skolariki et al., 2021). As the population ages, surgery is being performed more frequently, and in progressively older adults with MCI. The inappropriate perioperative management of patients with MCI might increase the incidence of PND and accelerate the progression of MCI to Alzheimer's disease (AD; Racine et al., 2018; Xin et al., 2021). So the choice of appropriate anesthetic drugs is important to reduce the incidence of PND in patients with MCI (Xin et al., 2021). We previously conducted preliminary research on the types, dosages, and compatibility of general anesthetics for patients with MCI and confirmed that the combination of 0.7 minimum alveolar concentration (MAC) sevoflurane with 1.2 μg/ml propofol (plasma target concentration) is safe for MCI patients undergoing surgery with general anesthesia. Closely related biomarkers contributed uniquely to predict the occurrence of PND.

Intraoperative cerebral ischemia and cerebral oxygen desaturation have been proposed as possible mechanisms of PND (Lei et al., 2007). Optimizing cerebral perfusion may allow for reducing the risk of PND. Cerebral oxygen saturation ($SctO_2$) based on near-infrared spectroscopy monitoring depends on the balance of cerebral oxygen supply and consumption. During noncardiac surgery, a well-maintained level of $SctO_2$ can help reduce the incidence of intraoperative cerebral ischemia in elderly patients (Casati et al., 2005). Studies have shown that the change in intraoperative $SctO_2$ has important implications in PND (Ni et al., 2015). Intraoperative $SctO_2$ less than 50% or a greater than 20% decline from baseline is an independent risk factor for PND (Kim et al., 2016). However, the available literature regarding the relationship between $SctO_2$ and PND in elderly patients with MCI undergoing major surgeries is minimal. Further, whether the combined assessment of preoperative plasma biomarkers and $SctO_2$ is also effective to clinically reduce the incidence of PND is still unclear.

Given these limitations, we hypothesized that screening biomarkers closely correlated to MCI, and limiting the fluctuation in $SctO_2$ based on near-infrared spectroscopy monitoring might further prevent PND in elderly patients with MCI. Hence, the present study was designed to elucidate the predictive capability of preoperative biomarkers and $SctO_2$ for PND in elderly patients with MCI undergoing surgery.

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Methods

Study design

The randomized clinical study was conducted with the approval of the Institutional Human Research Ethics Committee of the Third Central Clinical College of Tianjin Medical University (IRB2019-011-01) and is in compliance with the Helsinki Declaration. The study was registered at the Chinese Clinical Trials Registry Center before patient enrollment (Registration No.: ChiCTR2000038307). Written informed consent for participation in this study was obtained from all patients.

Participants

Patients aged 65–80 years and scheduled for spinal surgery were enrolled between June 2019 and July 2020. Sex, body mass index (BMI), and American Society of Anesthesiologists (ASA) physical status II or III were recorded. MCI was diagnosed clinically based on the following criteria (Radtke et al., 2013): subjective memory loss stated by self or family members preoperatively, Montreal Cognitive Assessment (MoCA) score in the range of 15–24, mini-mental state scale (MMSE) score less than 27 points (it depends on the level of education), Clinical Dementia Rating (CDR) of 0.5 points, and activities of daily living (ADL) score less than 26 points. Patients were excluded from the study if they met any of the following criteria: preoperative neurological diseases (such as vascular dementia), severe liver and renal insufficiency, autoimmune diseases, recent use of sedatives, antidepressants, or immunosuppressive drugs, traumatic brain injury or history of alcoholism, and previous participation in the study. In all, 224 patients were enrolled and randomly divided into three groups (n = 70): propofol group (group P), sevoflurane group (group S), and propofol/sevoflurane group (group PS; **Figure 1**).

Anesthetic management

Heart rate (HR), blood oxygen saturation (SpO_2), invasive arterial pressure, electrocardiogram (ECG), bispectral index (BIS), and $SctO_2$ were continuously monitored in all patients during the perioperative period. Two sensors of a cerebral oximeter were placed on the left and right sides of the forehead for continuous $SctO_2$ monitoring until the end of administration of anesthesia. When $SctO_2$ is a greater than 20% oxygen saturation reduction from baseline or an absolute value of less than 50%, the patient's head position was checked, and the mean arterial pressure (MAP) was optimized. Surgeons and anesthesiologists were blinded to the patients' group allocations and the measurement of $SctO_2$ to exclude subjective bias.

All patients received intravenous anesthesia following the same induction protocol with 0.05–0.2 mg/kg midazolam, 0.3–0.6 μg/kg sufentanil, 0.3 mg/kg etomidate, and 0.2 mg/kg cisatracurium. Mechanical ventilation was initiated after endotracheal intubation, and the ventilator parameters were adjusted: inhaled oxygen concentration 60%, oxygen flow rate 1 L/min, respiratory rate 12–14 /min, tidal volume 8–10 mL/kg, inhalation and exhalation ratio 1:2, and maintenance of partial expiratory carbon dioxide pressure between 35 and 45 mmHg (1 mmHg = 0.133 kPa). Group P was administrated target-controlled infusion of propofol, and the plasma target-controlled concentration was 4 μg/ml. Group S was administered 1.3 MAC of sevoflurane by inhalation. In the PS group, propofol was injected with a target-controlled plasma concentration of 1.2 μg/ml, accompanied by sevoflurane inhalation of 0.7 MAC. Remifentanil (0.2–0.5 $g{\cdot}kg^{-1}{\cdot}min^{-1}$) was continuously administered to all patients, and cisatracurium was intermittently administered to maintain muscle relaxation. During the surgery, the anesthetic was adjusted according to the BIS value (controlling BIS 40–60) and hemodynamic parameters, and symptomatic treatment was used to maintain the vital signs within the normal range by administering vasoactive medications if necessary.

Physiologic variables and index detection

Physiologic variables including HR, MAP, SpO_2, BIS, and $SctO_2$ were recorded in the three groups. Data were analyzed at six time points: induction of anesthesia (T_0), 10 min after anesthesia induction (T_1), 20 min after anesthesia (T_2), 30 min after the start of surgery (T_3), one hour after the start of surgery (T_4), at the end of surgery (T_5), and before leaving the post-anesthesia care unit (PACU; T_6). $SctO_2$ was the average of left and right monitoring data. The duration of surgery, anesthesia, and PACU stay was recorded.

Blood samples were collected in ethylenediaminetetraacetic acid tubes 1 day preoperatively. The levels of amyloidβ-40 (Aβ-40), Aβ-42, total tau protein (T-tau), phosphorylated tau protein (P-tau), and triggering receptors on myeloid cells-2 (TREM2) were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions (BioVendor, Brno, Czechia). The absorbance was measured using VICTOR Nivo (PerkinElmer, USA) at 420 nm wavelength. Each plasma sample was double-blinded, resulting in an average concentration of two measurements.

Neurocognitive function assessment

MoCA test was used to assess the cognitive function on the day before surgery and on the 7th postoperative day by a trained senior anesthesiologist who was blinded to the clinical

information. MoCA test includes attention and concentration, executive function, memory, language, visual structure skills, abstract thinking, calculation, and orientation; it comprises a total of 11 items in eight cognitive fields, with a total score of 30, plus one point if the participant has 12 years or less of education.

The patients were grouped to calculate the standard deviation (SD) in the preoperative MoCA score, and the difference between the postoperative scores and preoperative scores was compared with the SD. If the score was reduced by ≥1 SD, it was considered that the patients had developed PND (He et al., 2019).

Sample size and statistical analysis

The incidence of PND in elderly patients with MCI (above 65 years old) has been reported as 33.3% in non-cardiac surgery on the 7th postoperative day (Tang et al., 2014). The appropriate depth of anesthesia can reduce the incidence of PND by 22% 7 days after surgery according to the results of the pre-experimental study. With the significance set at 0.05 and the power set at 90%, the sample size required to detect differences was 60 patients. Considering that the rate of loss to follow-up is about 10%–20%, a minimum sample size of 70 patients was assigned to each group for the randomized controlled study.

Statistical analyses were performed using IBM SPSS Statistic 21.0 (SPSS Inc., Chicago, IL). The normality of the continuous variables was tested with the Shapiro–Wilk test. Differences in the continuous variables were determined by the independent *t*-test or Mann-Whitney *U* test and expressed as mean ± standard deviation, or the median (interquartile range). Categorical variables were analyzed through the χ^2 test or Fisher exact test. All reported *P* values are 2-tailed. A $p < 0.05$ was considered statistically significant, and all p-values were two-tailed. A single factor linear regression analysis was performed to evaluate the correlation between the biomarkers and MoCA score. The predictive value of $SctO_2$ for PND was evaluated using the receiver operating characteristic (ROC) curve and the area under the curve (AUC).

Results

The flow diagram is shown in **Figure 1**. A total of 257 patients were screened for eligibility, and 224 patients were ultimately enrolled and randomized. Five patients were excluded from data analysis for the following reasons: operation time not

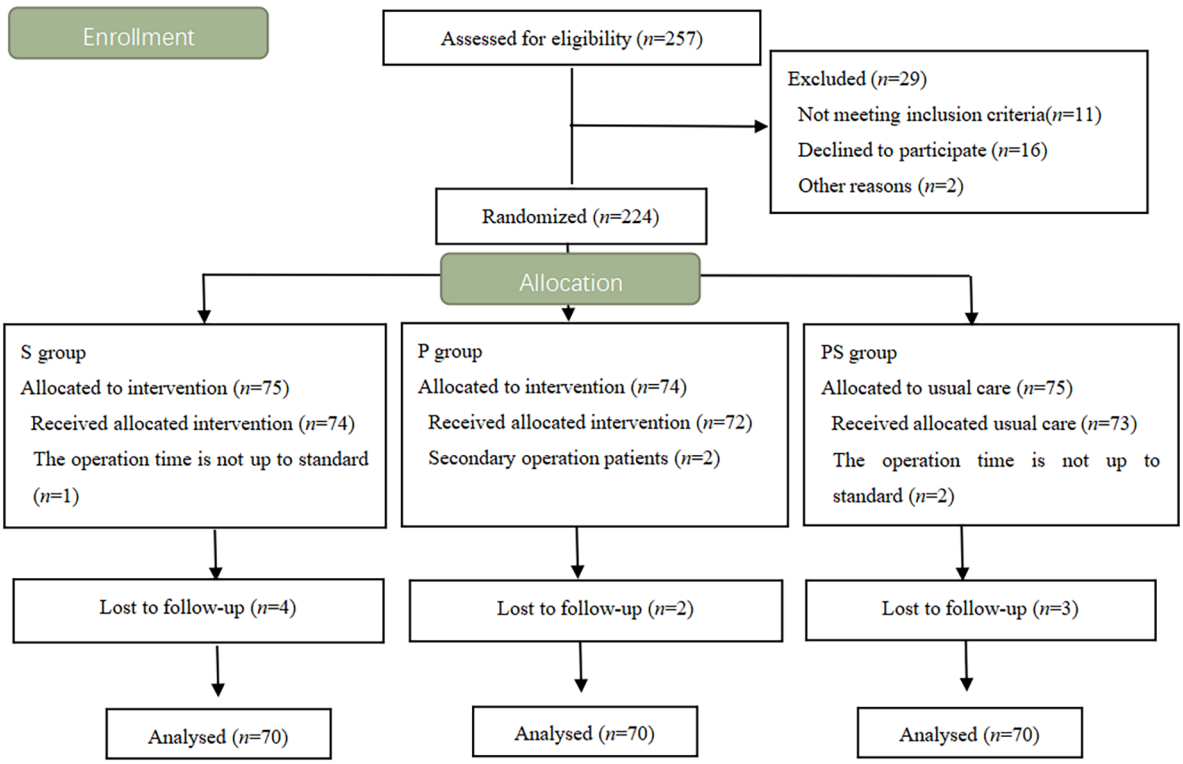

FIGURE 1
Flow chart of patients included in the study.

up to standard, and a secondary operation. Nine patients were lost to follow-up. Finally, 210 patients were randomly assigned to the three groups.

Basic characteristics and the incidence of PND

There were no statistically significant differences in gender, age, BMI, ASA classification, and years of education among the groups (Table 1). No differences in anesthesia duration, surgery duration, and PACU duration were found among the three groups. The incidence of PND in the P, S, and PS groups was 14% (10/70), 33% (23/70), and 7% (5/70), respectively, with statistically significant differences ($\chi^2 = 16.643$, $p < 0.05$).

Predictive ability of preoperative plasma biomarkers for PND

Univariate linear regression analysis showed that Aβ-42 had the strongest significant correlation with preoperative MoCA score ($R = 0.697$, Table 2). The preoperative value of Aβ-42 predicted PND byROC curve analysis, and the critical cutoff value of Aβ-42 predictive of PND was 28.34 pg/ml. The predicted area under the PND curve was 0.711 (95% CI, 0.644–0.777), and the sensitivity and specificity were 0.599 and 0.947, respectively (Figure 2).

Predictive ability of Aβ-42 and $SctO_2$ for PND

Based on the cognitive assessment, all patients with a preoperative Aβ-42 value below 28.34 pg/ml were divided into the PND and non-PND groups based on the MoCA score. The differences were found to be significant in age and education years between the group PND and group non-PND ($p < 0.05$).

TABLE 1 Characteristics of the patients, surgery, and anesthesia.

	Group P	Group S	Group PS	*P* value
Age (years)	69.5 ± 3.3	70.1 ± 3.5	69.8 ± 3.3	0.412
Sex (Male/Female)	33/37	35/35	30/40	0.698
ASA score (II/IV)	39/31	32/38	41/29	0.280
BMI (kg/m^2)	24.3 ± 3.8	24.2 ± 3.3	24.3 ± 3.5	0.992
Education years	6.9 ± 2.0	7.1 ± 1.8	7.3 ± 1.7	0.511
Surgery time (min)	176.5 ± 32.4	183.9 ± 36.8	183.6 ± 32.7	0.348
Anesthesia time (min)	206.7 ± 33.5	211.4 ± 36.8	207.3 ± 34.7	0.696
PACU stay time (min)	42.6 ± 8.1	43.9 ± 8.9	44.4 ± 7.7	0.420

The normally distributed data were presented as mean ± SD, the comparison was evaluated by *t*-test; categorical variables were analyzed through the χ^2 test or Fisher exact test. ASA: American Society of Anesthesiologists; BMI: body mass index; PACU: post-anesthesia care unit.

TABLE 2 Correlation between P-tau, T-tau, TREM2, Aβ-42, Aβ40, and preoperative MoCA values.

	R	F	*P*value
P-tau	−0.281	17.831	**0.000**
T-tau	−0.345	28.191	**0.000**
TREM2	−0.084	1.488	0.224
Aβ-42	0.697	196.391	**0.000**
Aβ-40	0.253	14.243	**0.000**

Bold data indicate $p < 0.05$ was considered statistically significant. P-tau, phosphorylated tau protein; T-tau, total tau protein; TREM2, triggering receptors on myeloid cells-2; Aβ-42, amyloidβ-42; Aβ-40, amyloid β-40.

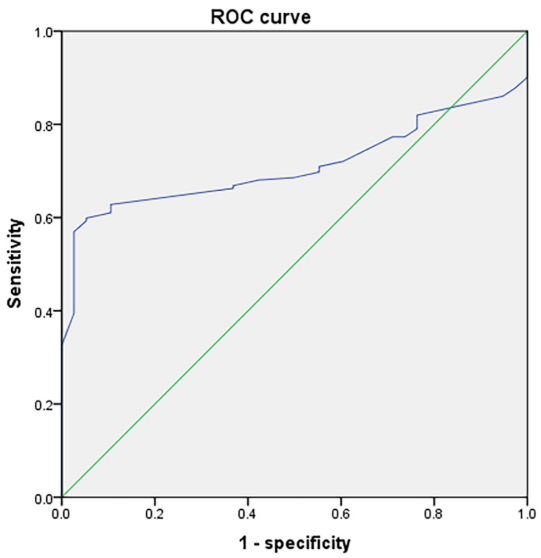

FIGURE 2
Receiver operating characteristic (ROC) curve analysis of preoperative measurement values of Aβ-42 for postoperative neurocognitive dysfunction (PND).

The duration of PACU stay of the group PND was longer than the group non-PND ($p < 0.05$), and the difference in surgery and anesthesia time between the groups was not statistically significant. There were no significant differences in SpO_2, BIS, HR, and MAP between the two groups (Table 3).

$SctO_2$ in the group PND was lower than that in the group non-PND at time points T_3, T_4, T_5, and T_6 (Figure 3). The percentage decrease of $SctO_2$ at T_{3-6} compared with the baseline value is predictive of PND. When the preoperative value of Aβ-42 was less than the critical value of 28.34 pg/ml, the cutoff value of change in $SctO_2$ was determined to be 9.92% according to AUC and Jordon index. The combination predicted AUC for PND was 0.872 (95% CI: 0.797–0.947), and the sensitivity and specificity were 0.833 and 0.841 respectively (Figure 4).

There was no significant difference in $SctO_{2max}$% of male and female in the group PND and group non-PND (Table 4). When the preoperative Aβ-42 value was less than 28.34 pg/ml, the cutoff value of $SctO_2$ change in male was 11.94% according

TABLE 3 Characteristics of the patients, surgery, and anesthesia in the group PND and group Non-PND.

		Group PND ($n = 36$)	Group Non-PND ($n = 69$)	*P* value
Age (years)		70.5 ± 3.4	69.9 ± 2.4	**0.007**
Sex (Male/Female)		14/22	33/36	0.382
ASA score (II/III)		20/16	32/37	0.372
BMI (kg/m^2)		24.5 ± 3.1	24.0 ± 3.1	0.670
Education years		6.6 ± 1.5	7.4 ± 2.0	**0.045**
Surgery time (min)		180 ± 34	180 ± 35	0.959
Anesthesia time (min)		211 ± 31	204 ± 36	0.370
PACU stay time (min)		47 ± 7	42 ± 7	**0.001**
SpO_2 (%)	T_0	97.3 ± 1.7	97.1 ± 1.4	0.415
	T_1	99.6 ± 1.1	99.9 ± 0.4	0.075
	T_2	99.8 ± 0.6	99.9 ± 0.3	0.162
	T_3	99.9 ± 0.4	99.9 ± 0.3	0.346
	T_4	99.9 ± 0.4	99.9 ± 0.4	0.651
	T_5	99.9 ± 0.3	99.9 ± 0.3	0.861
	T_6	96.4 ± 1.4	96.4 ± 1.1	0.921
BIS	T_0	95.3 ± 1.5	95.4 ± 1.7	0.706
	T_1	48.3 ± 6.1	49.7 ± 5.0	0.229
	T_2	49.7 ± 5.1	49.6 ± 5.1	0.924
	T_3	49.4 ± 5.1	49.0 ± 5.1	0.712
	T_4	50.0 ± 5.1	50.5 ± 5.0	0.683
	T_5	62.9 ± 6.1	61.4 ± 7.0	0.291
	T_6	92.7 ± 1.8	92.6 ± 2.2	0.765
HR	T_0	69.4 ± 7.0	70.9 ± 6.6	0.272
	T_1	67.4 ± 8.1	68.6 ± 8.6	0.494
	T_2	64.8 ± 8.8	66.5 ± 7.4	0.299
	T_3	62.5 ± 9.3	64.6 ± 7.4	0.201
	T_4	63.4 ± 8.1	64.3 ± 7.6	0.581
	T_5	66.1 ± 7.6	66.6 ± 8.0	0.787
	T_6	72.7 ± 7.2	71.8 ± 7.4	0.585
MAP	T_0	91.2 ± 7.2	90.3 ± 6.8	0.534
	T_1	78.7 ± 7.0	81.0 ± 6.6	0.107
	T_2	77.6 ± 7.9	79.3 ± 7.0	0.241
	T_3	77.1 ± 7.7	80.0 ± 6.9	0.054
	T_4	77.5 ± 6.1	79.6 ± 6.4	0.111
	T_5	79.2 ± 7.4	81.1 ± 5.6	0.152
	T_6	92.4 ± 10.3	92.2 ± 9.7	0.911

Bold data indicates $p < 0.05$ was considered statistically significant. PND, Postoperative neurocognitive dysfunction; ASA, American Society of Anesthesiologists; BMI, Body mass index; PACU, Postanesthesia care unit; BIS, Bispectral index; HR, Heart rate; MAP, Mean arterial pressure.

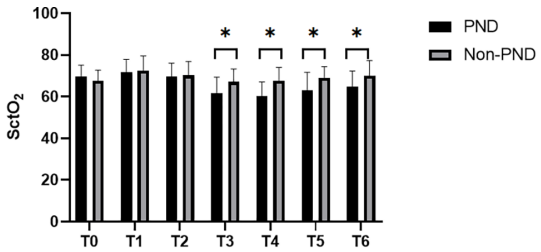

FIGURE 3
Comparison of $SctO_2$ at different time points between group PND and group non-PND. *$p < 0.05$.

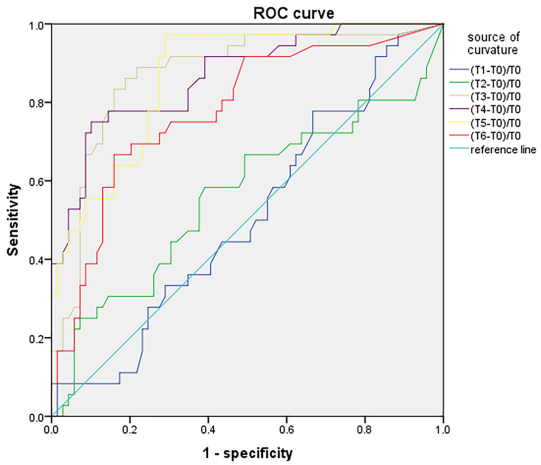

FIGURE 4
ROC curve analysis of PND predicted by the maximum percentage decline of $SctO_2$ at each time point.

to the AUC and Jordon index. The AUC of combined prediction of PND was 0.917 (95% CI: 0.833–1.000), and the sensitivity and specificity were 0.929 and 0.879, respectively (Figure 5). The cutoff value of $SctO_2$ change in females was 6.79% according to the AUC and Jordon index. The AUC of the combined prediction of PND was 0.884 (95% CI: 0.797–0.970), and the sensitivity and specificity were 0.864 and 0.955, respectively (Figure 6).

Discussion

The essential finding of the prospective randomized study was that when preoperative Aβ-42 was less than 28.34 pg/ml, the intraoperative fluctuation in $SctO_2$ not exceeding 9.92% could reduce the incidence of PND in elderly patients with MCI. Thus, we confirmed the predictive ability of preoperative plasma biomarkers and $SctO_2$ for patients with MCI.

PND is a common complication of perioperative neurocognition in elderly patients undergoing major surgery. Spinal surgery is performed in the prone position accompany a variety of hemodynamic and respiratory alterations. Deiner and colleagues indicated that elderly spine surgery patients in the prone position were more than twice as likely to experience mild cerebral desaturation as patients in the supine position (Deiner et al., 2014). Notably, it is essential to monitor $SctO_2$ in the prone spinal surgery. In our study, elderly patients undergoing spinal surgery were selected as subjects.

Sevoflurane and propofol are both commonly used anesthetics during general anesthesia. Anesthetics could theoretically also contribute to cognitive deficits by altering central cholinergic transmission through nicotinic and muscarinic receptors. The effective chamber concentration of propofol for general anesthesia is 4 μg/ml (Mahli et al., 2011), and the maintenance concentration of sevoflurane alone is 1.3 MAC (Hu et al., 2014). Studies have shown that sevoflurane anesthesia alone can aggravate PND in patients with MCI (Liu et al., 2013), while propofol with neuroprotection can improve postoperative cognitive function (Kalimefis et al., 2013). The use of compatible anesthetic drugs may reduce the dosage and

TABLE 4 Comparison of $SctO_{2max}$% between males and females at different time points in the group PND and group Non-PND.

$SctO_{2max}$%	Group male ($n = 47$)	Group female ($n = 58$)	*P* value
$(T_1-T_0)/T_0$	7.50 ± 6.29	7.28 ± 7.07	0.870
$(T_2-T_0)/T_0$	6.66 ± 5.70	7.78 ± 5.80	0.324
$(T_3-T_0)/T_0$	8.74 ± 8.02	8.83 ± 7.69	0.951
$(T_4-T_0)/T_0$	11.46 ± 8.07	9.88 ± 6.60	0.271
$(T_5-T_0)/T_0$	9.43 ± 8.45	7.78 ± 6.88	0.272
$(T_6-T_0)/T_0$	6.77 ± 5.74	7.13 ± 6.81	0.778

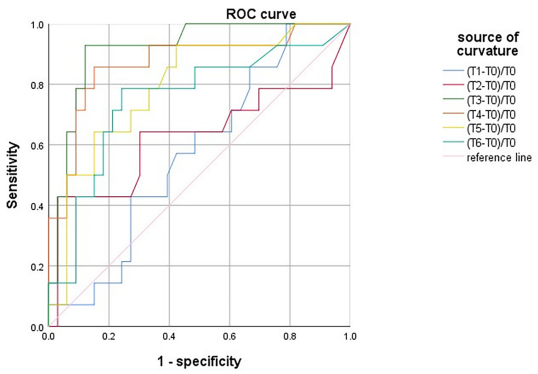

FIGURE 5
ROC curve analysis of PND predicted by the maximum percentage decline of $SctO_2$ in males at each time point.

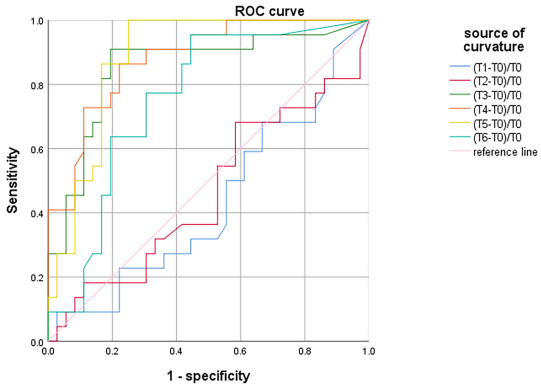

FIGURE 6
ROC curve analysis of PND predicted by the maximum percentage decline of $SctO_2$ in females at each time point.

the adverse reaction of a single drug. Hence, we divided the subjects into three groups for observation. Additionally, there is evidence suggesting that depth of anesthesia may be related to the risk of cognitive impairment (Radtke et al., 2013), and the intraoperative BIS value was maintained at 40–60 to eliminate the interference. In the study, the incidence of PND in the group PS significantly decreased compared with group S and group P. Thus, it is extremely necessary to optimize the anesthesia strategy for elderly patients with MCI.

The pathophysiology of PND is still unknown, although various pathophysiological mechanisms have been suggested in different situations. Preoperative cognitive impairment has been previously shown to negatively affect surgical outcomes. Adogwa found patients with preoperative cognitive impairment were at a greater than a two-fold risk of developing postoperative delirium (Adogwa et al., 2018). MCI, characterized by a transitional state on the continuum of cognitive function between normal aging and dementia, has been associated with biomarkers for AD. Identification of optimal-related biomarkers and development of monitoring strategies could enhance PND prevention and treatment practices.

Aβ has neuronal toxicity and induces neuronal apoptosis, which is a potential sign of nerve damage and persistent neuroinflammation (Wilczyńska and Waszkiewicz, 2020). Aβ-42, the fragment with 42 amino acid residues, is the main component of amyloid plaques found in the brain of patients with AD (Wilczyńska and Waszkiewicz, 2020). A second dominant isoform of Aβ is a peptide with a length of 40 amino acid residues. Neurofibrillary tangles (NFTs) formed by tau protein hyperphosphorylation and senile plaques formed by the accumulation of Aβ in the brain are the initial pathological changes in patients with AD. MCI, the early stage of AD, shows similar pathophysiological changes. Phosphorylated tau protein (P-tau) aggregates in the neurons to form NFTs, which eventually lead to neurodegeneration (Zhao et al., 2020). The elevated level of abnormal P-tau suggests the formation of NFTs in the brain parenchyma. Studies have shown that the level of P-tau protein in the CSF of patients with AD is significantly higher than that in the normal control group, and similar changes are found in patients with MCI (Ahmad et al., 2020; van Maurik et al., 2021). What's more, high levels of plasma Aβ-42 and T-tau in MCI are associated with cognitive decline (Chen et al., 2019). Triggering receptors on myeloid cell-2 (TREM2) can stimulate the production of inflammatory cytokines during an immune response and chronic inflammation (Shi and Holtzman, 2018; Katsel and Haroutunian, 2019). Therefore, the study explored the association of these biomarkers with MCI preoperatively.

In the univariate linear regression analysis, Aβ-42 had the strongest significant correlation with the preoperative MoCA score. Studies have found a relationship between $SctO_2$ and cerebral hypoxia-ischemia, resulting in cognitive decline (Mailhot et al., 2016). Optimizing $SctO_2$ would potentially improve neurologic outcomes. In our study, patients were divided into the PND and non-PND groups based on the critical cutoff value of Aβ-42. $SctO_2$ in the group PND was lower than that in the group non-PND at time points T_{3-6}. This result was in line with that of a previous research (Colak et al., 2015), indicating that minimizing the $SctO_2$ fluctuation to maintain

normal cerebral oxygen supply might reduce the occurrence of PND. However, the scope of intraoperative $SctO_2$ in elderly patients with MCI lacks guidance. This study enrolled geriatric patients with MCI, preoperative screening of the valuable markers and intraoperative dynamically monitoring $SctO_2$ were used to determine the predictive value for PND. In this study, the maximum decrease of $SctO_2$ was 9.92%, in the case of Aβ-42 less than 28.34 pg/ml. This finding provided guidance for the application of $SctO_2$ to reduce PND in elderly patients with MCI.

The study demonstrated that patients in the group PND were older and had less education than the group non-PND. Consistent with previous studies, advanced age is a risk factor for PND (Scott et al., 2014), because of the decline in the central nervous system function reserve. Xu stated that the intraoperative $SctO_2$ value could increase with a MAP elevation (Xu et al., 2020). In the study, there were no significant differences in MAP between the group PND and group non-PND. Thus, there are potential factors affecting $SctO_2$.

With aging, the brain undergoes important structural and physiological changes. Cerebral blood flow significantly declines with age which is most prominent in the prefrontal and frontal cortex. A prospective study indicated that advanced age negatively influences baseline $SctO_2$ which is otherwise influenced by sex, with women showing significantly lower values (Robu et al., 2020). Similar to previous reports, we found that males and females had similar rates of postoperative cognitive decline (Hogue et al., 2003). In addition, there was no significant difference in $SctO_{2max}$% between males and females at different time points. Interestingly, we found that the cutoff value of change in $SctO_2$ was different in gender. When the preoperative Aβ-42 value was less than 28.34 pg/ml, the cutoff value of $SctO_2$ change was 11.94% and 6.79% in males and females respectively. These data suggest that, although the frequency of PND of geriatric patients with MCI is similar for males and females, females appear more likely to suffer injury to intraoperative change of $SctO_2$. Gender differences in $SctO_{2max}$% are important for the development and implementation of individualized therapeutic interventions to improve the prognosis of elderly with MCI.

MMSE scores are a widely used tool for the assessment of cognitive status in elderly subjects (Zhu et al., 2020). MoCA score is a comprehensive evaluation (including short-term memory, attention, language, time and space orientation, etc.) and can identify a wide range of changes in cognitive function with high sensitivity and specificity. Recent studies have shown that the MoCA score is the best assessment to identify MCI (Smith et al., 2007) and can sensitively identify PND (Tsoi et al., 2015). Therefore, the evaluation for PND was performed on the seventh day after surgery in this study by MoCA score.

There are limitations to the study. The sample size is relatively small, which can lead to random errors impacting the results. Due to individual and clinical scenarios differences in $SctO_2$, a larger study is required to validate the relationship between the intraoperative thres hold of $SctO_2$ with PND and determine the optimal $SctO_2$ value that has a prognostically important relationship with PND. We only observed the cognitive function 7 days after surgery, and long-term rehabilitation was not monitored. It is recommended to evaluate all perioperative neurocognitive disorders until 12 months after surgery. The follow-up time should be extended to at least 1 year after surgery to verify less risk of group non-PND in conversion from mild cognitive impairment to AD than the group PND. Moreover, we measured the plasma levels of the biomarkers; however, the biomarkers in cerebrospinal fluid may be more representative. Nevertheless, the study has clinical implications for the prevention of PND.

Conclusion

In summary, the randomized controlled study showed that under the conditions of preoperative Aβ-42 less than 28.34 pg/ml, the intraoperative fluctuation range of cerebral oxygen saturation should be maintained within 9.92% to reduce the occurrence of PND in geriatric patients with MCI. Preoperative plasma biomarkers along with $SctO_2$ is a novel predictive strategy for the occurrence of PND in elderly patients with MCI. Biomarkers closely correlated to MCI are a predictive factor for the rapid progression of dementia. It is necessary to strengthen the monitoring of $SctO_2$ undergoing surgery, especially for the elderly with preoperative cognitive decline. At the same time, multicenter studies with larger samples are needed to further accurately confirm the safety range of $SctO_2$ and its relationship with PND.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Human Research Ethics Committee of The Third Central Hospital of Tianjin. The patients/participants

provided their written informed consent to participate in this study.

Author contributions

YL and XX equally contributed to recruitment and initial screening of participants, data collection, and the first draft of the manuscript. HoW was responsible for data acquisition, data interpretation, and blood parameters analysis. WH contributed to study design, and revising the manuscript. XW and YW contributed to statistical analysis and revising the manuscript. PL and TZ were in charge of perioperative management, data collection, and critical manuscript revision. HaW contributed to study design, supervision, critical manuscript revision, and final approval of the version to be published. 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.

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Utilizing apolipoprotein E genotypes and associated comorbidities for the assessment of the risk for dementia

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Introduction: Dementia is associated with many comorbidities while being related to Apolipoprotein E (*ApoE*) polymorphism. However, it is unclear how these clinical illnesses and genetic factors modify the dementia risk.

Methods: We enrolled 600 dementia cases and 6000 matched non-dementia controls, with identified *ApoE* genotype (ε4/ε4, ε4/ε3, and ε3/ε3). Eight comorbidities were selected by medical records, and counted if occurring within 3 years of enrollment.

Results: The dementia group had a higher ratio of carrying ε4 allele and prevalence of comorbidities than the non-dementia group. Homozygous ε4 carriers presented the broken line of dementia risk with the peak age at 65–75 years and odds ratio (OR) up to 6.6. The risk only emerged after 65 years of age in ε3/ε4 subjects with OR around 1.6–2.4 when aged > 75 years. Cerebrovascular accident (CVA) is the commonest comorbidity (14.6%). CVA, sleep disorder, and functional gastrointestinal disorders remained as significant risk comorbidities for dementia throughout all age groups (OR = 1.7–5.0). When functional gastrointestinal disorder and ε4 allele both occurred, the dementia risk exceeded the summation of individual risks

(OR = 3.7 and 1.9 individually, OR = 6.0 for the combination). Comorbidities could also be predictors of dementia.

Conclusion: Combining the genetic and clinical information, we detected cognitive decline and optimize interventions early when the patients present a specific illness in a particular age and carry a specific *ApoE* allele. Of comorbidities, functional gastrointestinal disorder is the strongest predicting factor for dementia in ε4 allele carriers.

KEYWORDS

dementia, comorbidity, apolipoprotein E (*ApoE*), cerebrovascular accident (CVA), functional gastrointestinal disorder

Introduction

Dementia is the most common neurodegenerative disease in the world, and the reported incidence and prevalence are around 8.6/1,000 person-years and 5.1%, respectively (Ponjoan et al., 2019). The classification of dementia depends on the neuropathological signs, but such features are usually overlapping and presenting as the spectrum (Raz et al., 2016). Demented patients have poor memory, impaired executive function, psycho-behavioral problems, and higher morbidity and mortality (Wolters and Ikram, 2018). Due to worldwide aging, the dementia population is gradually increasing. However, dementia remains incurable at present times. Only early intervention seems to delay dementia progression and prevent disability. Nevertheless, there is still a lack of standard guidelines in clinical practice on how to identify patients at risk of dementia early.

In addition to environmental and socioeconomic status (Jia et al., 2020), genetics is also an important risk factor for dementia. Apolipoprotein E (*ApoE*) polymorphism is the most susceptible genetic factor for late-onset Alzheimer's disease (AD), which is located on chromosome 19, composed of 299 amino acids (Mahley and Rall, 2000). The amino acids at positions 112 and 158 determine three alleles (ε2, ε3, and ε4) (Mahley and Rall, 2000). The *ApoE* genotypes present differential effects on lipid-binding ability, amyloid-β (A-β) aggregation and clearance, and the susceptibility of the blood-brain barrier (Liu et al., 2013), and are linked to a neurodegenerative process (Mahley, 2016), including AD, vascular dementia, and mixed dementia (Liu et al., 2012). The heterozygous ε4 allele increased the AD risk by 2.6–3.2 times (Farrer et al., 1997; Houlden et al., 1998; Harold et al., 2009), while the homozygous ε4 allele further increased the risk by 14.9 times in Caucasians (Farrer et al., 1997). The ε4 allele effect seemed to possess ethnic differences, presenting 2.7–3.5 times (Lai et al., 2003) and 5 times (Zheng et al., 2016) higher dementia risk in the Chinese population, respectively. Besides, it is unclear how age modifies the risk related to *ApoE* polymorphism. Only one western cohort study demonstrated the reverse U-shaped dementia risk curve with the peak age at 70–75 years in both ε4 homozygotes and heterozygotes (Bonham et al., 2016). However, whether the Asian population has the same risk curve of *ApoE* polymorphism has not been disclosed.

Dementia subjects carry around two to eight chronic medical illnesses (Sanderson et al., 2002). Headache (Wang et al., 2018), ischemic stroke (Vijayan and Reddy, 2016), coronary artery disease (CAD) (Deckers et al., 2017), depression (Bennett and Thomas, 2014), sleep disturbance (Shi et al., 2018), fibromyalgia (Tzeng et al., 2018), and epilepsy (Sen et al., 2018) have been described as common comorbidities of dementia. In Taiwan's cross-sectional study (Chen et al., 2017), diabetes mellitus (DM), cerebrovascular disease, liver cirrhosis, and asthma were found to be highly associated with cognitive decline. These chronic diseases seemed to have a bidirectional relationship with dementia because they might share similar risk factors, biological pathways, and environmental backgrounds. Besides, the *ApoE* genotype also affects the susceptibility to develop these illnesses (Afroze et al., 2016; Konialis et al., 2016; Burns et al., 2020). Oxidative stress, neuroinflammation, vascular risk factors, and blood-brain barrier breakdown are responsible for the development of both dementia and associated comorbidities (Raz et al., 2016). The relationship among *ApoE*, dementia, and comorbidities seems to be complex. How comorbidities interact with and influence cognitive function in the timeline has not been well-established. The role of these diseases, acting as a prodrome or as a predictor of dementia, remains unclear.

Therefore, we used a 3-year longitudinal case-control study to explore the interaction between *ApoE* polymorphism and clinical presentations. This is the first study that combined genetic and clinical information to identify the riskiest comorbidities to predict cognitive decline. Thus, the occurrence of such diseases in the subjects can be considered a red flag or

a marker to inform clinicians to screen the cognitive function within a certain age population.

Materials and methods

Participants

The Taiwan Precision Medicine Initiative (TPMI) project began in June 2020. It has been promoted by Academia Sinica in Taiwan, and executed in 15 hospitals, including Taichung Veteran General Hospital (TCVGH). This study was approved by the ethics committee of Taichung Veterans General Hospital's Institutional Review Board (CE16270B-1) in our hospital. We enrolled the outpatients who had the will to join this project and signed the informed consent. They received the genetic study using a customized SNP chip (Axiom Genome-Wide TWB 2.0 Array Plate) and provided their electrical medical records in our hospital. There were 46,020 participants enrolled from our hospital until the date we began the analysis in April 2021.

Because our main purpose is to study the risk of the ε4 allele of the *APOE* gene, we first identified the subjects carrying ε3/ε3, ε3/ε4, and ε4/ε4. There were 37,135 subjects which were enrolled among 46,020 participants. There were 600 newly diagnosed dementia cases between June 2013 and April 2021. Besides, 6,000 gender- and age-matched controls without dementia were selected, whose matched age was calculated by the year 2020.

Apolipoprotein E genotyping

The DNA was extracted when the blood samples were obtained and then genotyped by the Genome-wide association study (GWAS) technique. We used the Axiom Genome-Wide TWB 2.0 Array Plate (Affymetrix, Santa Clara, CA, USA), which is designed for the Taiwan Han Chinese population. Affymetrix Power Tool was used for standard quality control. After excluding markers that failed Hardy–Weinberg equilibrium tests for controls ($P < 1.0 \times 10^{-4}$), minor allele frequency ($P < 0.01$), and the low call rate SNPs (<99%), there were 591,048 SNPs for analysis. This array contained two alleles of the *ApoE* gene: rs429358 and rs7412, which defined the three main *ApoE* alleles (ε2, ε3, and ε4). The ε3/ε3 carriers were used as a reference because it was the most common *ApoE* genotype.

Disease identification

We included medical records from both inpatients and outpatients in the TCVGH from December 2009 to April 2021. The disease diagnosis was based on the International Classification of Disease-nine (ICD-9) code. All causes of dementia and cognitive decline disorders were collected (ICD-9 290.*, 294.0–294.1, 331.*). Because we set the development of dementia or cognitive decline as the endpoint, only the cases with their first diagnostic date between 1 January 2013 and 30 April 2021 were enrolled. The controls were selected from the subjects who were not diagnosed with dementia until 30 April 2021, who were age- (according to the year 2020) and gender-matched with dementia cases using the 1:10 ratio.

We selected eight common diseases presented as dementia-associated comorbidities and their first diagnosed date was recorded: headache (ICD-9 307.81, 346.*), epilepsy (ICD-9 345.*), cerebrovascular accident (CVA, ICD-9 433-438), cardiovascular disease (CAD, ICD-9 410-414), sleep disorders (ICD-9 307.4*, 780.5*), psychiatric disorders (ICD-9 290.8, 290.9, 294.8, 294.9, 295–297, 300.*, 311), functional gastrointestinal disorder (ICD-9 536.8, 536.9, 564.0, 564.1), and fibromyalgia (ICD-9 729.1). Besides, other vascular risk factors were also collected: diabetes (ICD-9 250.**) and hypertension (ICD-9 401.9). When dementia cases had any of these diseases diagnosed 3 months–3 years (90–1,095 days) before the diagnosis of dementia, it was defined as having this disease. In control groups, they were defined as having these diseases when the diseases occurred between 2018 and 2020. Because hypertension and diabetes are considered non-curable diseases, both were counted when they occurred before the diagnosis of dementia or the calculated year.

Statistical analysis

SPSS version 20.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Categorical variables were analyzed with a Chi-squared test for group comparison, while the student *t*-test was used for continuous variables. Clinical variables were analyzed by logistic regression. First, the presence of each comorbidity was applied to predict whether cognitive decline would occur after 3 months–3 years later. Then, the dementia risk was also calculated in three different age groups: younger than 65, 65–75, and older than 75 years. The statistical significance was defined as a p-value < 0.05.

Results

The dementia group had a higher prevalence of comorbidities

The baseline demographic characteristics and the presence of comorbidities in the two groups are shown in **Table 1**. *ApoE* genotypes are significantly different between the two groups. The dementia group showed a higher ratio of ε3/ε4 (23.8 vs. 16.2%) and ε4/ε4 (3.2 vs. 0.7%) than the control group. Within 3 years of dementia occurrence, the prevalence of most selected comorbidities and hypertension was higher in the dementia group than in the control group, except for a headache. Averagely, each dementia patient carried at least 0.6

TABLE 1 Demographic characteristics and associated diseases, which occurred 3 months–3 years before the diagnosis of dementia, in dementia, and matched non-dementia groups.

	Non-dementia (n = 6000)		Dementia (n = 600)		P-value[$]
	N	Percentage	N	Percentage	
Gender (M:F)		1:1.12		1:1.10	0.827
Male	2,832	47.2%	286	47.7%	
Female	3,168	52.8%	314	52.3%	
Age	73.0	±9.3	73.0	±9.4	0.942[#]
Genotype					
ε3/ε3	4,984	83.1%	438	73.0%	<**0.001**
ε3/ε4	974	16.2%	143	23.8%	<**0.001**
ε4/ε4	42	0.7%	19	3.2%	<**0.001**
Comorbidities:					
Headache	75	1.3%	10	0.2%	0.388
Epilepsy	29	0.5%	8	1.3%	**0.008**
CVA	345	5.8%	88	14.6%	<**0.001**
CAD	338	5.6%	54	9.0%	**0.001**
Sleep disorder	168	2.8%	62	10.3%	<**0.001**
Psychiatric disorder	200	3.3%	34	5.6%	**0.003**
Functional GI disorder	214	3.6%	68	11.3%	<**0.001**
Fibromyalgia	156	2.6%	49	8.2%	<**0.001**
Hypertension	2,714	45.2%	301	50.2%	**0.021**
Diabetes	2,384	39.7%	249	41.5%	0.400

[$]chi-squared test.
#Mann-Whitney U test.
CVA, cerebrovascular accident; CAD, coronary artery disease; GI, gastrointestinal; SD, standard deviation; M, male; F, female.

comorbidities within 3 years before the diagnosis of dementia, which was 2.4 times of the age-comparable control group. The most common comorbidity was CVA, and it happened in at least one of the seven dementia cases (14.6%). Although CVA still showed the highest prevalence (5.8%) in the control group, it was only half of the value in the dementia group. For chronic diseases, diabetes presented a similar percentage in both groups (41.5% in the dementia group and 39.7% in the non-dementia group). The diagnostic ages of each disease are listed in Supplementary Table 1, which showed no significant differences between the demented and non-demented groups. In addition, the frequency of homozygous ε4 and ε3 cases were 0.63% (288/37,135) and 65.98% (30,363/37,135) in our hospital cohort, respectively (Supplementary Table 2).

Dementia was associated with the ε4 allele and some comorbidities

In the univariate analysis, almost all selected comorbidities were associated with dementia occurrence within 3 months–3 years later, except for headache and diabetes (Table 2). By applying multivariate regression, the odds ratio (OR) of cognitive decline for carrying any *ApoE* ε4 allele was 1.89 in contrast to the ε3/ε3 polymorphism. In the clinical aspect, we found that CVA, CAD, sleep disorders, functional gastrointestinal disorders, and fibromyalgia had a significantly higher OR for the occurrence of dementia within 3 months–3 years, ranging from 1.43 to 3.09. Among them, sleep disorders were the most dangerous factor, presenting 3.09 OR for dementia occurrence, followed by functional gastrointestinal disorders with OR of 2.73. Neither these two common chronic diseases, hypertension and diabetes, showed a higher risk for dementia after multivariate regression.

Dementia risk related to comorbidities and the ε4 allele varies with age

After age stratification, homozygous *ApoE* ε4 carriers presented the highest risk among all factors for dementia occurrence throughout all age groups (Table 3). Its OR reached the peak in the group of 65–75 years up to 6.63, and then, the risk declined after 75 years to 4.06. Heterozygous ε4 carriers showed dementia risk only when they were older than 65 years. The OR gradually increased with the age, from 1.61 to 2.40 at the age older than 65 years. Among comorbidities, CVA, sleep disorders, and functional gastrointestinal disorders remained significant risk factors for dementia occurrence within 3 years throughout all age groups, and the OR ranged from 1.74 to 5.05. Both CVA and sleep disorders showed the highest risk in the relatively younger age group (<65 years) than in the older group (>65 years). Functional gastrointestinal disorder showed the highest risk within the 65–75 years group. Besides, the presence of fibromyalgia only demonstrated dementia risk in the age of 65–75 years. CVA (OR 5.05), functional gastrointestinal dysfunction (OR 3.04), and sleep disorders (OR 3.29) were the most dangerous comorbidities in the younger than 65, 65–75, and older than 75 years age groups, respectively.

Functional gastrointestinal disorders have a further higher risk for dementia in ε4 allele carriers

We took the four most important risk factors (ε4 allele, CVA, sleep, and functional gastrointestinal disorders), which were selected by multivariate analysis from Table 3, to explore the combined effect of genes and comorbidities on dementia (Figure 1). The ε4 allele carriers had a persistently higher risk for dementia compared with those not carrying ε4, with OR around 1.9–2.0. These three comorbidities alone were also related to the risk of dementia, and OR ranged from 3.4 to 5.3. When combining the ε4 allele and the comorbidities, these three comorbidities presented different patterns of dementia

TABLE 2 Use of logistic regression to predict dementia development between 3 months and 3 years of presence of comorbidities.

	Univariate			Multivariate		
	Odds ratio	**95% CI**	***P*-value[#]**	**Odds ratio**	**95% CI**	***P*-value[#]**
Age (years)	1.00	(0.99–1.01)	0.975	1.00	(0.99–1.01)	0.842
APOE genotype (any e4 allele)						
ε 4/ε 4 &ε 4/ε 3 vs. ε 3/ε 3	1.81	(1.50–2.20)	<0.001	**1.89**	**(1.55–2.31)**	**<0.001**
Headache	1.34	(0.69–2.60)	0.390	0.82	(0.40–1.69)	0.587
Epilepsy	2.78	(1.27–6.11)	**0.011**	1.82	(0.80–4.17)	0.155
CVA	2.82	(2.19–3.62)	**<0.001**	2.22	(1.69–2.90)	**<0.001**
CAD	1.66	(1.23–2.24)	**0.001**	1.43	(1.05–1.96)	**0.023**
Sleep disorder	4.00	(2.95–5.42)	**<0.001**	**3.09**	**(2.23–4.28)**	**<0.001**
Psychiatric disorders	1.74	(1.20–2.53)	**0.004**	1.22	(0.82–1.82)	0.334
Functional GI disorder	3.46	(2.59–4.60)	**<0.001**	**2.73**	**(2.01–3.71)**	**<0.001**
Fibromyalgia	3.33	(2.39–4.65)	**<0.001**	2.12	(1.48–3.04)	**<0.001**
Hypertension	1.22	(1.22–1.44)	**0.021**	1.12	(0.93–1.35)	0.216
Diabetes	1.08	(0.91–1.28)	0.400	1.04	(0.86–1.24)	0.715

[#]Logistic regression.
CVA, cerebrovascular disease; CAD, coronary artery disease; GI, gastrointestinal. Bold values indicated that this item presented statistical significance.

TABLE 3 Multivariate logistic regression predicts dementia development between 3 months and 3 years of presence of comorbidities in different age groups.

	<65 year-old (*n* = 1,122) [Dementia = 102, 9.1%]			**65–75 year-old (*n* = 2,795) [Dementia = 225, 8.1%]**			**>75 year-old (*n* = 2,683) [Dementia = 243, 9.1%]**		
	Odds ratio	**95% CI**	***P*-value[#]**	**Odds ratio**	**95% CI**	***P*-value[#]**	**Odds ratio**	**95% CI**	***P*-value[#]**
APOE genotype			**0.002**			**<0.001**			**<0.001**
ε 4/ε 3 vs.ε 3/ε 3	0.73	(0.38–1.40)	0.345	**1.61**	**(1.18–2.22)**	**0.003**	**2.40**	**(1.76–3.254)**	**<0.001**
ε 4/ε 4 vs.ε 3/ε 3	**6.53**	**(2.20–19.36)**	**0.001**	**6.63**	**(3.09–14.22)**	**<0.001**	**4.06**	**(1.06–15.48)**	**0.040**
Headache	0.43	(0.07–2.58)	0.357	0.71	(0.22–2.28)	0.561	1.23	(0.41–3.72)	0.717
Epilepsy	4.35	(0.64–29.44)	0.132	2.59	(0.86–7.78)	0.090	0.67	(0.08–5.44)	0.711
CVA	**5.05**	**(2.60–9.81)**	**<0.001**	**1.74**	**(1.11–2.72)**	**0.016**	**2.18**	**(1.45–3.27)**	**<0.001**
CAD	1.26	(0.56–2.85)	0.574	1.56	(0.98–2.50)	0.061	1.40	(0.85–2.30)	0.193
Sleep disorder	**4.32**	**(1.99–9.37)**	**<0.001**	**2.56**	**(1.54–4.26)**	**<0.001**	**3.29**	**(1.93–5.59)**	**<0.001**
Psychiatric disorders	2.58	(0.99–6.75)	0.053	1.01	(0.52–1.98)	0.977	1.16	(0.63–2.12)	0.638
Functional GI disorders	**2.43**	**(1.06–5.54)**	**0.035**	**3.04**	**(1.91–4.84)**	**<0.001**	**2.51**	**(1.53–4.12)**	**<0.001**
Fibromyalgia	2.21	(0.88–5.57)	0.092	**2.55**	**(1.50–4.34)**	**0.001**	1.72	(0.93–3.20)	0.085
Hypertension	0.74	(0.44–1.24)	0.253	1.07	(0.81–1.41)	0.651	1.29	(0.96–1.72)	0.093
Diabetes	1.33	(0.82–2.15)	0.253	1.05	(0.79–1.40)	0.721	0.97	(0.73–1.30)	0.849

[#]Logistic regression.
CVA, cerebrovascular disease; CAD, coronary artery disease; GI, gastrointestinal. Bold values indicated that this item presented statistical significance.

risk. Subjects having CVA with or without the ε4 allele show a similar risk of dementia (OR 2.9 vs. 3.4). When functional gastrointestinal disorders and the ε4 allele both occurred, the risk of dementia exceeded the summation of individual risks (OR 3.7 and 1.9 individually, OR 6.0 for the combination), demonstrating a further higher dementia risk. In contrast, the presence of sleep disorders alone showed the highest risk (OR 5.2) compared to the ε4 allele with and without sleep disorders (OR 2.4 and 2.0).

Discussion

This hospital-based case-control study demonstrated the impact of specific diseases/events and the *ApoE* polymorphism on dementia occurrence. This is the first study to explore how clinical and genetic factors affect dementia risk individually and collectively. We combined the ε4 allele and comorbidities as factors to establish a model to predict the occurrence of dementia within 3 years. Therefore, it allows us to screen

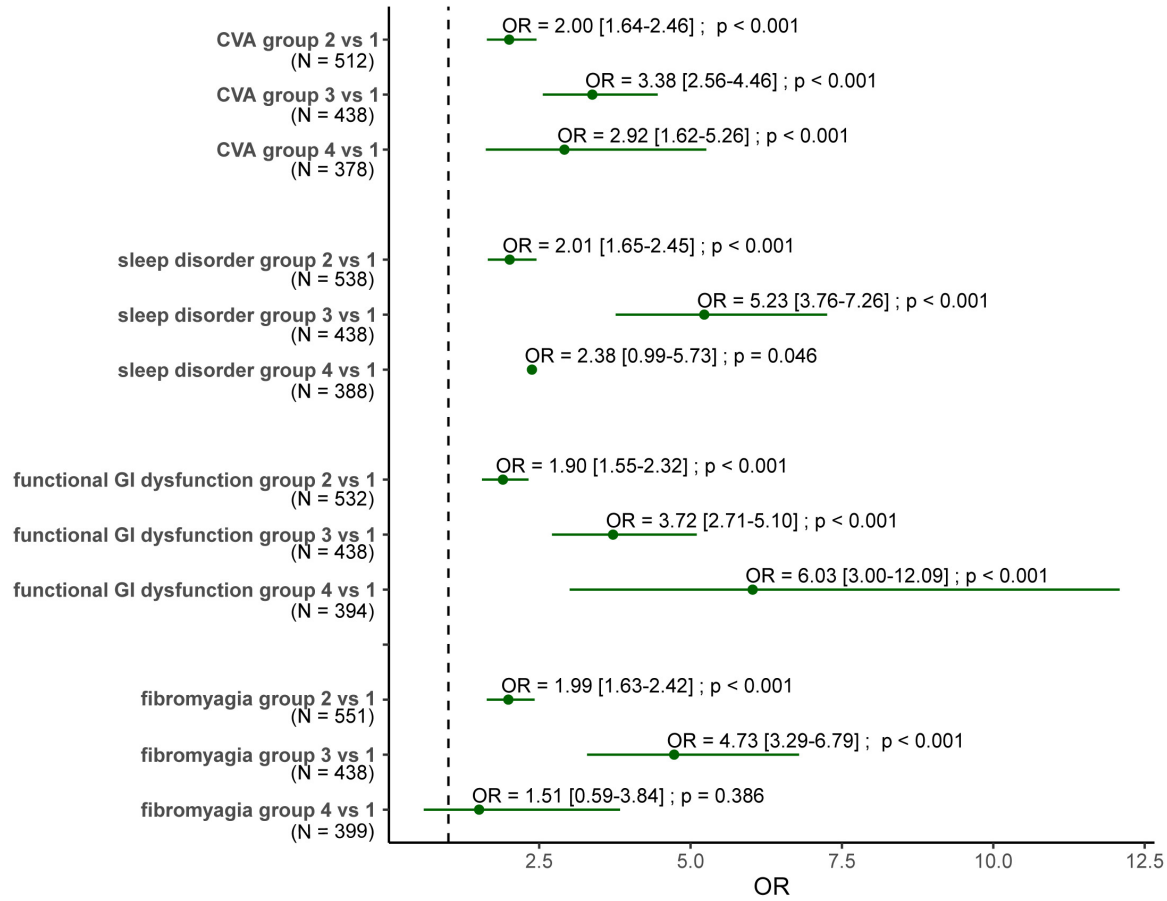

FIGURE 1
Forest plot for the odds ratio of dementia for subjects carrying ε4 allele with/without comorbidities. Group 1: subjects without the ε4 allele nor the comorbidity. Group 2: subjects carrying the ε4 allele without the comorbidity. Group 3: subjects carrying the comorbidity without the ε4 allele. Group 4: subjects carrying both the ε4 allele and the comorbidity. OR, odds ratio; N, the number of the demented cased of these two groups. The numbers within the square brackets are confidence interval.

for dementia and provide early intervention once these risky comorbidities occur in ε 4 carriers.

Among the studied risk factors, the ε4 allele of the *ApoE* genotype is the persistently significant factor to predict dementia throughout all age groups. Our ε4 allele carriers had around two times higher risk for all-cause dementia within 3 years compared with ε3/ε3 carriers, which seemed to be relatively lower compared with previous case-control studies. The OR for subjects carrying at least one ε4 allele was 3.84 for late-onset AD and 1.70 for vascular dementia in Han Chinese (Yang et al., 2001). In Caucasians, the odds ratio for AD was 3.2 in ε3/ε4 and 14.9 in ε4/ε4 compared with the ε3/ε3 allele (Farrer et al., 1997). In contrast to such studies, our non-demented controls were selected, enrolling age- and gender-matched subjects, instead of only non-demented cases. This method avoided the confounding effect of age since the dementia group might be older.

After age stratification, the risk of dementia in ε3/ε4 carriers only emerged after 65 years. However, the ε4/ε4 carriers had a persistently higher dementia risk in all age groups. This phenomenon suggested the additive effect of the ε4 allele, for which the number of ε4 alleles is correlated with the dementia risk and the onset age (Liu et al., 2013). Although *ApoE* polymorphism had been told to show extensive influence from the age of 40–90 years (Farrer et al., 1997), the strength of the risk seemed to change with age. Instead of a reverse U-shaped risk curve for dementia observed in the western literature (Bonham et al., 2016), our heterozygous and homozygous ε4 carriers demonstrated separate trends of the age-dependent risk. The ε3/ε4 carriers presented a slowly upgoing line. In contrast, we found that the dementia risk seemed to reach the peak at the age of 65–75 years in ε4/ε4 carriers, and then the genetic effect declined after 75 years. However, because only 19 dementia cases were diagnosed in our ε4/ε4 carriers, we could not confirm whether the presence of the broken line in homozygous ε4 subjects was due to a smaller sample size. The ε3/ε4 and the ε4/ε4 had different routes for dementia occurrence in Han Chinese. The strategy of risk assessment for dementia screening could

not be the same for these two groups. The ε4 homozygotes need more active surveillance and earlier prevention.

The dementia subjects were reported to carry around 2.4 comorbidities (Schubert et al., 2006), and we found 0.6 comorbidities occurred within 3 years of dementia being diagnosed. Among the listed comorbidities, CVA, sleep disorders, and functional gastrointestinal disorders seemed to be the important risk factors to predict dementia occurrence within 3 years.

Occurrence of CVA or stroke was found to have 1.7–5.0 times of risk for cognitive decline. Stroke has been considered the major risk factor for vascular dementia and AD for a long time (Vijayan and Reddy, 2016). The risk of post-ischemic stroke dementia was 1.7–3.8 (Desmond et al., 2002), and the risk was much higher in ε4 homozygotes (Pendlebury et al., 2020). The incidence of new-onset dementia gradually increased after the index stroke, reporting from 7% in the first year to 10% in the third year, and 23% in the tenth year (Zhu et al., 2000). Stroke leads to intracranial hypoxia, provoking oxidative stress (Slevin et al., 2015), inflammation, and microRNA alteration, which then facilitate and accelerate A-β protein accumulation (Vijayan and Reddy, 2016), and finally, dementia pathogenesis (Brenowitz et al., 2021). We found that the post-stroke dementia risk is highest in the relatively younger group, which appeared in the reverse direction of the stroke incidence and prevalence that increases with age (Sedova et al., 2021). We postulated that the younger brain is more susceptible to the ischemic insult, which might be related to a more fulminant inflammatory response. *ApoE* ε4 also disturbs lipid homeostasis in astrocytes and microglia and then leads to blood-brain barrier failure in stroke patients (Duong et al., 2021). Furthermore, the ε4 allele is also related to a younger stroke age (Lagging et al., 2019) and a higher ischemic stroke risk (Konialis et al., 2016). We found that the ε4 allele and stroke would individually increase the dementia risk, but this risk was not summative when both factors were simultaneously present. Therefore, when subjects had the first event of a stroke, especially at a younger age, it is necessary to keep an eye on the subtle signs of dementia occurrence.

Sleep disturbance showed a 2.5–4.3-times higher risk for future cognitive decline while it was reported to increase the risk of dementia by 50–80% in previous studies (Shi et al., 2018; Brenowitz et al., 2021). On the molecular level, abnormal sleep duration is associated with amyloid-β accumulation (Winer et al., 2021) and sleep-disordered breathing increased tau deposition (Bubu et al., 2019). The dementia risk was also relatively higher in subjects aged less than 65 years in our study, reflecting that the younger brain is vulnerable to such abnormal protein accumulation. However, there was no evidence that *ApoE* genotypes have differential effects on sleep disorders (Palpatzis et al., 2021). This phenomenon explained our finding in **Figure 1** that subjects who had only sleep disorders presented with a significantly higher dementia risk regardless of carrying the ε4 allele. Therefore, sleep disorder itself seems to jeopardize dementia risk more than the *ApoE* genotype does. Thus, sleep disturbance might be a potentially modifiable risk factor to prevent dementia, even if there has been insufficient evidence to confirm it yet.

Gastrointestinal dysfunctions, such as constipation and delayed gastric emptying, are associated with a 2.4–3.0-times higher dementia risk throughout all age groups in our study. Gut microbiota alteration occurred several years before the appearance of cognitive decline (Li et al., 2019), which indicated that the subjects had been exposed to systemic disturbances related to metabolites of the gut microbiota, such as lipopolysaccharide and trimethylamine. They influence the neurological system by affecting neurotransmitters (Zhuang et al., 2020), such as serotonin and GABA, and increasing amyloid-β deposition (Nagpal et al., 2019). Consequently, the theory of the gut-brain axis had been proposed in many neurodegenerative disorders (Martin et al., 2018). However, whether probiotic treatment will prevent dementia development after a change in the intestinal ecology is still unknown (Santiago and Potashkin, 2021). Besides, *ApoE* polymorphism is also associated with the compositions of the gut microbiome (Parikh et al., 2020). This phenomenon is also reflected in our observation. When ε4 allele carriers presented any functional gastrointestinal disorder, their risk for developing dementia within 3 years further increased, exceeding those having any one of the two factors. Among comorbidities, functional gastrointestinal disorders might act as an important red flag sign in ε4 allele carriers. Accordingly, even though gastrointestinal symptoms are usually non-specific, they might be used as a biomarker for early detection and prediction of cognitive decline in ε4 allele carriers in clinical practice.

Cerebrovascular accident, sleep disorders, functional gastrointestinal dysfunctions, and fibromyalgia seem to be the predictors of dementia with variable risk based on age, suggesting the susceptibility of the brain changes with age. Although we could not confirm the cause-effect relationship between dementia development and comorbidity occurrence, the interaction of *ApoE* polymorphism and these clinical illnesses certainly modifies the dementia risk. Combining the genetic and clinical information, we have the odds to early detect cognitive decline and optimize interventions when the subjects first present with a specific illness at a particular age and carry a specific *ApoE* allele.

There were some limitations in our study. First, the diagnosis of dementia and associated diseases was based on the medical records only from our hospital. We could miss the diagnoses which had been made in other hospitals. Second, the illness with a minor severity, such as headaches or gastrointestinal discomforts, could also be ignored if the subjects did not search for medical assistance. Underestimation of the risk related to these illnesses might be possible. Besides, instead of further classifying dementia subtypes, such as vascular dementia and AD, we took all-cause cognitive decline as the

single group for analysis. Furthermore, our sample size was not large enough because only 600 dementia cases were identified. It made the statistical model unstable. We had the preservation to conclude the interaction between the *ApoE* genotype and comorbidities, as well as the dementia risk related to individual comorbidities in three age groups.

Conclusion

Our study demonstrated that dementia patients not only had a higher chance to carry the ε4 allele, but also had a higher prevalence of many chronic illnesses within 3 years before the cognitive decline, including hypertension, headache, epilepsy, CVA, CAD, sleep disorders, psychiatric disorders, functional gastrointestinal disorders, and fibromyalgia. Although *ApoE* polymorphism seems to be the riskiest factor for dementia, the effects of the number of ε4 alleles differ. The ε4 homozygotes presented a persistently high risk for dementia in all age groups, while the risk only emerged after 65 years in ε3/ε4 subjects. Among these comorbidities, the occurrence of CVA, sleep disorders, functional gastrointestinal disorders, and fibromyalgia seemed to be predictive of cognitive decline within 3 years. Besides, functional gastrointestinal disorders might be an important predicting factor for dementia occurrence in ε4 allele carriers.

Data availability statement

The clinical data presented in this study are available on request from the corresponding authors. The genetic data from the Taiwan Precision Medicine Initiative are not publicly available because some access restrictions may apply to the data underlying the findings. The data used in this study cannot be made available in the manuscript, the supplemental files, or in a public repository due to the Personal Information Protection Act executed by Taiwan's government, starting in 2012. Requests for data can be sent as a formal proposal to obtain approval from the ethics review committee of the appropriate governmental department in Taiwan.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Taichung Veterans General Hospital (TCVGH) (CE20316A). The patients/participants provided their written informed consent to participate in this study.

Author contributions

HT, C-HL, Y-MC, and T-HH: work conception and study design. HT and W-JL: data acquisition and collection. T-HS and C-SL: conducting NGS and genomic data analysis. HT and L-SC: clinical data analysis. C-HL and Y-MC: interpretation of data. HT and T-HH: drafting the work. Y-YL and T-HH: revising the work for valuable intellectual content. HT, Y-YL, and T-HH: final approval of the version. 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.

The reviewer Y-CL declared a shared parent affiliation with one of the authors Y-YL to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.927656/full#supplementary-material

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A novel dual-task paradigm with story recall shows significant differences in the gait kinematics in older adults with cognitive impairment: A cross-sectional study

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Objective: Cognitive and motor dysfunctions in older people become more evident while dual-tasking. Several dual-task paradigms have been used to identify older individuals at the risk of developing Alzheimer's disease and dementia. This study evaluated gait kinematic parameters for dual-task (DT) conditions in older adults with mild cognitive impairment (MCI), subjective cognitive decline (SCD), and normal cognition (NC).

Method: This is a cross-sectional, clinical-based study carried out at the Zhongshan Rehabilitation Branch of First Affiliated Hospital of Nanjing Medical University, China.

Participants: We recruited 83 community-dwelling participants and sorted them into MCI (n=24), SCD (n=33), and NC (n=26) groups based on neuropsychological tests. Their mean age was 72.0 (5.55) years, and male–female ratio was 42/41 (p=0.112). Each participant performed one single-task walk and four DT walks: DT calculation with subtracting serial sevens; DT naming animals; DT story recall; and DT words recall.

Outcome and measures: Kinematic gait parameters of speed, knee peak extension angle, and dual-task cost (DTC) were obtained using the Vicon Nexus motion capture system and calculated by Visual 3D software. A mixed-effect linear regression model was used to analyze the data.

Results: The difference in gait speed under DT story recall and DT calculation was −0.099 m/s and −0.119 m/s (p=0.04, p=0.013) between MCI and SCD, respectively. Knee peak extension angle under DT story recall, words recall, and single task was bigger in the MCI group compared to the NC group, respectively (p=0.001, p=0.001, p=0.004). DTC was higher in the DT story recall test than all other DT conditions (p<0.001).

Conclusion: Kinematic gait parameters of knee peak extension angle for the DT story recall were found to be sensitive enough to discriminate MCI individuals from NC group. DTC under DT story recall was higher than the other DT conditions.

KEYWORDS

mild cognitive impairment, subjective cognitive decline, motor dysfunctions, dual-tasking, kinematics, gait

Introduction

Aging is associated with an increased risk of physical and cognitive decline, which can lead to cognitive and motor dysfunction (Anton et al., 2015, p. 58). By 2050, the number of people of aged 65 and older with Alzheimer's disease and dementia that will significantly contribute to disability and loss of independence is projected to reach 12.7 million worldwide (Wollesen et al., 2019). The human gait pattern is affected by age and cognitive decline. For example, older individuals walk slowly, have a shorter step and stride length, wider steps, and high gait variability (Li and Lindenberger, 2002; Herssens et al., 2018). In older people, safe walking and maintaining a proper speed require intact cognition and executive control and is an indicator of general health and survival. This is because the sensorimotor aspect of walking requires a high degree of attention and cognitive control (Cullen et al., 2019). A growing body of evidence suggests that gait impairment is clinically significant and can predict cognitive decline earlier than cognitive tests (Montero-odasso et al., 2005; Porta et al., 2020).

Understanding the relationship between gait and cognitive impairment has broad public health implications for the aging population (Al-Yahya et al., 2011). The activities of daily life usually involve simultaneous cognitive and motor performance or dual-tasking. Such activities like walking while talking and avoiding obstacles or making turns, become challenging with advancing age (Mancioppi et al., 2020). Dual-task performance can predict the deterioration of gait and cognitive decline in people with neurological deficits. Studies have shown that the slowing of gait during dual-tasking can differentiate healthy individuals from people with neurological problems such as pre-dementia or mild cognitive impairment (MCI; Dubost et al., 2006). Poor dual-task (DT) performance was also found to be associated with an unstable gait and a high risk of fall in the frail elderly, and has been considered a predictor of future fall (Fuentes-Abolafio et al., 2020). Recently, it has been found that motor impairments precede cognitive impairment and that early motor changes such as gait speed and dual-task cost (the percentage difference between single and dual-task performance in cognitive and/or motor tasks) are potential biomarkers for the progression of cognitive decline from MCI to Alzheimer's disease (Montero-Odasso and Perry, 2019; Bishnoi and Hernandez, 2021). With advancing age and deteriorating physical functions, older adults become heavily dependent on cognitive reserve (Bishnoi and Hernandez, 2021). Many studies suggest that increased cognitive demand under DT conditions increases the sensitivity of gait assessment (Ramírez and Gutiérrez, 2021). Thus, gait dysfunction in combination with memory, execution, and attention-demanding tasks may be used to predict and distinguish individuals with pathological cognitive decline from healthy individuals. Several DT paradigms, such as walking and simultaneously performing arithmetic (counting, subtracting), verbal (calling animal names), and memory (words recall) tasks, have been used to investigate the interaction between gait and cognition (Montero-Odasso et al., 2017; Åhman et al., 2020). A recent systemic review showed the mental tracking tasks including serial subtraction and verbal fluency were the most sensitive in detecting MCI-related changes in older adults (Bishnoi and Hernandez, 2021). Although the "words per time unit" outcomes of DT tests including Timed-Up-and-Go (TUGdt), i.e., "animals/10 s" and "months/10 s" were found to have high levels of discrimination between dementia, MCI, subjective cognitive decline (SCD), and normal cognition (NC) groups, the DTC showed no difference among groups (Åhman et al., 2020). Another study found that DT parameters under words recall cannot distinguish MCI from normal elderly either (Jayakody et al., 2020).

Studies indicated that functional changes in gait can be easily identified through kinematic analysis (Muir et al., 2012; Beauchet et al., 2016). They have found that gait kinematics of the lower limb changes with cognitive decline and become worse with the progression of the disease. Another study has found that gait speed was associated with immediate recall memory in older adults (Sebastiani et al., 2020). Spatiotemporal gait variations using the DT

paradigm are well studied in MCI patients, and most studies have reported changes in gait speed under those conditions (Mintun et al., 2021), (Montero-Odasso et al., 2020). Fuentes-Abolafio et al. (2021) have reported that MCI patients have higher variability in kinematic parameters compared to healthy adults. However, further studies are needed to find which kinematic parameters are sensitive enough to discriminate people with MCI from healthy individuals. Furthermore, changes in joint kinematics between single and dual-tasking have not been reported, and such observations could be relevant for targeting specific interventions for the prevention of functional and cognitive decline. Since memory is typically impaired in people with MCI, dual-tasking involving memory tasks may help to distinguish MCI patients from healthy individuals. In our previous study, story recall has a higher DTC compared to words recall in MCI and normal cognitive elderly, and DTC of words recall in MCI group was significantly higher than it in the NC group (Zhu et al., 2020). The difference of DTC under DT story recall failed to reach a significance level, which may be due to a small sample size. We therefore hypothesize that (1) a novel dual-tasking with story recall can distinguish MCI patients from healthy individuals better than the other DTs including calculation, naming animals, and words recall, and (2) joint kinematic parameters under a DT conditions are different in MCI and SCD patients compared to healthy older adults. The aims of this study were to identify the significance of DT paradigm with story recall in older adults and to assess whether kinematic gait parameters such as gait speed, knee peak extension angle, and DTC can differentiate patients with MCI from SCD and cognitively normal older adults.

Methodology

Participants selection criteria

Older adults from the local community were recruited if they: (1) were 55–85 years old; (2) had no neurological disease such as stroke, severe head injury, or cerebral tumor; (3) had no lower limb functional mobility issues, fractures, diabetic foot, or severe arthritis; (4) had no severe cardiopulmonary problems; (5) had no serious liver or kidney dysfunction; and (6) had received primary education or above. Participants were excluded if they had any of the following conditions: (1) had structural abnormalities such as brain tumor, subdural hematoma, head trauma, or a neurological or psychiatric disorder that could impact cognitive functions; (2) had severe depression or were unable to participate in cognitive function tests or gait analysis; or (3) had communication problems such as deafness, blindness, or language problems.

Screening and recruitment

Sample size calculation

The sample size was calculated using PASS 15 with repeated measures analysis procedure. The outcome was DTC. The mean DTC of DT calculation, DT naming animals, DT story memory, and DT words memory were 0.14, 0.14, 0.19, and 0.12 which were between subject effect, the mean DTC of MCI, SCD, and NC group were 0.15, 0.1, and 0.1 which was within-subject effect. The standard deviation of effects was set 0.02, the between-subject standard deviation was 0.1 and the auto correlation was 0.2. To achieve 80% power at a 2-sided significance level of 5%, the sample size of each group was 25, and the overall sample size was 75. Considering 5% of withdraw from the study, the sample size of each group was 26, and the overall sample size was 78.

Participants were screened by a neuropsychologist from July 2020 to June 2021 at the memory clinic of the First Affiliated Hospital of Nanjing Medical University. For this cross-sectional study, the screened individuals were recruited if they met the diagnostic criteria for MCI, SCD, or NC and provided written consent. The Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR) scores were used to exclude dementia and Alzheimer's disease patients (Folstein et al., 1975; Morris, 1993; Lam et al., 2008). The Hachinski ischemic score (HIS) was also administered to exclude vascular mild cognitive impairment or dementia (Hachinski et al., 2012).

The cognitive status of the participants was assessed on three cognitive domains: (1) memory (delayed recall and delayed recognition score based on the Huashan version of the auditory-verbal learning test, AVLT-H; Zhao et al., 2012); (2) speed/executive function (time spent on Trial Making Tests, TMT-A, and TMT-B; Salthouse, 2011); and (3) language function (verbal fluency test and Boston Naming Test, BNT; Stålhammar et al., 2015). Furthermore, depression was assessed using the Chinese version of the Geriatric Depression Scale (GDS-30; Chau et al., 2006).

The diagnostic criterion for MCI was based on the above neuropsychological tests (Bondi et al., 2014), recommendations for diagnosis and treatment of preclinical Alzheimer's disease in China, and having memory complaints for more than 6 months (Han, 2018). In addition, a self-reported questionnaire was used to distinguish SCD from NC individuals according to the suggestions of the SCD-Initiative working group.

Participants were considered to have MCI if they had at least one of the following: (1) two impaired scores on any two scales of the three cognitive domains (memory, speed/executive function, or language of >1 SD below the age-corrected normative means) or (2) one impaired score in each of the three scales of cognitive domains (memory, speed/executive function, or language, >1 SD below the age-corrected normative mean in each of the three cognitive domains). The normative means selected in this study are taken from Chinese population studies as described by Li et al. (2019).

Individuals were considered to be SCD if they met the following criteria (Slot et al., 2018; Cullen et al., 2019): (1) had a self-reported persistent decline in the memory domain of cognition for more than 6 months; (2) had concerns about memory loss and feeling of deteriorating performance compared to individuals of the same age group; (3) had worse performance on standard cognitive tests adjusted for age, gender, and education; and (4) did not meet MCI or dementia diagnostic criteria.

The inclusion criteria for healthy individuals (NC) were as follows: (1) they had no complaints of cognitive impairment or memory loss, and (2) they did not meet SCD or MCI diagnostic criteria.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (also named Jiangsu Province Hospital; Approval Number: 2019-SR-015). All of the participants provided written consent.

Motion capture and gait assessment of the ST and DT walking

All participants completed one ST and four DT walking tasks. For the ST, participants were asked to walk at their usual pace in a quiet, well-lit room wearing comfortable footwear and without the use of any mobility aids. For the DTs, participants walked at their usual pace while also performing the following cognitive tasks aloud: DT calculation, DT naming animals, DT story recall, and DT words recall. In DT calculation, participants were required to count down from 100, 90, 80, and 70 by serial 7 s while walking. In DT naming animals, participants were asked to say out loud as many names of animals as possible while walking. In DT story recall, participants were required to repeat a short story while walking, narrated to them at the beginning of the test. In DT words recall, participants were asked to repeat five Chinese words (narrated at the beginning of the test) during walking. These four DT paradigms were repeated three times for each participant to obtain at least ten gait cycles of data for each participant.

Gait observation of the participants was carried out at the Gait Lab in the Zhongshan Rehabilitation Branch of First Affiliated Hospital of Nanjing Medical University. A Vicon Nexus 2.8 (with 12 cameras, Vantage5, Vicon Nexus2.8, Oxford Metrics, Oxford, United Kingdom) motion capture system was used to collect movement data. The Conventional Gait Model 2 (CGM 2.3 vision), an open-source biomechanical model with 51 markers, was used to capture the gait data. These markers were attached to different parts of the body, the details of which have been previously published (Zhong et al., 2021). Participants were instructed to walk at their usual speed on a 10 m walking path. To reduce the impact of acceleration/deceleration and turning on walking speed, the 2-m window at the beginning and end of the walking test was not included in the final data collection. To minimize the effects of fatigue, participants were allowed 2–3 min rest between the tasks. Time taken by the subjects during the middle 6 m window was noted and retained by the motion capture system to obtain gait kinematics for further analysis.

Kinematic analyses of gait data

Gait kinematic parameters and average speed were processed using Visual 3D software (C-motion Inc., Rockville, MD, United States), and kinematic variables were recorded for right and left legs separately. We further used the captured motion to define heel contact and toe-off for stride and step identification, as well as joint angle identification between the shank and thigh in the sagittal plane. We also used it to calculate the average level waking speed, knee peak extension angle, and DT cost. Dual-task cost (DTC) was obtained using gait speed for each individual in all dual-task conditions. DTC is the measure of reduced walking performance (slowing of gait speed) due to cognitive-motor interference while dual-tasking. It is the percentage of decrement in performance between ST and DTs. DTC was calculated using the gait speed under ST and DT with the following formula: DTC = [(ST gait speed – DT gait speed)/ST gait speed] (Cullen et al., 2019).

Statistical analysis

The demographic characteristics of the participants are described in Table 1. Categorical variables are presented as proportions and were compared using the χ^2 test. Continuous variables are shown as the mean, median and interquartile range, as well as standard deviation and confidence interval (minimumand maximum), and their distribution was examined using the Wilcoxon rank-sum test. We used a linear mixed, random-effects model, a random slope (for different tasks), and unstructured correlation to estimate change in gait parameters under different tasks and cognitive status. Gait parameters, i.e., gait speed, knee peak extension angle, and DTC were considered as dependent variables, while various tasks and cognitive status as independent variables. We had pre-selected, gender, age, body mass index (BMI), diabetes, GDS score, and years of education as potential covariates, based on the literature review and our previous findings. A two-sided $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the statistical software SAS 9.4.

Results

Figure 1 shows the recruitment flow chart. At recruitment, 181 older adults were screened and 136 met the inclusion criteria. A total of 83 men and women (50% each) aged 65–83 years old initially signed up for the study. However, 53 people were excluded due to loss of contact ($n = 20$) and refused to sign the consent ($n = 33$). The reasons of not signing the consent are (1) lived too far away ($n = 14$), (2) moving to another place ($n = 2$), and (3) short of time ($n = 17$). The descriptive statistics of participants' cognitive status and demographic characteristics are presented in Table 1. Out of 83 individuals recruited for this study, 24 were diagnosed with MCI, 33 with SCD, and 26 had normal cognition. There was no significant difference in age among the three groups, and the average age for each group was as follows: MCI 71.0 (6.42), SCD 72.7 (5.25), and NC 71.9 (5.09) ($p = 0.497$). Gender was generally balanced among the three groups, with men making up 50% of the MCI group, 45.5%

TABLE 1 Baseline characteristics of the participants.

Variables	MCI ($N=24$)	SCD ($N=33$)	NC ($N=26$)	Total ($N=83$)	Value of p
Sex, *n* (%)					
Male	12 (50.0)	15 (45.5)	15 (57.7)	42 (50.6)	0.112
Female	12 (50.0)	18 (54.5)	11 (42.3)	41 (49.4)	
Age, years					
N (Nmiss)	24 (0)	33 (0)	26 (0)	83 (0)	
Mean (STD)	71.0 (6.42)	72.7 (5.25)	71.9 (5.09)	72.0 (5.55)	0.497
Median	69.0	72.0	71.0	71.0	
p_{25} ~ p_{75}	67.5 ~ 76.0	70.0 ~ 76.0	69.0 ~ 75.0	68.0 ~ 76.0	
BMI，kg/m^2					
N (Nmiss)	24 (0)	33 (0)	26 (0)	83 (0)	
Mean (STD)	24.8 (2.94)	24.0 (2.76)	24.9 (3.18)	24.5 (2.94)	0.416
Median	24.4	24.0	23.8	24.0	
p_{25} ~ p_{75}	22.7 ~ 27.8	22.6 ~ 25.1	23.3 ~ 26.1	22.6 ~ 26.0	
Diabetes, *n*(%)					
No DM	21 (87.5)	25 (75.8)	22 (84.6)	68 (81.9)	0.553
DM	3 (12.5)	8 (24.2)	4 (15.4)	15 (18.1)	
GDS score					
N (Nmiss)	24 (0)	33 (0)	24 (2)	81 (2)	
Mean (STD)	8.3 (6.00)	9.1 (4.84)	5.4 (4.87)	7.8 (5.39)	0.029
Median	6.0	8.0	4.0	6.0	
p_{25} ~ p_{75}	5.0 ~ 10.0	6.0 ~ 11.0	2.5 ~ 7.0	4.0 ~ 10.0	
Education, years					
N (Nmiss)	24 (0)	33 (0)	26 (0)	83 (0)	
Mean (STD)	11.8 (2.94)	13.2 (2.35)	12.9 (2.52)	12.7 (2.62)	0.119
Median	10.5	14.0	12.0	12.0	
p_{25} ~ p_{75}	9.0 ~ 15.0	12.0 ~ 15.0	12.0 ~ 15.0	9.0 ~ 15.0	
AVLT-H delayed recall					
N (Nmiss)	24 (0)	33 (0)	26 (0)	83 (0)	
Mean (STD)	2.1 (2.12)	4.3 (2.45)	4.4 (1.60)	3.7 (2.34)	.
Median	1.5	4.0	4.0	4.0	< 0.001
p_{25} ~ p_{75}	0.0 ~ 4.0	3.0 ~ 6.0	3.0 ~ 6.0	2.0 ~ 5.0	
AVLT-H recognition					
N (Nmiss)	24(0)	33 (0)	26 (0)	83 (0)	
Mean (STD)	18.0(3.20)	20.6 (2.38)	22.0 (1.59)	20.3(2.90)	
Median	18.0	21.0	22.0	21.0	< 0.001
p_{25} ~ p_{75}	15.5 ~ 20.5	19.0 ~ 22.0	21.0 ~ 23.0	19.0 ~ 22.0	
TMT-A					
N (Nmiss)	24(0)	33(0)	26 (0)	83 (0)	
Mean (STD)	93.5(38.22)	70.5(24.88)	56.7 (16.77)	72.8 (30.77)	
Median	89.0	64.0	52.5	65.0	<0.001
p_{25} ~ p_{75}	74.5 ~ 101.5	54.0 ~ 80.0	44.0 ~ 69.0	52.0 ~ 90.0	
TMT-B					
N (Nmiss)	23(1)	33(0)	26(0)	82(1)	
Mean (STD)	222.7(53.71)	182.5(57.91)	147.7(49.70)	182.7(60.96)	
Median	220.0	189.0	136.0	180.0	<0.001
p_{25} ~ p_{75}	182.0 ~ 260.0	141.0 ~ 200.0	112.0 ~ 173.0	134.0 ~ 216.0	
BNT					
N (Nmiss)	24(0)	33(0)	26 (0)	83 (0)	
Mean (STD)	20.7(4.03)	23.2(3.61)	24.6 (3.02)	22.9 (3.85)	
Median	20.5	23.0	26.0	23.0	0.002
p_{25} ~ p_{75}	17.5 ~ 24.0	22.0 ~ 26.0	23.0 ~ 27.0	20.0 ~ 26.0	

(Continued)

TABLE 1 (Continued)

Variables	MCI ($N=24$)	SCD ($N=33$)	NC ($N=26$)	Total ($N=83$)	Value of p
VFT					
N (Nmiss)	24(0)	33(0)	26(0)	83(0)	
Mean (STD)	16.2(5.46)	18.0(4.29)	21.1 (3.69)	18.5 (4.85)	
Median	16.0	17.0	20.5	19.0	<0.001
$p_{25} \sim p_{75}$	12.0 ~ 20.0	14.0 ~ 21.0	19.0 ~ 24.0	15.0 ~ 21.0	
MMSE					
N (Nmiss)	24(0)	33(0)	26 (0)	83 (0)	
Mean (STD)	26.6(1.74)	27.0(1.95)	28.3 (1.61)	27.3 (1.90)	
Median	27.0	27.0	28.5	28.0	0.002
$p_{25} \sim p_{75}$	25.0 ~ 28.0	26.0 ~ 28.0	27.0 ~ 30.0	26.0 ~ 29.0	
MOCA					
N (Nmiss)	24 (0)	33 (0)	26 (0)	83 (0)	
Mean (STD)	22.4 (3.09)	23.2 (3.06)	27.0 (2.13)	24.1 (3.39)	
Median	22.5	23.0	27.0	25.0	<0.001
$p_{25} \sim p_{75}$	20.0 ~ 24.5	21.0 ~ 25.0	27.0 ~ 28.0	21.0 ~ 27.0	

MCI, mild cognitive impairment; SCD, subject cognitive decline; NC, normal cognition; BMI: body mass index; HIS, Hachinski Ischemic Scale; AVLT-H, Auditory Verbal Learning Test—Huashan version. TMT, Trail Making Test; BNT, Boston Naming Test; AVFT, Animal Verbal Fluency Test; MMSE, Mini-mental State Examination; MoCA, Montreal Cognitive Assessment.

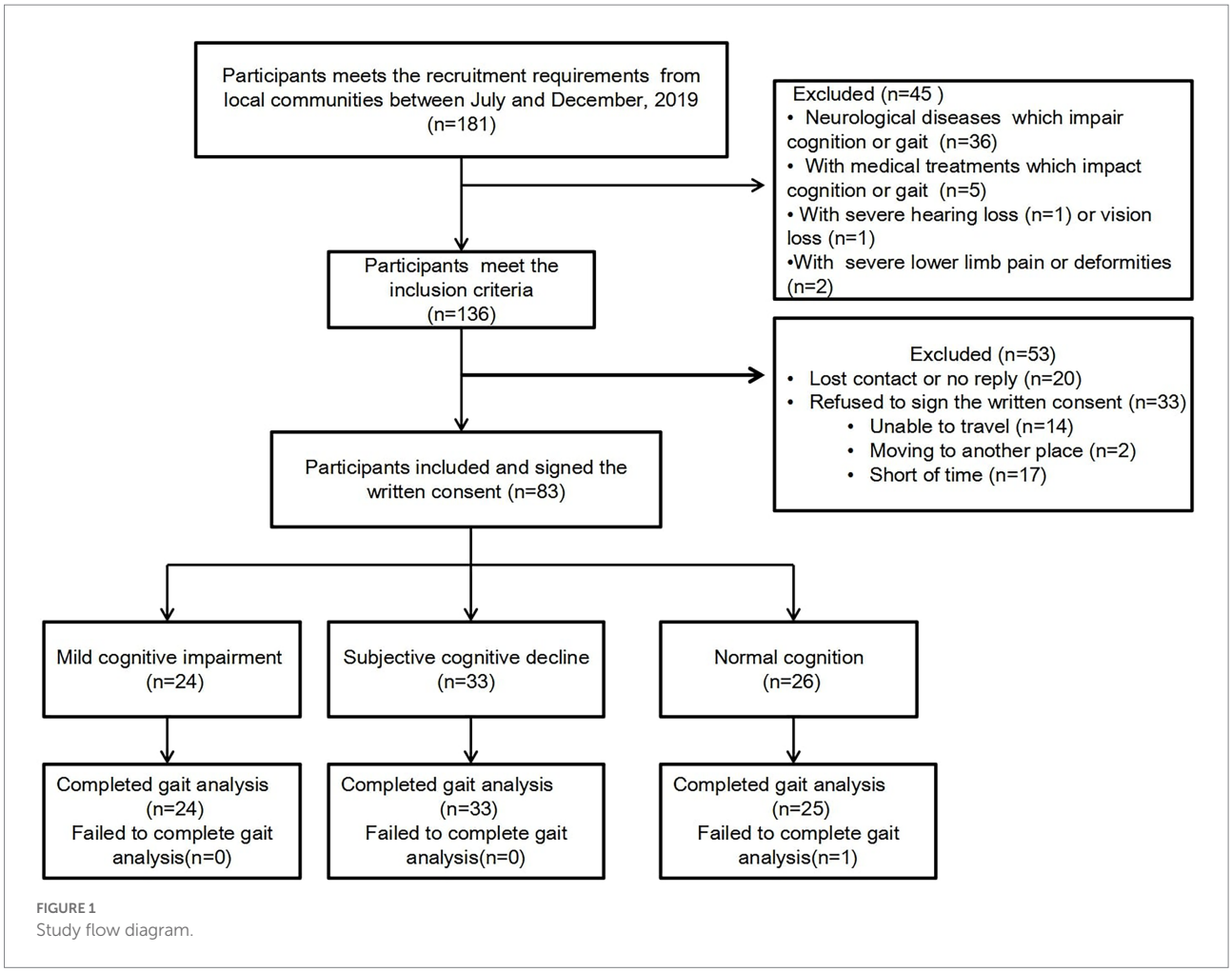

FIGURE 1
Study flow diagram.

of the SCD group, and 60% of the NC group ($p = 0.112$). The GDS scores were different among the groups: MCI 8.3 (6.00), SCD 9.1 (4.84), and NC 5.4 (4.87) ($p = 0.029$). The majority of the participants had 12 or more years of education. Demographic characteristics and comorbidities were balanced among the three groups. Finally, there were no significant differences with respect to age, gender, or BMI among the groups. All the cognitive assessments showed significant differences among three groups ($p < 0.05$; Table 1).

Gait kinematic parameters of participants with MCI, SCD, and NC

We estimated the adjusted mean value for gait speed, knee peak extension angle, and DTC of different interventions, as well as the severity of cognitive disorder by the mixed-effect linear regression model and the results are shown in Table 2.

Gait speed

The results of mixed-effect linear regression model analysis showed a significant effect ($p < 0.001$) of task-adjusted gait speed for DT story recall and was the slowest compared to all other DTs and ST in all the groups (MCI, SCD, and NC; Table 2). The adjusted gait speed under single task was faster than 1 m/s in all the three groups. Additionally, the adjusted gait speed for the MCI group was the slowest compared to the SCD and NC groups, under all DT walking conditions.

We also found a difference in gait speed under DT calculation between the MCI and NC groups [−0.103 (95%CI: −0.202, −0.004), $p = 0.043$], and between the MCI and SCD groups [−0.119 (95%CI: −0.213, −0.026), $p = 0.013$]. Although the statistical significance disappeared after adjusting for multiple comparisons, a difference in gait speed of more than 0.1 m/s can be considered clinically meaningful. Furthermore, the difference in gait speed of the DT story recall between the MCI and SCD groups was also significant (−0.099 (95%CI: −0.193, −0.005), $p = 0.04$). On the other hand, the difference in gait speed for the DT naming animals, DT words recall, and ST between the MCI, SCD, and NC groups was not significant (Table 2).

Knee peak extension angle

The knee peak extension angle was bigger in the MCI group compared to the SCD and NC groups under all DT and ST conditions (Table 2). We had also observed a significant difference in the knee peak extension angle under the DT story recall and DT words recall, which could distinguish MCI from NC ($p = 0.001$). Furthermore, a significant interaction effect of task and cognitive status was observed ($p = 0.021$). Figure 2 shows knee peak extension angles for the three groups under different task conditions. While there was no difference between the SCD and NC groups under the DT calculation and DT naming animals, we did observe a significant difference between the SCD and NC groups for the DT story recall and DT naming animals (3.901 (95%CI: 1.148, 6.655), $p = 0.006$), as well as a difference between the SCD and NC groups for the DT story recall and DT calculation (2.901 (95%CI: −0.302, 6.104), $p = 0.075$).

Dual-task cost

A significant effect of task on DTC was observed ($p < 0.001$) in all the groups, but no significant differences of DTC under each task were found among three groups. The difference of DTC under story recall was noticeable as −0.058 [95%CI: (−0.12, 0.004), $p = 0.066$] between SCD and NC group. Meanwhile, the DTC was higher under the DT story recall compared to DT calculation, DT naming animals, and DT words recall (Table 2). The difference in DTC between the story recall and calculation dual-tasks was 0.068 [95%CI: (0.047, 0.090), $p < 0.001$]. Furthermore, the difference in DTC between DT story recall and DT naming animals was 0.035 (95%CI: 0.019, 0.051, $p < 0.001$), and the difference between DTC for DT story recall and DT words recall was 0.067 (95%CI: 0.048, 0.086, $p < 0.001$; Figure 3).

Discussion

In this study, we found that a novel gait parameter under DT conditions was effective in discriminating MCI patients from healthy controls. Gait kinematics, especially knee peak extension angle, was significantly bigger in MCI group compared to NC group under the DT story recall, DT words recall, and ST. We also found that DTC was significantly higher under the DT story recall compared to all other DT paradigms. The key findings in gait kinematics could be an important step forward in developing clinically validated measures for MCI-related functional deficits, and could aid in the early diagnosis of cognitive disease (Ghoraani et al., 2021).

Our results showed slower gait speed under DT condition compared to ST condition in MCI, SCD, and NC individuals, which is in line with the previous findings. Ghoraani et al. (2021), Montero-Odasso et al. (2020), and Ramírez and Gutiérrez (2021) have all shown that slowed gait speed while dual-tasking can not only differentiate MCI from NC individuals, but can also predict its progression to dementia. A recent study showed that most of the spatiotemporal gait variables could discriminate between dementia and cognitively intact individuals under single and dual tasks (Bovonsunthonchai et al., 2022). The DT in this study is counting backward which is similar to the DT calculation in our study. We found gait speed under DT calculation and DT story recall could distinguish MCI from NC group as well. Furthermore, our new finding is that gait speed under DT calculation could also distinguish MCI from SCD group, and gait speed under DT story recall could distinguish MCI from NC group. However, our results showed that the gait speed under ST, DT naming animals, and DT

TABLE 2 The adjusted mean for speed, Knee extension angle, and DTC of different DT conditions and severity of cognitive disorder*.

Gait parameter	Task	MCI[a] (*N* = 24)	SCD[a] (*N* = 33)	NC[a] (*N* = 25)	Total[a] (*N* = 82)	MCI-SCD[b]	*p*	MCI-NC[b]	*p*	SCD-NC[b]	*p*
Speed (m/s)											
	DT calculation	0.865 (0.039)	0.984 (0.032)	0.968 (0.039)	0.939 (0.024)	−0.119 (−0.213,-0.026)	**0.013**	−0.103 (−0.202,-0.004)	**0.043**	0.016 (−0.08,0.113)	0.733
	DT naming animals	0.877 (0.039)	0.948 (0.033)	0.888 (0.039)	0.904 (0.024)	−0.07 (−0.165,0.025)	0.145	−0.011 (−0.112,0.09)	0.83	0.059 (−0.038,0.157)	0.23
	DT story recall	0.831 (0.039)	0.93 (0.032)	0.844 (0.039)	0.869 (0.024)	−0.099 (−0.193,-0.005)	**0.04**	−0.013 (−0.113,0.087)	0.794	0.085 (−0.011,0.182)	0.082
	DT words recall	0.897 (0.038)	0.974 (0.032)	0.945 (0.038)	0.939 (0.024)	−0.077 (−0.169,0.016)	0.103	−0.047 (−0.146,0.051)	0.338	0.029 (−0.066,0.124)	0.544
	ST	1.039 (0.044)	1.108 (0.037)	1.064 (0.044)	1.07 (0.026)	−0.069 (−0.177,0.039)	0.208	−0.025 (−0.14,0.09)	0.666	0.044 (−0.066,0.154)	0.431
	P for interaction	0.089									
	P for task	**<0.001**									
	P for cognitive status	0.1560									
Knee peak extension angle (degree)											
	DT calculation	−0.096 (1.318)	−2.939 (1.109)	−3.403 (1.319)	−2.146 (0.78)	2.843 (−0.444,6.131)	0.089	3.308 (−0.206,6.821)	0.065	0.464 (−2.881,3.809)	0.783
	DT naming animals	−0.183 (1.261)	−3.512 (1.06)	−2.976 (1.262)	−2.224 (0.751)	3.329 (0.198,6.46)	**0.037**	2.793 (−0.551,6.136)	0.1	−0.537 (−3.728,2.655)	0.738
	DT story recall	0.168 (1.24)	−1.978 (1.042)	−5.343 (1.254)	−2.384 (0.743)	2.146 (−0.925,5.217)	0.168	5.511 (2.211,8.811)	**0.001**	3.365 (0.209,6.521)	**0.037**
	DT words recall	−0.096 (1.11)	−2.425 (0.925)	−5.077 (1.11)	−2.533 (0.674)	2.33 (−0.375,5.034)	0.09	4.981 (2.097,7.866)	**0.001**	2.652 (−0.121,5.425)	0.061
	ST	−0.557 (1.121)	−3.404 (0.939)	−4.643 (1.137)	−2.868 (0.683)	2.847 (0.106,5.587)	**0.042**	4.086 (1.143,7.03)	**0.007**	1.24 (−1.596,4.076)	0.386
	P for interaction	**0.021***									
	P for task	0.79									
	P for cognitive status	**<0.01**									
DTC											
	DT calculation	0.146 (0.027)	0.094 (0.022)	0.093 (0.027)	0.111 (0.016)	0.052 (−0.013,0.118)	0.115	0.052 (−0.017,0.122)	0.138	0 (−0.067,0.067)	0.998
	DT naming animals	0.141 (0.026)	0.129 (0.021)	0.162 (0.026)	0.144 (0.016)	0.012 (−0.051,0.074)	0.71	−0.021 (−0.087,0.046)	0.541	−0.032 (−0.096,0.032)	0.32
	DT story recall	0.188 (0.025)	0.146 (0.021)	0.204 (0.025)	0.179 (0.015)	0.042 (−0.019,0.102)	0.172	−0.016 (−0.081,0.048)	0.616	−0.058 (−0.12,0.004)	0.066
	DT words recall	0.12 (0.025)	0.102 (0.021)	0.114 (0.025)	0.112 (0.015)	0.018 (−0.043,0.079)	0.563	0.006 (−0.058,0.071)	0.845	−0.011 (−0.074,0.051)	0.718
	P for interaction	0.055									
	P for task	**<0.001**									
	P for cognitive status	0.49									

*adjusted variable: gender, age, BMI Diabetes, GDS score, Education year. DT: dual-task, ST: single task, DTC: dual-task cost.

[a]Variable expressed as mean (se).

[b]Variable expressed as mean (95% CI).

Values in bold are statistically significant, i.e., *p*-values < 0.05.

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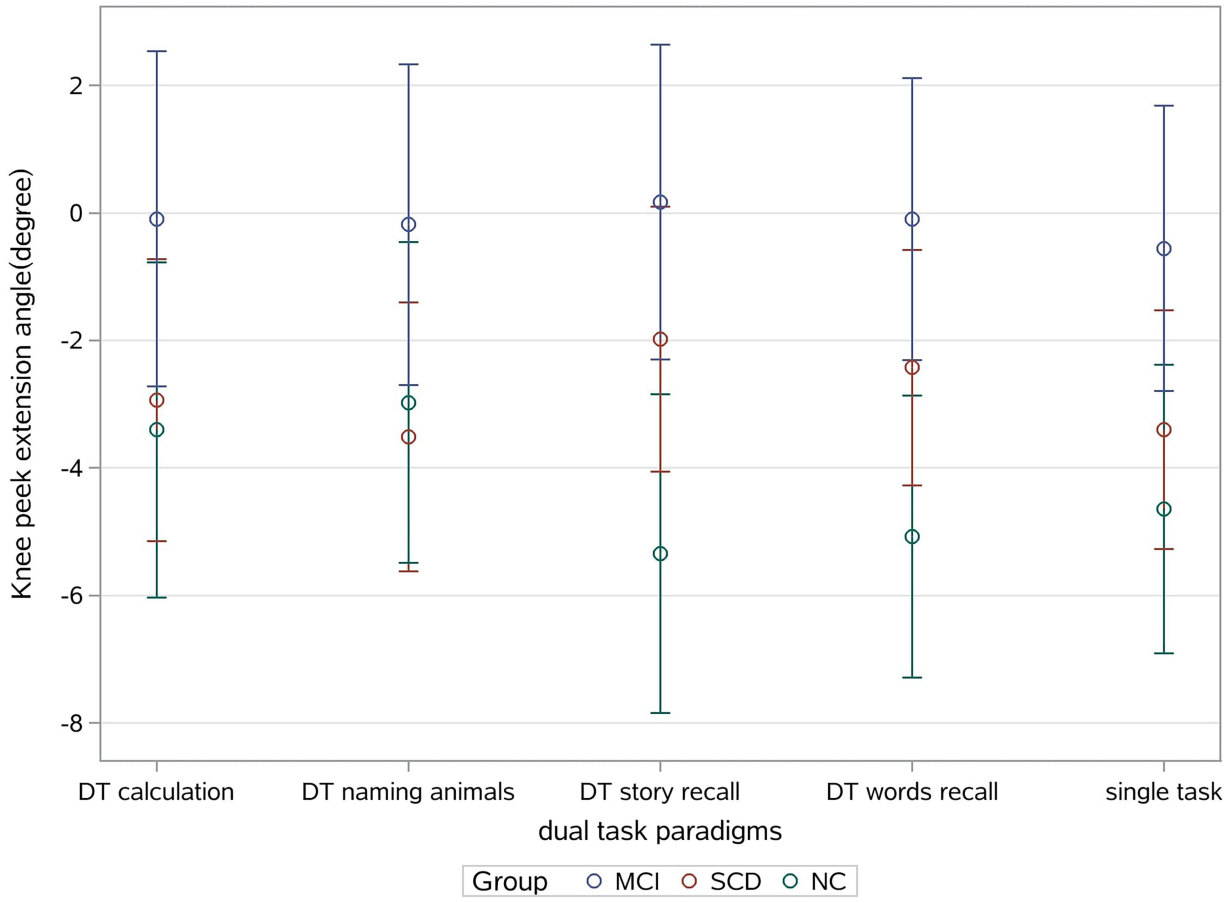

FIGURE 2
Knee peak extension angle and cognitive status of participants.

words recall could not distinguish different groups. Those findings indicated that different cognitive tasks have diverse interferences on walking performance, which could be affected by severity of cognitive impairment and the deficits of different cognitive domains. Further studies are needed to investigate the gait interference of memory tasks in Alzheimer's dementia and SCD population.

Our previous work has shown that knee kinematics during level walking are significantly different in patients with MCI and NC (Zhong et al., 2021), and our new finding regarding knee peak extension angle under DT story and words recall could significantly differentiate MCI from NC group. This differences of knee joint angle are around 5°, which is clinically noticeable and meaningful. A bigger knee extension angle indicated worse knee control during standing phase, which might aggravate the walking instability and increase the falling risk of MCI patients. Reduced knee extension during stance phase was found in elderly individuals, suggesting that they favored a flexed-knee gait possibly either to give assistance in weight acceptance or to increase knee joint stability (Begg and Sparrow, 2006). However, an impairment of cognition may eliminate this age-related adaptation, leading to worse knee control during DT walking. The peak knee extension angle was found to be highly correlated with walking performance and self-reported disability in elderly with osteoarthritis (Maly et al., 2006), and its clinical significance in patients with MCI was firstly reported by our team. Therefore, functional assessments for MCI should not only include cognitive performance but also consider gait kinematics, in order to improve their functional independence in clinical interventions. Attention should also be given to strength training of knee extensors and flexors to improve knee control during ST and DT walking.

A high DTC is associated with an increased risk of progression to dementia (Montero-Odasso et al., 2017). Whether the DTC could discriminate MCI from NC remains inconsistent. Our results are in line with previous studies that DTC under DTs cannot distinguish MCI from normal elderly (Åhman et al., 2020; Jayakody et al., 2020). While others found significant differences of DTC between MCI and normal group (Zak et al., 2021; Zheng et al., 2022). The conflict of findings may due to the different inclusion criteria of MCI participants and different cognitive tasks, which may have different interferences in walking performance.

Previous studies have found that the sensitivity of DT gait assessment differs depending on the difficulty of the cognitive task. Arithmetic tasks with high cognitive demand such as

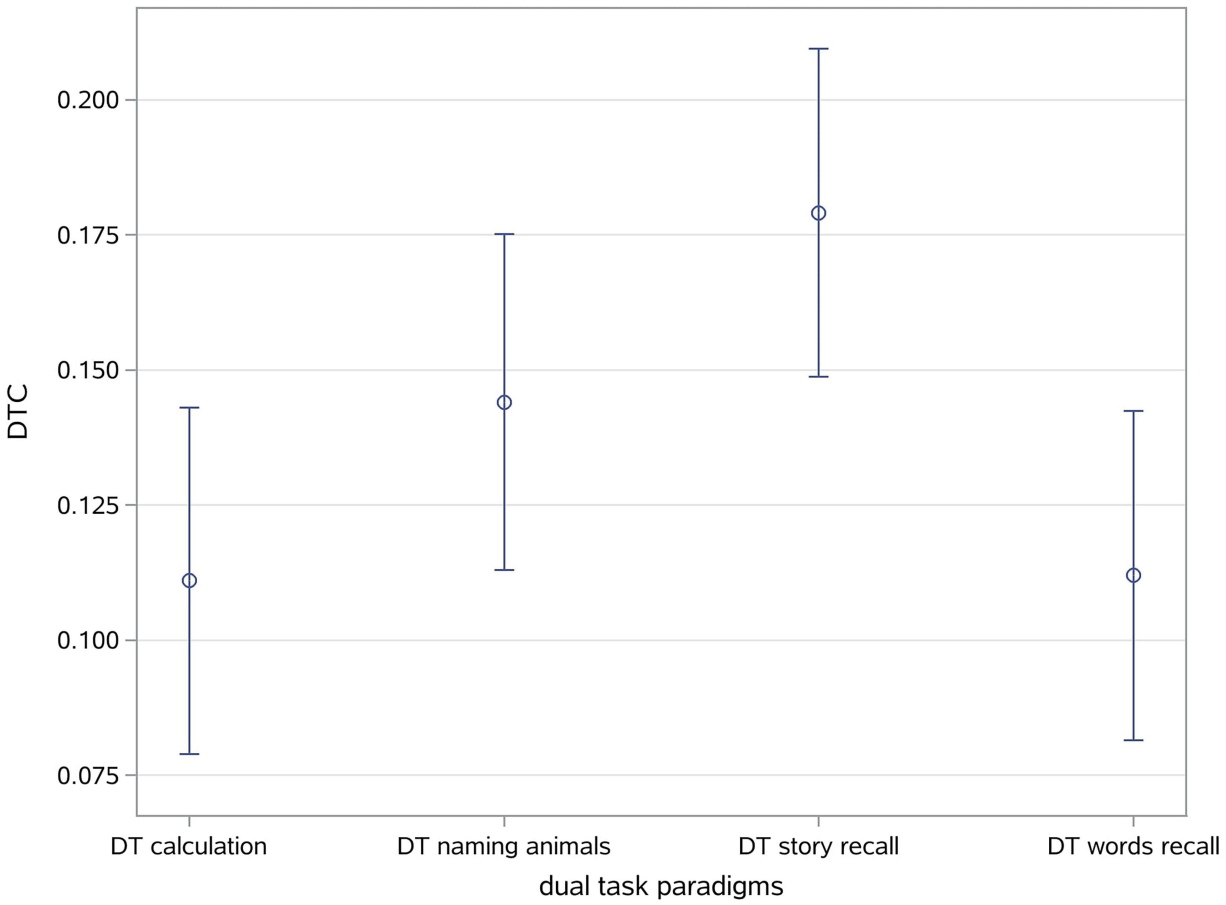

FIGURE 3
Mean difference in dual-task cost among various dual-task paradigms.

calculation (such as counting by serial 3 or 7 s) and verbal fluency (naming animals) tasks are often used to elicit certain MCI-specific gait patterns. These DT paradigms could potentially discriminate MCI patients from patients with Alzheimer's disease and healthy individuals (Åhman et al., 2020). Maintaining balance and speed while dual-tasking is a complex function that requires trunk stability, intact autonomic, and sensorimotor nervous systems. Therefore, dual-tasking requires a higher degree of balancing skills, attention, and executive function than single-tasking. As MCI is the transitional state from normal aging to Alzheimer's disease, the use of dual-tasking with memory tasks seems ideal for observing gait changes in this population.

Cognitive decline with age is primarily observed in the domains of working memory and executive functions which results in reduced attention, postural control, and processing speed (Ramírez and Gutiérrez, 2021). However, older adults also show declines in physical functions such as loss of muscle mass, motor control, and balance (Granacher et al., 2011; Cohen et al., 2016). Therefore, age-related physical and cognitive decline are related functions that can negatively impact the quality of life and independence at older ages (Martin et al., 2011). DT walking relies on a complex neuronal network that consists of primary/supplementary motor area, hippocampus, frontal cortex, occipital cortex, and cerebellum. Although the exact mechanism of gait speed reduction is not known, it is suggested that it might be due to reduced attention resources and is in direct correlation with gray matter volume in frontal cortical regions in MCI patients (Allali et al., 2019).

The use of the DT paradigm exposes cognitive deficits through the simultaneous use of attention-demanding resources (Bahureksa et al., 2017). The story recall test is similar to an everyday memory demand that requires more attention, better learning ability, and good language comprehension of the listener (Baek et al., 2011), and may therefore provide crucial information about the coding, storage, and retrieval process of the memory system. Loss of episodic memory may further be an indicator of the early cerebral atrophy and hippocampal shrinkage that occur during the early stages of cognitive decline. Studies have shown that certain gait parameters such as slowing of gait speed in older adults are associated with reduction in memory and processing speed and therefore can provide diagnostic insights into

specific cognitive domains (Chudoba and Schmitter-Edgecombe, 2020). For example, Toots et al. (2019) have found that gait speed is strongly associated with global cognition and executive functions in cognitively impaired individuals. A worse DT gait performance was found to be associated with volume reduction in the entorhinal cortex (Sakurai et al., 2019). Our previous findings have shown that gait kinematics in ST condition differ among older adults with MCI, SCD, and individuals with normal cognition (Zhong et al., 2021). In addition, our recent findings have shown that cognitive impairment can also impact DT gait kinematics in older adults. It is possible that cognition and gait share certain brain regions and control processes such as gray, white matter, and frontal brain regions and their deterioration impact on gait kinematics and kinetics. Further studies are recommended to explore the changes of DT-related brain functional network in cognitive impairment participants.

Strength and limitations

The strength of our study is that we have used well-studied DT gait parameters and our findings are clinically relevant for the assessments of MCI patients. In addition, the changes of knee peak extension angle we observed during dual-tasking indicated a worse knee control in MCI compared to NC individuals. The gait parameter under DT story recall showed more sensitive to discriminate MCI from normal elderly. One limitation of our study is that we did not randomize the order of DT paradigms but captured the gait data in a constant order for all the participants, which may lead to some learning effect in the second or third trial of each paradigm.

Another limitation of our study is that it is a cross-sectional study and whether DT performance is related to AD progression in MCI and SCD remains unknown. Future research utilizing larger sample size with a longitudinal approach will be crucial in addressing the long-term and large-scale effects of dual-tasking on cognition in the elderly population.

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 The First Affiliated Hospital of Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YZ and TW completed the funding application, managed and coordinated the study. NA, JL, and HT applied for the ethical application, completed the statistical analysis and drafted the manuscript. WP, YT, and HW screened and diagnosed the participants, collected the data, and analyzed the characteristics of the participants. QZ, YG, HW, and CS collected the gait analysis data and completed the gait parameters analysis. MX and TW provided the research ideas, guided the study design and study process and revised the manuscript. All the 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.2022.992873/full#supplementary-material

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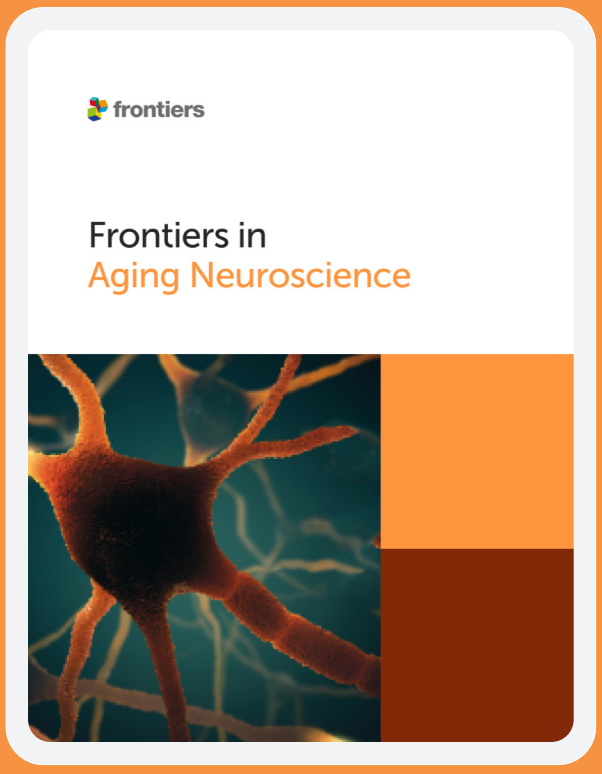

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