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Original Article

Multidimensional modeling of biological aging: integrating gait, eye movement, rest-state functional connectivity, and plasma biomarkers in non-dementia older adults

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ABSTRACT

Accurate modeling of biological age has clinical value for risk stratification, personalized prevention, and intervention planning, promoting proactive healthcare for aging and age-related diseases. Because aging is multidimensional, robust and interpretable multimodal approaches are needed. We studied 908 non-dementia older adults (> 60 years), collecting data on gait, eye movements, resting-state functional connectivity (rs-FC), and plasma biomarkers (neurofilament light chain, NfL, and glial fibrillary acidic protein, GFAP). Fourteen gait features, two eye movement features, 19 rs-FC features, and plasma GFAP levels were significantly correlated with age ($p < 0.05$). Among single-domain models, eye movement features showed the strongest predictive performance ($R^2 = 0.606$; MAE = 3.060). A combined multimodal model achieved markedly higher accuracy ($R^2 = 0.814$; MAE = 1.902). These findings demonstrate that integrating physiological, neurological, and biomarker data substantially improves biological age modeling, supporting the development of comprehensive frameworks to assess aging better and guide timely, targeted preventive strategies.

1. Introduction

Aging of the world's population represents the most significant global medical and social demographic challenge [1]. According to United Nations data, the problem is expected to escalate significantly by 2050, with up to 38 percent of a country's population aged 65 and over [2]. The phenomenon of human aging is intricate and varies from person to person, manifesting across biological, psychological, and social dimensions. Among these factors, biological aging is characterized by gradual alterations in cellular metabolism and physicochemical properties, resulting in diminished self-regulation and regenerative capacity, as well as structural modifications and functional decline in tissues and organs [3,4]. Unlike chronological age, biological age is crucial for objectively assessing a person's rate of aging and predicting age-related disease risk [5,6]. With a more precise biological aging predictive model, individuals will be able to receive early support for diseases, such as dementia, that cannot be adequately identified by chronological age [7–9]. With the growing need to predict biological age, recent research has recognized the feasibility of using biomarkers or physical features to construct predictive models [4,10,11]. Advances in molecular biology have expanded the range of potential biomarkers for predicting biological age, including epigenetic clocks (primarily based on DNA methylation), transcriptomic, proteomic, and metabolomics-based predictors, as well as composite biomarker predictors [12]. However, to date, no single biomarker or combination of biomarkers has been identified that meets the optimal standards for markers of healthy aging

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[9]. At the same time, there remains a lack of models with high interpretability and technical robustness that can comprehensively measure and predict the aging process [13,14].

Recent research has shown that, with age, gait features undergo detectable changes, leading to modifications in walking and overall mobility [15–17]. According to research by Hirono et al. and Jayakody et al., gait patterns, such as gait speed (both circular and straight-line), declined significantly as age increased [18,19]. Age-related alterations in the central nervous system, which are frequently observed in older adults even without diagnosed neurological disorders, are thought to negatively impact gait patterns [15,20]. In addition to gait features, eye movement parameters are also correlated with age [21,22]. Płomecka et al. found that older participants showed higher error rates, longer reaction times, more inhibition failures, and delayed prosaccades than younger adults [23]. Similarly, the results from Noiret et al. indicate that the gradual decline in processing speed and executive attention with age is linked to, and can be underscored by, saccadic eye movements in prosaccade and anti-saccade tasks [24]. At the same time, correlations between rs-FC and age-related differences in the brain, such as in the occipital lobe, have been reported in previous studies, suggesting that rs-FC may serve as a predictor of biological age [25,26]. Additionally, since aging has been demonstrated to be the primary risk factor for neurodegenerative diseases, and plasma biomarkers, such as neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP), have been widely studied for their association with these diseases [27,28]. In previous studies, NfL and GFAP demonstrated high diagnostic value for distinguishing neurodegenerative dementias with high accuracy and were significantly associated with age in both healthy controls and patients [29,30].

In this study, 908 non-dementia older adults were examined for eye movements, gait, rs-FC, and plasma GFAP and NfL levels. Based on the data collected, we identified several parameters that showed statistically significant correlations with aging. With these identified features, predictive models were built using both single measures and integrated assessments, employing logistic regression. Finally, the multidimensional model showed strong predictive power for biological age in the population.

2. Materials and methods

2.1. Participants

A total of 908 non-dementia older adult Chinese participants of Han ethnicity from Jili Subdistrict, Liuyang City, China, were enrolled in this study. Jili Subdistrict comprises three villages (Xihu, Daowu, and Dongsha) located in remote rural areas, and six urban communities (Gongjiaqiao, Xinwuling, Xihe, Baiyi, Shenxian'ao, and Jiliqiao) situated near the city center with convenient transportation and modern living conditions. Participants were recruited in 2022 using a cluster sampling design, and by 2023, the first year of follow-up had been completed. The age of participants ranged from 60 to 93 years.

The inclusion criteria were: (1) age $\geq$ 60 years at the time of enrollment, (2) completion of at least one of the following assessments: eye movement, gait, functional near-infrared spectroscopy (fNIRS), or plasma biomarkers, (3) cognitively normal defined by both MMSE [31] and Clinical Dementia Rating (CDR) [32], and (4) provision of informed consent form signed by the participant or their guardian. The exclusion criteria were: (1) history of stroke, Parkinson's disease, or other neurological conditions, affecting the motor and eye functions of participants, (2) history of other systemic diseases or conditions, such as osteoarthritis and limb or spinal trauma; a history of cataract, glaucoma, or other eye diseases and other systemic conditions or diseases that could have caused ocular symptoms affecting eye tracking. All participants provided written consent to participate in the study in accordance with the Declaration of Helsinki. The ethics committee of Xiangya Hospital of Central South University approved the research protocol for this study.

2.2. Eye movement assessment

Eye movements were recorded using an eye-tracking system (EyeKnow; Beijing CAS-Ruiyi Information Technology Co., Ltd.) at a sampling rate of 120 Hz. The EyeKnow system comprises a tablet computer paired with a VR headset equipped with an eye-tracking camera. The VR headset was used to guide and record eye movements. This system requires only a chair and a table for testing. An initial nine-point calibration procedure was used to ensure a maximum calibration error of 2° in radius. Stimuli were displayed on a monitor integrated with the eye-tracking system, and eye-movement parameters were analyzed by an embedded data-processing module. The assessment involved three tasks (the smooth pursuit, pro-saccade, and anti-saccade tasks), as described in our previous study [33]: a. Smooth Pursuit Task: a green dot moved along a sinusoidal trajectory with a horizontal amplitude, participants were asked to track the sinusoidal movement of the target dot continuously; b. Pro-saccade Task: A green target dot was presented at the center of the display and randomly shifted horizontally and vertically away from the center. Participants were asked to promptly and accurately direct their gaze towards the target dot; c. Antisaccade Task: Similar to the pro-saccade task, a green target dot was presented, but participants were asked to make a saccade to the opposite position as soon as possible.

The following features were extracted from the three tasks: (1) Pursuit accuracy (the ratio of the accurately tracked duration of the target dot to the total duration of the task, participant gaze points within a 2° radius from the center of the target were considered accurately tracked) and pursuit offset number (the number of instances during the test of the gaze point deviating from the center of the target by more than 2° before accurate tracking subsequently resumed); (2) Saccade accuracy (the ratio of the number of successful saccades to the total number of saccade tests performed, saccade offsets falling within a 2° radius from the target dot were considered successful saccades) and saccade latency (the duration between target onset and saccade initiation); (3) Anti-saccade accuracy (the proportion of successful and direct saccades in the direction opposite to that of the target relative to the total number of anti-saccade tests conducted, eye movements toward the target prior to executing the anti-saccade in the opposite direction were regarded as task failures), corrective latency (the duration between a participant failing to inhibit a reflexive saccade toward the target and subsequent initiation of a voluntary saccade in the opposite direction), The definitions of saccade latency and saccade velocity were the same as those used in the pro-saccade task.

2.3. Gait assessment

Gait parameters were assessed using a quantitative motor function assessment system (ReadyGo, Beijing CAS-Ruiyi Information Technology Co., Ltd.). The ReadyGo system utilizes only one set of cameras, comprising a single RGB (red, green, and blue) camera and a single-depth camera, to capture three-dimensional (3D) motion, along with deep learning for skeletal point positioning. Additionally, gait tests do not require a specific room setup, as the system occupies less than 1 m^2 of space and functions effectively within a 1×5 m^2 area directly in front of the camera, with no obstructions. This device can both observe and estimate 3D joints and landmarks through deep visual perception, uniquely track multiple skeletons, and eliminate the need for the subject to wear any sensors. For the gait test, participants were asked to walk at their usual pace on a 3 m walkway without using any mobility aids. Each participant performed one practice trial on the walkway [33].

The following spatiotemporal gait features were extracted for both left and right limbs under three task conditions (single-task walking, dual-task 3-back, and dual-task cost [DTC]): Speed (distance/time excluding turns), Stride (distance between two successive foot contacts), Height (maximum foot clearance during swing), Cadence (60/step time), Width (distance between left and right feet), Stride Speed (stride length/stride cycle time), Swing Speed (swing distance/swing time), Swing (swing time/stride time), Stance (stance time/stride time), Double Support (proportion of stride time with both feet in contact), Test Time (total time to complete the test), and Turn Time (time spent turning).

2.4. Rest-state functional connectivity assessment

Rs-FC can be analyzed by using functional near-infrared spectroscopy (fNIRS). The participants underwent a 5-min session using a multichannel fNIRS system (NirSmartII-3000A, Hui Chuang, China) with two wavelengths (730 nm and 850 nm) at a sampling rate of 11 Hz. The measuring channels consisted of 14 sources and 14 detectors, designed to form 35 channels covering the frontal, temporal, and parietal lobes. Participants were instructed to remain still and awake with their eyes open throughout the session. NirSpark (Huichuang, China) was used to pre-process the obtained signals. Pearson's correlation coefficient was calculated to determine the FC between each pair of measurement channels.

Channel positions were digitized in 3D space and mapped to standard Montreal Neurological Institute (MNI) coordinates using a four-point positioning algorithm. Each channel was then assigned to one of 12 regions of interest (ROIs) covering the frontal, temporal, and parietal cortices, including: BA1+2 L, BA1+2R, BA6L, BA6R, BA9L, BA9R, BA10L, BA10R, BA45R, BA46L, BA46R, BA47R.

2.5. Plasma GFAP and NFL level assessment

Venous blood of participants was collected in ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 3000 rpm for 15 min at 4 °C within 2 h after collection. The plasma samples were stored at −80 °C, and none underwent any freeze-thaw cycles before experiments. NfL, and GFAP were quantified on a fully automated single-molecule detection machine (AST-*Sc*-Lite; AstraBio, Suzhou, China) according to the manufacturer's instructions. The test kits, GFAP and NfL (R64040 and R64060; AstraBio), were used according to the product instructions for detection. A detailed inspection of the measuring tools was conducted, followed by quality checks.

2.6. Statistical analysis

Demographics and other characteristics were summarized using either means and standard deviations or frequencies and percentages as appropriate. Attributes in accordance with normal distribution were analyzed using a *t*-test or otherwise using the Mann–Whitney U test. Statistical significance was set at $P < 0.05$. The correlation between the indicators and age was calculated using Spearman's rank correlation.

Predictive models for biological age were developed using XGBoost regression. Features from each modality were selected based on their statistical significance in correlation analyses ($P < 0.05$). For Eye movement, only two parameters met this criterion. To avoid underrepresentation of this modality in the predictive models, we included additional parameters derived from established oculomotor paradigms, namely smooth pursuit, prosaccade, and antisaccade tasks. These tasks are widely reported in the literature to reflect age-related changes in saccadic control, pursuit accuracy, and inhibitory processes [24]. Specifically, parameters related to saccade latency, velocity, error rates, and pursuit gain were incorporated. The inclusion criterion for these additional features was based on their theoretical and empirical relevance to age-related oculomotor decline. All models were adjusted for sex and education level, with gait models additionally adjusted for body mass index. The dataset was randomly divided into training and validation sets in a 7:3 ratio. Within the training set, 10-fold cross-validation was conducted to assess model performance using the coefficient of determination (R^2) and mean absolute error (MAE). The final trained models were then evaluated on the independent validation set, and R^2 and MAE were reported as the primary performance metrics. All statistical analyses were performed using Python version 3.8.19.

3. Results

3.1. Demographic information of participants

A total of 908 non-demented older adults were recruited from the community, with a mean age of 68.4 years (±5.7), of whom 45.5% were male. Participants were aged 60–93, with 520 (57.3%) aged 60–69, 360 (39.6%) aged 70–79, and 28 (3.1%) aged 80–93. The average years of education were 6.3 (±3.5), and the mean MMSE score was 26.5 (±2.3). The demographic characteristics of the cohort, including age, sex, years of education, MMSE score, comorbidities, and plasma biomarker levels, are summarized in Table 1. The study design and the construction flow of the diagnostic models are shown in Fig. 1.

3.2. Gait and age correlational analysis

665 individuals completed the gait analysis. To further identify which features correlate with the aging process, 60 gait features were measured and analyzed in 665 healthy control individuals. Of these, 14 features showed significant correlation with age ($P < 0.05$), including Gait-Stance_Left, Gait-Stride_Left, Gait-Stride_Right, Gait-Swing_Left, Gait-Width, Gait-Turntime, Gait-TestTime, Gait-Speed, Gait-SwingSpeed_Left, Gait-SwingSpeed_Right, Gait-DoubleSupport_Left, Gait-DoubleSupport_Right, Gait-StrideSpeed_Right, and Gait-StrideSpeed_Left. The correlational models of these features are represented in Fig. 2A.

3.3. Eye movement and age correlational analysis

For eye movement parameters, 388 healthy individuals were tested in both saccade and anti-saccade tasks, as well as in smooth pursuit tasks. Among the measured parameters, Prosaccade_Latency_AVG and Prosaccade_Latency_Max showed statistically significant correlations with age ($P < 0.05$, Fig. 2B).

3.4. Rest-State functional connectivity and age correlational analysis

409 individuals completed the fNIRS assessment. Considering the potential correlation between the rs-FC and age. In total, 35 channels were detected in this study and assigned to 12 brain regions: the right and left parts of Brodmann areas 1 + 2 (BA1 + 2R and BA1 + 2L), BA6R

Table 1
Demographic characteristics of participants.

		Overall (N = 908)
Age	Mean (SD)	68.4 (0.2)
Sex (%)	Male	413 (45.5)
	Female	495 (54.5)
Education (years)	Mean (SD)	6.3 (0.1)
MMSE	Mean (SD)	26.5 (0.1)
BMI	Mean (SD)	28.2 (3.5)
Smoke (%)	Never	713 (78.5)
	Former	52 (5.7)
	Current	143 (15.7)
Alcohol (%)	Never	812 (89.4)
	Former	44 (4.8)
	Current	40 (4.4)
Comorbidities (%)	Hypertension	456 (50.2)
	Diabetes	170 (18.7)
Drug use (%)		464 (51.1)
Plasma NfL (pg/mL)	Mean (SD)	71.9 (8.8)
Plasma GFAP (pg/mL)	Mean (SD)	33.7 (6.2)

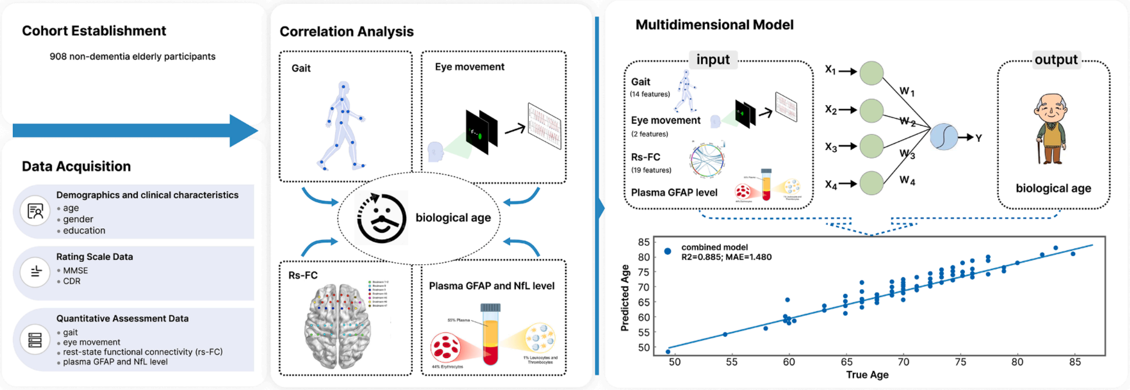

Fig. 1. Study design and predictive model construction.

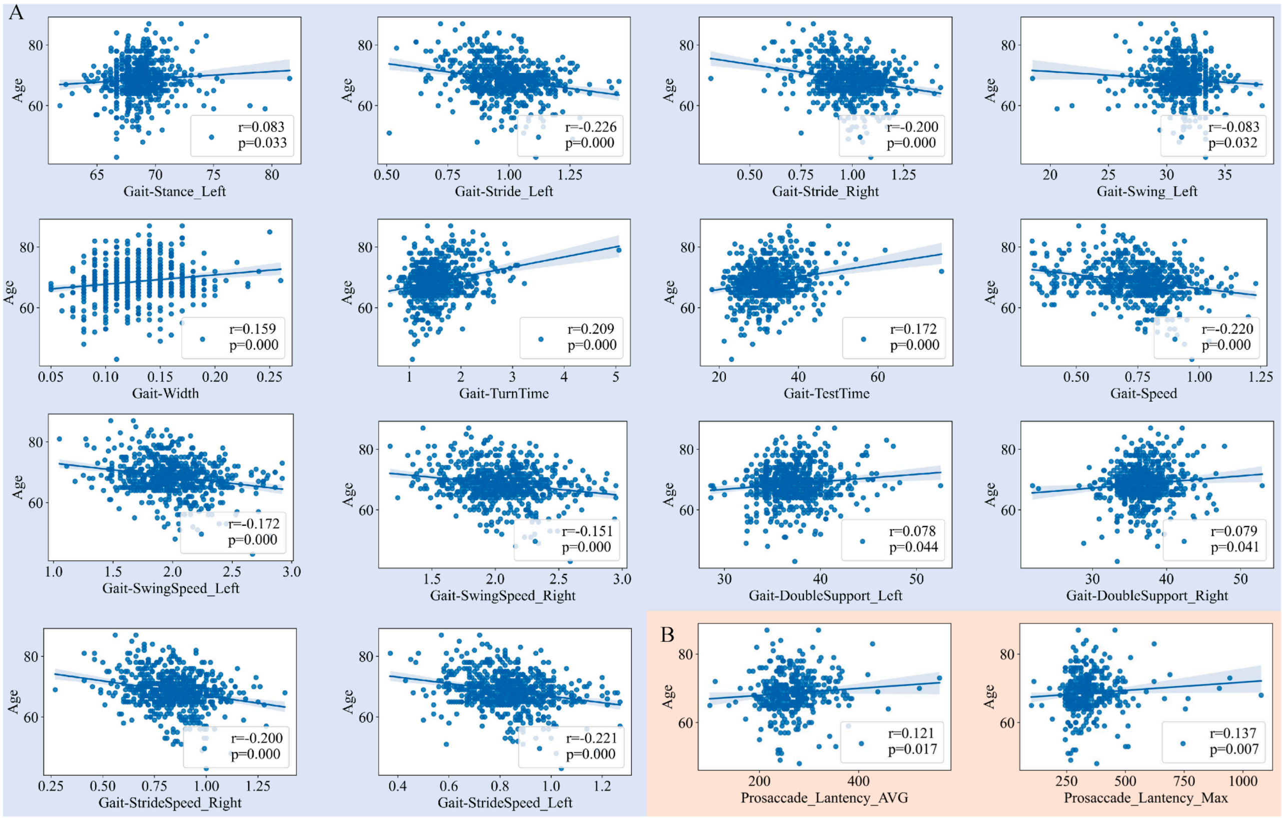

Fig. 2. Gait and eye-movement parameters highly correlate with aging ($p < 0.05$).

and BA6L, BA9R and BA9L, BA10R and BA10L, BA45R, BA46L, and BA47R. The corresponding locations were color-coded in the dorsal view of the brain shown in Fig. 3A. The 19 statistically significant correlations between rs-FC and age were observed and shown in Fig. 3B, including BA46L.vs.BA47R, BA46L.vs.BA45R, BA6L.vs.BA9R, BA47R.vs.BA46R, BA47R.vs.BA45R, BA47R.vs.BA10R, BA47R.vs.BA10L, BA46R.vs.BA9L, BA46R.vs.BA9R, BA46R.vs.BA10R, BA46R.vs.BA10L, BA6R.vs.BA9R, BA1+2R.vs.BA9R, BA9L.vs.BA9R, BA9L.vs.BA10R, BA9L.vs.BA10L, BA9R.vs.BA10R, BA9R.vs.BA10L, and BA10R.vs.BA10L ($P < 0.05$) .

3.5. *GFAP and NFL biomarkers and age correlational analysis*

Given the strong correlation between neurodegenerative diseases and aging, we developed a model based on two common plasma biomarkers: GFAP and NfL. The biomarker levels were measured in 70 nondemented older adults, and only GFAP showed a statistically significant positive association with aging ($P < 0.05$). The estimates for GFAP and NfL are shown in Fig. 4, with r = 0.336 and $P = 0.009$, suggesting that as GFAP increases, age also increases.

3.6. *Multi-Dimensional modeling of biological aging*

After examining the correlational relationships between each feature and chronological age, features with statistically significant associations ($P < 0.05$) were selected for model construction. For eye movement, only two parameters met this significance threshold, so additional parameters from the Prosaccade test were included to enhance model robustness. Using these selected features, four separate predictive models were built for each modality: gait, eye movement, resting-state

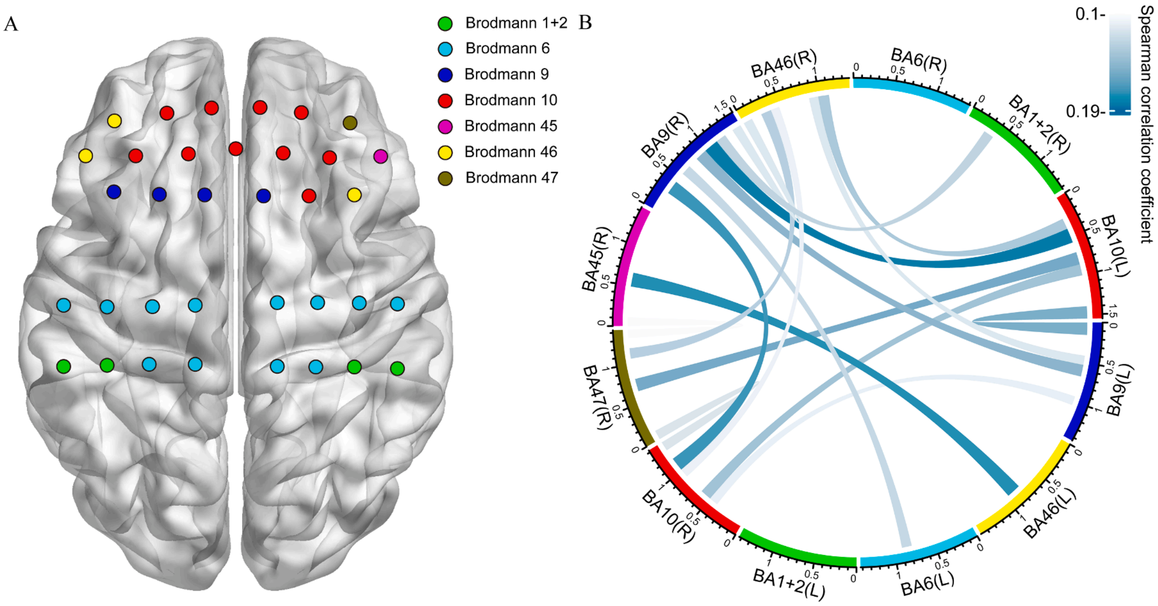

Fig. 3. Correlation between rest-state functional connectivity (rs-FC) and aging. (A): Color-coded regions for measuring rs-FC. (B)The strength of the correlational relationship between rs-FC and aging is depicted with the width and color of blue lines. The areas of the brain are laid out around the circle in the same colors as in the brain map, with the left hemisphere at the bottom and the right hemisphere at the top. The strength of the correlation between age and rs-FC across regions is indicated by the thickness and color of the lines connecting them. A thicker line indicates a stronger correlation between age and the rs-FC between the two areas. In contrast, the absence of a line between any two regions suggests no significant correlation.

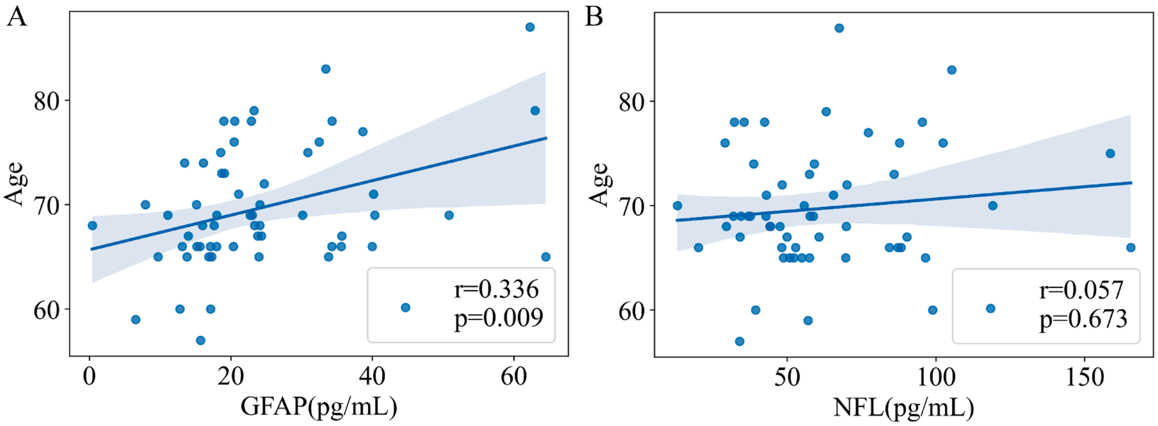

Fig. 4. Linear correlation between GFAP (A), NFL (B), and aging.

functional connectivity (rs-FC), and GFAP.

In the 10-fold cross-validation, the gait-based predictive model achieved an R-squared of 0.505 with a mean absolute error (MAE) of 2.795. The eye-movement feature showed higher predictive accuracy, with an R-squared of 0.566 and a lower MAE of 2.791, indicating that eye-movement parameters were more strongly associated with chronological age. In contrast, rs-FC ($R^2 = 0.220$, MAE = 4.104) and GFAP ($R^2 = 0.155$, MAE = 5.237) did not present substantial predictive value when used alone (Table 2).

Table 2
Performance of the models based on 10-fold cross-validation.

Features	Mean R^2	Mean MAE
Gait	0.505	2.795
Eye-movement	0.566	2.791
rs-FC	0.220	4.104
Gait + Eye-movement + rs-FC	0.719	1.874
GFAP	0.155	5.237
Gait + Eye-movement + rs-FC + GFAP	0.759	1.852

Considering potential interactions among modalities, combined predictive models were then developed. Integration of gait, eye-movement, and rs-FC features improved predictive performance ($R^2 = 0.719$, MAE = 1.874), and adding GFAP further enhanced the model ($R^2 = 0.759$, MAE = 1.852), highlighting the incremental value of multimodal integration.

External validation confirmed these trends, supporting the robustness of the models. Eye-movement remained the strongest single predictor ($R^2 = 0.606$, MAE = 3.060), followed by gait ($R^2 = 0.502$, MAE = 3.163), while rs-FC ($R^2 = 0.189$, MAE = 4.100) and GFAP ($R^2 = 0.171$, MAE = 4.799) alone provided limited predictive power. The multimodal model, which combines gait, eye movement, and rs-FC, achieved an R^2 of 0.735 and a MAE of 1.757. The addition of GFAP further improved performance ($R^2 = 0.814$, MAE = 1.902), demonstrating both the generalizability and superiority of multimodal feature integration (Fig. 5).

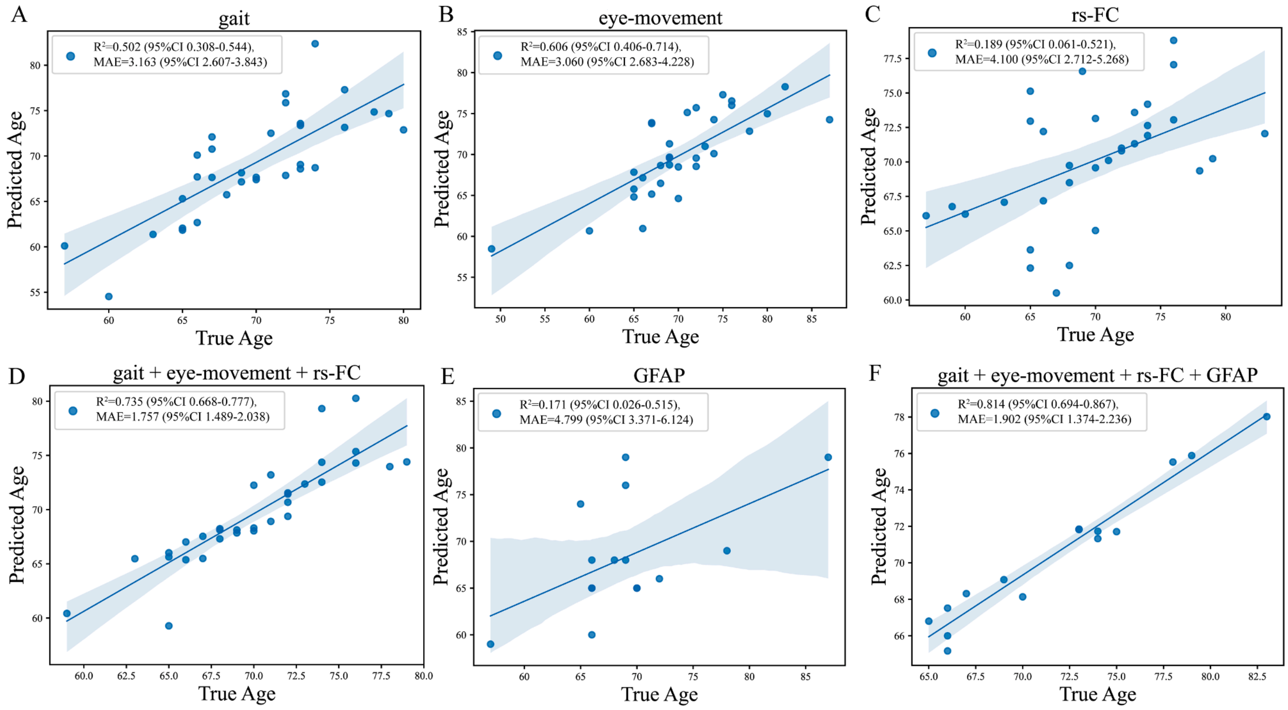

Fig. 5. Predictive models for age with different traits. (A): Model based on statistically significant gait features ($R^2 = 0.502$); (B): Model based on statistically significant eye-movement features ($R^2 = 0.606$); (C): Model based on statistically significant rs-FC features ($R^2 = 0.189$); (D): Integrated model based on gait, eye movement, and rs-FC features ($R^2 = 0.735$); (E): Models based on biomarker GFAP ($R^2 = 0.171$); (F): Integrated model based on gait, eye movement, rs-FC, and GFAP features ($R^2 = 0.814$).

3.7. Discussion

In this study, we developed a comprehensive predictive model for biological aging by integrating multidimensional parameters, including gait features, eye movement metrics, brain functional connectivity, and plasma levels of neurodegenerative biomarkers. Our results revealed that although individual parameter categories showed different correlations with age, the integrated model significantly improved the accuracy of predicting biological age. This model achieved an R^2 of 0.814 and an MAE of 1.902, demonstrating that a multifaceted approach provides a more precise assessment of biological aging than single-parameter models.

The analysis of gait parameters revealed that 14 features were significantly correlated with age ($P < 0.05$), confirming the impact of aging on gait patterns in non-dementia older adults. Among these features, the stride length on both sides showed the strongest negative correlation with age. Additionally, walk speed and several related indices showed a generalized negative correlation with age, with lower R-squared values, indicating that walking speed may be a non-specific characteristic of the aging process. Stride length tends to be more sensitive to biological aging because it directly reflects underlying neuromuscular, musculoskeletal, and biomechanical integrity, which often undergo subtle yet progressive decline with advancing age [34,35]. While overall walking speed can be influenced by a variety of cognitive and behavioral characteristics, stride length is more tightly coupled to the body's structural and functional capacity. Previous research has linked gait parameters to multiple aging phenotypes, including declines in muscle function, cognitive impairment, and an increased risk of cardiovascular disease [36–38]. Our results provide further evidence of a direct relationship between gait changes and aging. The improved detection of significant gait features can be attributed to the use of advanced equipment, highly automated parameter-collection techniques, and the inclusion of a broader range of parameters than in traditional wearable devices [39]. Overall, this study is the first to conduct a comprehensive assessment of subtle gait changes associated with biological aging, which may have been overlooked in previous studies.

The eye-movement analysis revealed significant age-related correlations in prosaccade latency parameters. Prosaccade tasks, which assess the ability to rapidly shift gaze toward a new stimulus, are less cognitively demanding and may be particularly sensitive to age-related declines in basic sensorimotor processing [40,41]. Conversely, anti-saccade error rates correlated with neuropsychological measures of dementia severity, suggesting that anti-saccade tasks are a more robust measure of inhibitory control deficits than pro-saccade tasks [42]. The anti-saccade parameters showed significant age correlations only in specific age groups, likely because of the greater cognitive effort required to suppress a reflexive response and initiate a voluntary movement in the opposite direction. This task may be more sensitive to declines in executive function that manifest differently across age groups. Some key brain regions have been reported to be involved in smooth pursuit, including the visual cortex, the middle temporal area, the medial superior temporal area, and motor control centers such as the cerebellum and frontal eye fields [43]. However, the lack of significant correlations between smooth pursuit parameters and age suggests that the neural mechanisms underlying smooth pursuit eye movements may remain relatively intact during normal aging, or the tasks employed may not have detected subtle changes. Specifically, smooth pursuit primarily relies on a continuous visual feedback loop involving the extrastriate visual cortex and the cerebellum, neural networks that may exhibit greater compensatory plasticity during healthy aging than the frontal-parietal networks heavily taxed by the inhibitory and executive demands of saccadic tasks.

We identified 19 significant correlations between rs-FC and age, indicating that functional connectivity between specific brain regions changes with aging [44]. BA10 covers the most anterior part of the frontal lobes and is located at the rostral end of the superior and middle frontal gyri [45]. It is one of the largest Brodmann areas, thought to be involved in strategic processes in memory retrieval and in integrating information from multiple sources to facilitate complex decision-making and problem-solving [46]. Changes in functional connectivity among these areas are associated with cognitive impairment, affecting personality, decision-making, and social behavior. They thus can be a sensitive indicator of subtle cognitive changes in the aging process. Previous research has identified these brain regions using MRI neuroimaging. However, the lack of portability and the lengthy scanning time of MRI equipment limit its convenience for early diagnosis at the community level and its utility in hospital settings. A recent study has reported that fNIR demonstrates high consistency with functional MRI (fMRI) [47]. For rs-FC, we chose fNIR over functional magnetic resonance imaging (fMRI) because fNIR requires only 2 min to assess rest-state functional connectivity [48]. Compared with fMRI, fNIR offers advantages such as portability, cost-effectiveness, and reduced sensitivity to movement artifacts, which are particularly beneficial for community-based studies in older adults, especially during tasks involving movement [49].

Our analysis demonstrated that plasma GFAP levels were significantly positively correlated with age, whereas NfL levels were not. Although previous studies have linked both GFAP and NfL to neurodegenerative diseases [50,51], our study is among the first to investigate their potential as biomarkers of biological aging in a non-dementia older adult population. GFAP, an indicator of astrocyte activation, may reflect age-related neuroinflammatory processes, whereas NfL, found in nerve axons, is typically associated with neurodegeneration [52,53]. A previous study showed a progressive increase in GFAP mRNA and protein levels with age, contributing to higher baseline plasma GFAP levels in older adults, even in the absence of neurodegenerative disease [54]. Our findings indicate that GFAP, rather than NfL, significantly correlates with aging, suggesting that neuroinflammatory processes, rather than neurodegeneration, may more accurately reflect biological aging in non-dementia older adults [55]. While NfL is a sensitive marker of active axonal damage in neurodegenerative diseases, its levels may remain relatively stable during normal biological aging in the absence of significant structural neurodegeneration. In contrast, astrocyte activation and the subsequent release of GFAP are prominent features of inflammaging, a chronic, low-grade inflammatory state characteristic of the aging brain. Therefore, GFAP elevation indicates a subclinical neuroinflammatory state even in individuals without dementia.

The integrated model, which combines gait, eye movement, rs-FC, and GFAP parameters, significantly outperformed these separate models based solely on individual parameters. The R^2 value increased from 0.606 in the eye movement model and 0.502 in the gait model to 0.814 in the integrated model, while MAE decreased. This marked improvement highlights the complex and multifaceted nature of aging, which involves intricate interactions among motor functions, cognitive processes, brain connectivity, and molecular biomarkers [56–58]. Aging is not solely a musculoskeletal phenomenon, nor purely a cognitive or neural vascular event. Instead, it involves intricate, interwoven changes across multiple physiological and neurological systems. By combining these aspects, we can capture subtle declines in executive function, sensorimotor integration, and neuromuscular control that may not be evident when examined separately. By encapsulating multiple dimensions of aging, the integrated model offers a more accurate and comprehensive prediction of biological age [6,13,59]. Traditional methods for predicting biological aging often rely on single biomarkers or limited physiological measures, such as telomere length or epigenetic clocks [60–63]. Our approach provides an economical, effective, and comprehensive alternative that better reflects the complexity of the biological aging process.

Despite the promising results, our study has limitations. Higher-order feature interactions are not explicitly modeled in this study, as the primary aim was to evaluate the predictive contributions of each modality and their integration within an exploratory framework. To minimize overfitting, we employed 10-fold cross-validation on the training dataset and further validated our models with independent external data, both of which consistently demonstrated stable predictive performance. Regarding model complexity, we used relatively simple regression-based models rather than highly parameterized or deep learning frameworks, thereby reducing the risk of overfitting and facilitating interpretability. Advanced modeling techniques, such as machine learning algorithms including polynomial regression [64], Ridge regression [65], and Lasso regression [66], could potentially enhance predictive accuracy by addressing intricate patterns in the data. Additionally, our analysis was constrained to two plasma biomarkers, GFAP and NfL, both of which are associated with neurodegeneration. This focus may limit the generalizability of our findings to broader populations. It is also important to note that the majority of the data was gathered from a Han Chinese cohort, which may restrict the generalisability of the results to other populations. Additionally, the age distribution of our sample is somewhat imbalanced, with a small proportion of participants aged over 80 (3.1%). This underrepresentation of the oldest-old population may limit the model's predictive performance and robustness in advanced age groups, underscoring the need for future studies with more evenly distributed age cohorts.

Recent studies have highlighted multiple routes to biological age estimation, encompassing molecular, clinical, and imaging modalities. For instance, a five-CpG DNA methylation clock developed in Chinese cohorts achieved $R = 0.95$, $MAE = 3.90$ in the Quzhou validation set and $R = 0.82$, $MAE = 5.37$ in the CAS cohort [67]. A second study combining ELOVL2 DNA methylation and sjTREC reported an overall $MAE \approx 4.4$ years, with exceptionally low error in younger adults ($MAE < 2.5$ years) but larger error in very old individuals, underscoring age-dependent performance and the potential value of immune markers in older populations [68]. A third model using nine clinically relevant biomarkers (e.g., HDL, HbA1c, VO₂max, suPAR) and PCA achieved $R^2 = 0.73$ in women and $R^2 = 0.65$ in men [4]. Similarly, a recent large-scale proteomic study analyzing 2920 plasma proteins across 48,728 participants identified 71 distinct proteins significantly associated with multidimensional aging phenotypes, such as PhenoAge acceleration and frailty index [69]. Furthermore, evaluating global health across 13 bodily systems in large longitudinal cohorts led to the development of the "Health Octo Tool" and "Body Clock," which capture multidimensional aging heterogeneity across organs and predict disability and mortality with high accuracy, underscoring the consensus that aging cannot be captured by a single metric [70]. In comparison, our multimodal approach (gait with left/right and single-task/3-back/DTC conditions, eye-movement metrics, fNIRS rs-FC ROI correlations, and plasma GFAP) achieves $R^2 = 0.814$ and $MAE = 1.902$, indicating competitive or superior age-regression accuracy among non-epigenetic modalities, while remaining non-invasive and deployable in community settings.

Future research should consider more sophisticated predictive models and utilize modern analytical techniques, such as proteomics and metabolomics, to discover non-invasive biomarkers that integrate gait and eye movements, thereby improving the applicability and accuracy of the results. Furthermore, Trabassi et al. demonstrated that combining conditional tabular generative adversarial networks (GANs) with explainable AI (SHAP) improved classification performance and clinical interpretability in patients with rare gait disorders associated with Hereditary Cerebellar Ataxia [71], which indicates that generative AI, data balancing, and explainable machine learning have been applied to gait analysis in rare neurological conditions. Thus, our ultimate goal is to expand the multidimensional dataset for this research and develop a generative AI model that can be applied across various scenarios to predict biological age.

4. Conclusions

Our study presents a novel, multidimensional approach to predicting biological age by integrating gait, eye movements, rs-FC, and plasma biomarkers. This integrated model surpasses the predictive performance of previous studies that relied on single biomarkers or specific physiological measures, as it better captures the complex and multifaceted nature of the aging process. By demonstrating that incorporating diverse phenotypic indicators can enhance the accuracy of biological age predictions, our research offers new insights into the interconnected systems that drive aging. This approach could inform future research to develop more comprehensive assessments of biological aging and to design interventions that target various aspects of the aging process. Future research should focus on refining predictive models through advanced analytics, broadening the range of biomarkers and functional measures, and applying the models to diverse populations to improve their practical utility in both clinical and research settings.

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Ethics approval and consent to participate

All participants provided written consent to participate in the study in accordance with the Declaration of Helsinki. The ethics committee of Xiangya Hospital of Central South University approved the research protocol for this study.

Declarations conflict of interest

All authors declare no financial or non-financial competing interests.

Declaration of generative AI and AI-assisted technologies

AI-assisted technologies were only used to assist with grammar editing. All scientific content, data analysis, and conclusions were developed and verified by the authors, who take full responsibility for the manuscript.

CRediT authorship contribution statement

Jingyi Lin: Writing – original draft, Investigation, Data curation. **Yiliang Liu:** Methodology, Data curation. **Ziyu Ouyang:** Methodology, Data curation. **Xuan Yang:** Data curation. **Sizhe Zhang:** Data curation. **Tianyan Xu:** Data curation. **Qijie Yang:** Data curation. **Yuan Zhu:** Data curation. **Meidan Wan:** Formal analysis, Data curation. **Xuewen Xiao:** Supervision, Data curation. **Xiangmin Fan:** Validation, Software, Formal analysis. **Beisha Tang:** Validation, Supervision. **Lu Shen:** Validation, Supervision, Investigation, Funding acquisition. **Bin Jiao:** Supervision, Investigation. **Shilin Luo:** Writing – review & editing, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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