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Biological age and brain age in midlife: relationship to multimorbidity and mental health

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ABSTRACT

Biological age and brain age estimated using biological and neuroimaging measures have recently emerged as surrogate aging biomarkers shown to be predictive of diverse health outcomes. As aging underlies the development of many chronic conditions, surrogate aging biomarkers capture health at the whole person level, having the potential to improve our understanding of multimorbidity. Our study investigates whether elevated biological age and brain age are associated with an increased risk of multimorbidity using a large dataset from the Midlife in the United States Refresher study. Ensemble learning is utilized to combine multiple machine learning models to estimate biological age using a comprehensive set of biological markers. Brain age is obtained using Gaussian processes regression and neuroimaging data. Our study is the first to examine the relationship between accelerated brain age and multimorbidity. Furthermore, it is the first attempt to explore how biological age and brain age are related to multimorbidity in mental health. Our findings hold the potential to advance the understanding of disease accumulation and their relationship with aging.

1. Introduction

Multimorbidity, coexistence of 2 or more chronic conditions in an individual, is increasingly common especially among older adults and poses substantial public health challenges (Suls et al., 2016). Multimorbidity differs from a related term comorbidity in the sense that comorbidity has a primary condition of interest and examines the co-occurring of other conditions with this primary condition (Johnston et al., 2019). Medical research has been primarily single-disease focused, which considers 1 disease at a time and sometimes the comorbidity of a particular disease of interest (Suls et al., 2016). Multimorbidity offers an effective framework for examining the co-occurrence of multiple chronic conditions without the need to define a specific primary condition (Fabbri et al., 2015).

Aging is a universally experienced process, which underlies the development of most chronic conditions. The mechanism that drives the aging process may also drive multiple age-related chronic conditions. In this sense, aging represents a key risk factor for multimorbidity and a shared pathway across many chronic diseases (Fabbri et al., 2015; Liu et al., 2018). In recent years, biological age has been proposed as a surrogate biomarker to capture the process of aging (Klemera and Doubal, 2006; Levine, 2012). At the individual level, biological age can deviate from chronological age, with elevated biological age indicating higher risk for aging-related diseases (Wu et al., 2021). More importantly, this type of surrogate aging biomarker allows researchers to examine health at the whole person level, not unique to a specific health condition.

However, currently only 3 studies have investigated the relationship between biological age and multimorbidity. Biological age obtained from regression-based models with a set of clinical markers was found to explain a significant amount of variability in multimorbidity defined based on 5 chronic diseases in middle-aged and older adults (Crimmins et al., 2021). Another study reported a significant correlation between multimorbidity and a biological age metric estimated based on blood immune biomarkers in older adults (Sayed et al., 2021). Additionally, a strong association was reported between the number of diseases and a summary aging measure obtained by regressing the hazard of mortality on a set of clinical markers and chronological age in adult (Liu et al., 2018). More

Abbreviations: AIDS, Acquired immunodeficiency syndrome; BMI, Body Mass Index; CI, Confidence Interval; CV, Cross Validation; FOV, Field of View; GED, General Education Development; HDL, High-Density Lipoprotein; HIV, Human Immunodeficiency Virus; IGF1, Insulin-like Growth Factor 1; LDL, Low-Density Lipoprotein; MAE, Mean absolute error; MIDUS, Midlife in the United States Study; MRI, Magnetic Resonance Imaging; IL10, Interleukin 10; OR, Odds ratio; SD, Standard deviation; TE, Echo time; TR, Repetition time

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research is needed to examine biological age estimated from a broad range of clinical/biochemical markers and multimorbidity defined based on more categories of chronic conditions. In addition, a number of important methodological issues need to be clarified, such as how to address the estimation bias that biological age tends to be overestimated for younger participants and underestimated for older participants in regression-based models.

Brain age estimated by machine learning models and neuroimaging data is another surrogate biomarker on aging (Niu et al., 2020, 2022). Similar to biological age, brain age allows us to examine health as an integrative process, not specific to a particular health condition. A comparison of an individual's brain age and chronological age can inform us whether an individual's brain ages faster or slower than it should. Studies have shown that brain age is powerful in predicting a broad range of health outcomes, such as cognitive functioning, stroke, diabetes, Alzheimer's disease, smoking, alcohol, and mortality (Cole, 2020; Cole et al., 2017a, 2017b; Wang et al., 2019). However, no study has examined the relationship between brain age and multimorbidity. In addition, it is unclear whether the relationship between these aging biomarkers (i.e., biological age, brain age) and multimorbidity is moderated by sex. Studies have reported that the prevalence of multimorbidity differs by sex, notably a higher rate in women (Marengoni et al., 2011). Understanding how the influence of biological age and brain age on multimorbidity differs by sex may allow us to develop more personalized strategies to prevent or delay multimorbidity.

To answer these questions, we first estimate biological age using machine learning models and a comprehensive set of clinical and biochemical markers including BMI (Body Mass Index), blood hemoglobin, and C-reactive protein. We then investigate whether the elevated biological age is related to an increased risk of multimorbidity defined based on 13 different categories of chronic conditions. Then, we examine whether accelerated brain age estimated by machine learning models and neuroimaging data is associated with an increased risk of multimorbidity. In addition, we test whether the relationship between multimorbidity and the surrogate aging biomarker (e.g., biological age and brain age) is moderated by sex. Lastly, multimorbidity in mental health has been relatively under-researched, despite its advantage in understanding clinical complexity in psychiatry (i.e., having multiple psychiatric and/or addictive disorders in an individual) (Bhalla et al., 2018; Langan et al., 2013). To this end, we explore the relationship between these surrogate aging biomarkers and mental health multimorbidity. Findings from our study hold the potential to improve our understanding of multimorbidity and its major risk factor aging, which may shed the light on new strategies to improve the treatment and clinical management of multimorbidity.

2. Materials and methods

2.1. MIDUS Refresher Study

We used data from the Midlife in the United States (MIDUS) Refresher study (Ryff et al., 2017). A national sample of 4085 adults, aged 25–74 years, was studied between 2011 and 2016. Participants recruited through random digit dialing and a separate Black/African American sample from Milwaukee were included in the analyses. The MIDUS Refresher Biomarker Project obtained comprehensive biological assessments from 863 respondents who participated in the MIDUS Refresher project. Additionally, the MIDUS Refresher Neuroscience Project collected neuroscience assessments from 138 respondents who also completed the Biomarker Project. Detailed sample descriptions can be found in other published papers (Boylan et al., 2020; Radler, 2014). To estimate biological age, we used data from all participants in the MIDUS Refresher Biomarker Project (n = 863). To obtain brain age, we included data from all participants in the MIDUS Refresher Neuroscience Project (n = 138).

2.2. Multimorbidity

We assessed multimorbidity using a dichotomous variable, indicating whether having 2 or more of the following conditions. Similar to other studies that examined multimorbidity using MIDUS data, we considered 13 different categories of chronic conditions including diabetes, asthma, hypertension, HIV or AIDS, tuberculosis, neurological disorders, stroke, ulcer, arthritis, ever had cancer, heart trouble, obesity, and/or high cholesterol levels (Friedman et al., 2015; Shorey and Friedman, 2018). This dichotomous variable was coded as 0 if the subject had single or no condition and 1 if the subject had 2 or more of the 13 chronic conditions in the past 12 months. We computed multimorbidity for all participants in the Biomarker Project. Additionally, we examined multimorbidity in mental health, a binary variable indicating whether having 2 or more of the psychiatric and addictive conditions, including depression (Rottenberg et al., 2019), anxiety disorder (Disabato et al., 2021), alcohol abuse (Ransome et al., 2017), and drug misuse (Kim et al., 2020).

2.3. Biological measures

Based on studies that estimated biological age using clinical and biochemical markers (Belsky et al., 2015; Crimmins et al., 2021; Klemera and Doubal, 2006), we identified a set of fairly standard markers that are commonly collected in a clinical exam. Here is the complete list of biological measures used: BMI, waist-hip ratio, blood pressure (systolic), HDL cholesterol, LDL cholesterol, total cholesterol, triglycerides, HbA1C, blood-fasting glucose, blood-fasting insulin-like growth factor 1 (IGF1), C-reactive protein, creatinine, peak flow, blood serum MSD IL10, and bone-specific alkaline phosphatase.

2.4. Biological age

We built machine learning models to estimate biological age with the list of biological measures described above. To do so, we first identified a subset of healthy participants without any of the physical and mental health conditions listed above (n = 193, a mean age of 45.60 years, SD = 12.73 years, age range 25–74 years). The healthy cohort was randomly split in half with 50% of the data as a training set and the remaining 50% as an independent test set. We then built machine learning models with the list of biological measures to predict chronological age using the training set. We took an ensemble learning approach, which is an effective tool to combine multiple machine learning models to achieve improved prediction accuracy, compared to a single machine learning model. In particular, we combined 3 popular machine learning models including support vector regression (Smola and Schölkopf, 2004), elastic net (Zou and Hastie, 2005), and Gaussian processes regression (Rasmussen and Williams, 2006) using a general linear model to create a linear combination of models as implemented in the R package caretEnsemble version 2.0.1 (Deane-Mayer and Knowles, 2016; Kuhn, 2008). Support vector regression generalizes the idea of support vector machine to a regression setting. In this study, we used a linear kernel and tuned the cost parameter using 10-fold cross-validation (CV) within the training set. Elastic net is a type of regularized regression model with both L_1 and L_2 norms. The tuning parameters alpha and lambda were chosen to minimize CV error. We conducted Gaussian processes regression with radial basis function kernel and optimized the tuning parameter sigma using 10-fold CV. Biological measures were standardized before running machine learning models. For each of the biological measures, we subtracted the mean and then divide by its standard deviation in the training set. For the test set, we standardized each biological measure based on its mean and standard deviation computed from the training set. All 15

biological measures listed in Section 2.3 were used in the ensemble model. The trained model was then applied to the independent test set of healthy participants to predict their chronological age. The predicted chronological age is the so-called biological age. The underlying assumption is that the biological age is on average the same as the chronological age for healthy participants. In this sense, we would expect an accurate model to achieve a small mean absolute error (MAE) from a test set of healthy participants. One important methodological issue that has not been made clear in the literature of biological age estimation is that a valid metric for evaluating model performance needs to be computed from an independent test set. This is because a model can easily overfit a training set. Another key methodological issue to note is that biological age can deviate from chronological age in disease groups. Thus, models for predicting biological age should be built with healthy participants. Though model training should be done with a healthy cohort, the trained model for estimating biological age can be applied to disease groups. We also applied our trained model to predict biological age for participants with at least 1 of the 13 categories of chronic conditions (n = 630). We computed a difference score by subtracting chronological age from the estimated biological age, called the biological age gap. A positive biological age gap means accelerated biological aging. To correct for age-related bias, we conducted a general linear model to regress the biological age gap on chronological age and computed the residualized/adjusted biological age gap.

2.5. Image acquisition

All structural scans were acquired using a 3T scanner (MR750, GE Healthcare, Waukesha, WI) with an 8-channel head coil. These data were derived from BRAVO T1-weighted structural images with TR (Repetition Time) = 8.2 ms, TE (Echo Time) = 3.2 ms, flip angle = 12°, matrix = 256 × 256, FOV (Field of View) = 256 mm, slices = 160, slice thickness = 1 mm, and inversion time = 450 ms.

2.6. Brain age

The estimates of brain age were computed using the brainageR (https://github.com/james-cole/brainageR) model as described by Cole et al. (2017a, 2017b). In brief, Gaussian processes regression was built using raw T1-weighted MRI (Magnetic Resonance Image) data from a large set of healthy adults (n = 2001, age range 18–90 years) to predict chronological age, which was reported to achieve high prediction accuracy. The software brainageR takes raw MRI data, which is then minimally preprocessed automatically by the software itself. The predicted chronological age based on brain imaging data is the so-called brain-predicted age, or brain age in short. As discussed in previous publications from our group and others, brain age is often overestimated in younger individuals and underestimated in older individuals (Cole et al., 2017a, 2017b; Liang et al., 2019; Niu et al., 2020, 2022; Smith et al., 2019). This brainageR model also corrects for age-related prediction bias by regressing brain age on chronological age. The trained model from Cole et al. (2017a, 2017b) was applied to raw T1-weighted MRI data collected in the Neuroscience Project (n = 138) to obtain their brain age. Moreover, we calculated a difference score between brain age and chronological age, called the brain age gap. A positive brain age gap indicates accelerated brain aging.

2.7. Statistical analysis

To examine whether accelerated biological aging was associated with an increased risk of multimorbidity, we conducted logistic regression with the residualized biological age gap as the independent variable and multimorbidity as the outcome variable. In this analysis, we pooled the test set of healthy subject and the remaining participants with at least 1 chronic condition together (total sample size n = 726). We also controlled for chronological age, sex, race, and education. Race was coded as a binary variable, representing white and non-white. The non-white group included a few race categories including African American, native American, Asian, native Hawaiian or Pacific Islander, and other. These categories were combined due to their relatively small sample sizes. Education was categorized as 3 levels including high school or General Educational Development (GED), some college, and college degree or more. Similarly, logistic regression was used to examine whether advanced brain aging (i.e., a positive residualized brain age gap) was related to an increased risk of multimorbidity. Since the brain age prediction model was trained using a different data set, we included all participants from the MIDUS Refresher Neuroscience Project with structural MRI data available (n = 127) in the logistic regression. The same set of covariates was controlled for in this analysis. In addition, we examined the potential moderating effect of sex on the relationship between multimorbidity and biological age gap. We also conducted separate moderation analyses to test whether the association between multimorbidity and brain age gap depended on sex. Lastly, we conducted exploratory analyses to examine the relationship between mental health multimorbidity and each of the 2 surrogate aging biomarkers (i.e., biological age gap, brain age gap). To account for the potential confounding effect of chronological age, we used residualized biological and brain age gap and included chronological age as a covariate in all analyses examining multimorbidity.

3. Results

3.1. Sample characteristics

Descriptive statistics for the study sample is shown in Table 1. Participants in this study aged between 25 and 76 years with a mean age of 50.84 years (SD = 13.41 years). The sample was primarily non-Hispanic white, and 52.1% were female. Of the study sample, 52.2% had a college degree or more and 42.8% had 2 or more chronic conditions (i.e., multimorbidity). The proportion of mental health multimorbidity was 9.7%. Among the participants in the Neuroscience Project (n = 138, age range: 25–74 years), 40.6% had 2 or more chronic conditions and 10.1% had mental health multimorbidity.

3.2. Biological age

In the training set, biological age estimated by the machine learning model was correlated with chronological age (r = 0.81, MAE = 7.09 years) before bias adjustment. As shown in Fig. 1A, biological age was overestimated for younger participants and underestimated for older participants. Due to this systematic difference between the estimated biological age and chronological age, the biological age gap was negatively associated with chronological age (Fig. 1B). However, we expect

Table 1
Subject characteristics, the MIDUS Refresher Biomarker Project (n = 863)

Variable	Mean (SD)	Range	Percent
Age	50.84 (13.41)	25–76	
Sex (% female)			52.1%
Race (% non-white)			29.4%
Education			
High school or GED			17.3%
Some college			30.5%
College or more			52.2%
Number of chronic conditions	1.58 (1.56)	0–8	
Multimorbidity (% yes)			42.8%
Number of mental health conditions	0.42 (0.74)	0–4	
Mental health multimorbidity (% yes)			9.7%

Key: GED, General Educational Development.

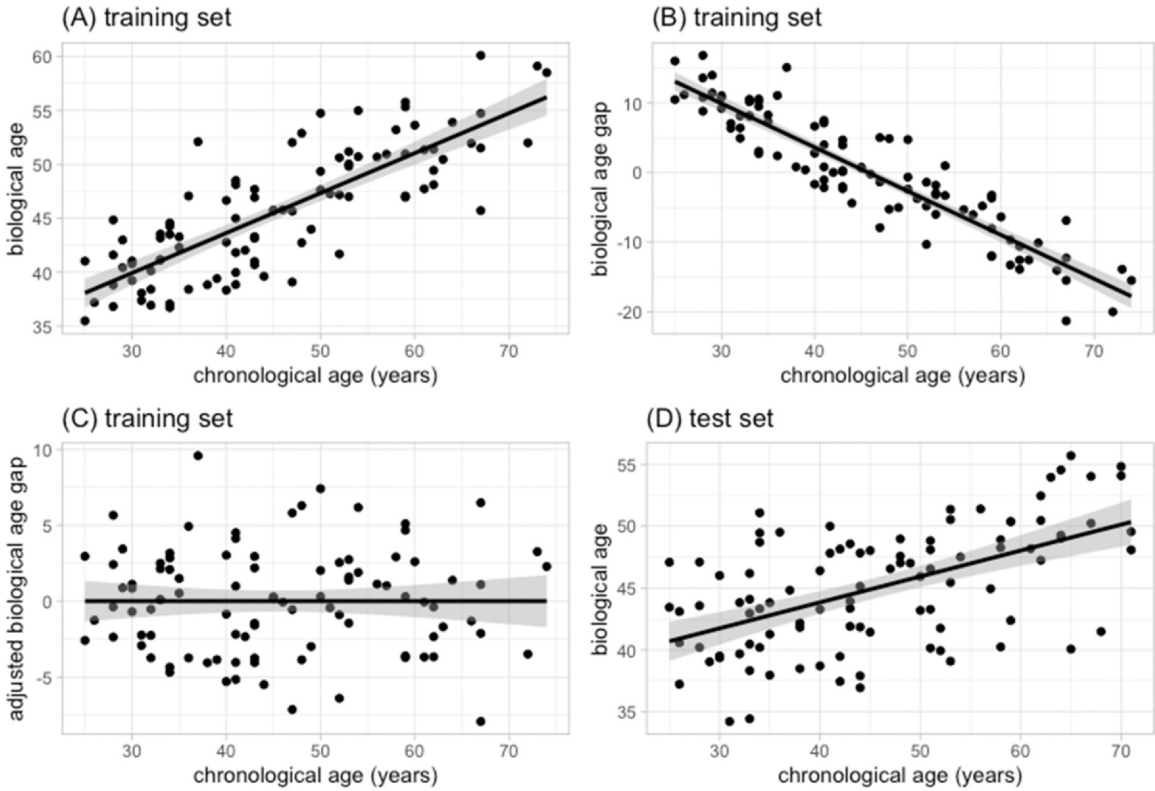

Fig. 1. Scatterplots of biological age/(adjusted) biological age gap (y-axis) and chronological age (x-axis) with fitted regression lines and 95% confidence bands for the training and test sets of healthy adults.

the biological age gap is on average 0 among healthy participants. To account for this systematic negative association between biological age gap and chronological age, we regressed biological age gap on chronological age and computed the residualized/adjusted biological age gap (i.e., bias correction step). As shown in Fig. 1C, the adjusted biological age gap was not associated with chronological age and was on average very close to 0. When investigating biological age or biological age gap as a potential biomarker for aging, it is essential to account for this systematic bias by either computing the residualized biological age (gap) and/or controlling for chronological age. Otherwise, the effect of biological age (gap) can be confounded by chronological age.

The trained machine learning model was then applied to a test set of healthy adults to evaluate model performance. The systematic difference in the estimated biological age and chronological age was also observed in the test set (Fig. 1D). The model prediction performance on the test set ($r = 0.54$, MAE = 9.08 years) was less accurate than what was achieved on the training set, which is to be expected in a train-test split. The difference in prediction performance between the training and test sets is common in machine learning evaluations and can be attributed to the fact that a trained model is evaluated on new, unseen data during testing. This highlights the importance of reporting model performance from both the training and test sets since reporting the training MAE alone would not allow researchers to evaluate the model generalizability to new, unseen data. It is also worth noting that a high correlation coefficient and a small MAE imply better prediction performance only when we examine a cohort of healthy participants.

Feature importance value was computed from the ensemble learning model to rank the biological markers in terms of their contribution for estimating biological age (Fig. 2). Feature importance for each machine learning model was calculated using the root mean squared error. These metrics were then scaled to collectively sum up to 100, enabling standardized comparison. The overall variable importance was computed by a weighted average, where feature importance from each individual model was averaged based on their corresponding weight in the overall ensembled model, as implemented in the function varImp in the R package caretEnsemble version 2.0.1 (Deane-Mayer and Knowles, 2016; Kuhn, 2008). This results in a standardized metric representing variable importance, considering both individual model contributions and their weights in the ensemble. The top predictors were total cholesterol, blood-fasting IGF1, bone-specific alkaline phosphatase, and blood pressure. Creatinine was ranked as the least informative biological marker for estimating biological age.

3.3. Brain age

The brainageR model trained with a large set of healthy adults by Cole and colleagues (2017) was applied to the MIDUS Refresher Study Neuroscience Project. The resulting model performance of the trained brainageR model on our MIDUS test data was $r = 0.79$ and MAE = 6.1 years. Given that participants in the Neuroscience Project included the ones with chronic health conditions, the deviation from chronological age could indicate accelerated aging, which was examined in the following sections. Similar to what we observed for biological age, brain age was overestimated in younger participants and underestimated in older participants (Fig. 3 left panel). This systematic bias has been discussed in our previous publications (Liang et al., 2019; Niu et al., 2020). Though a regression-based bias correction step was conducted automatically by the brainageR model to account for a statistical dependency on chronological age (Cole et al., 2017a, 2017b), we still observed some overestimation and underestimation in the bias-adjusted brain age (Fig. 3 right panel, $r = -0.31$). Therefore, in the subsequent analyses examining multimorbidity, we included chronological age as a covariate to further adjust for age dependency and control for the potential confounding effect of chronological age.

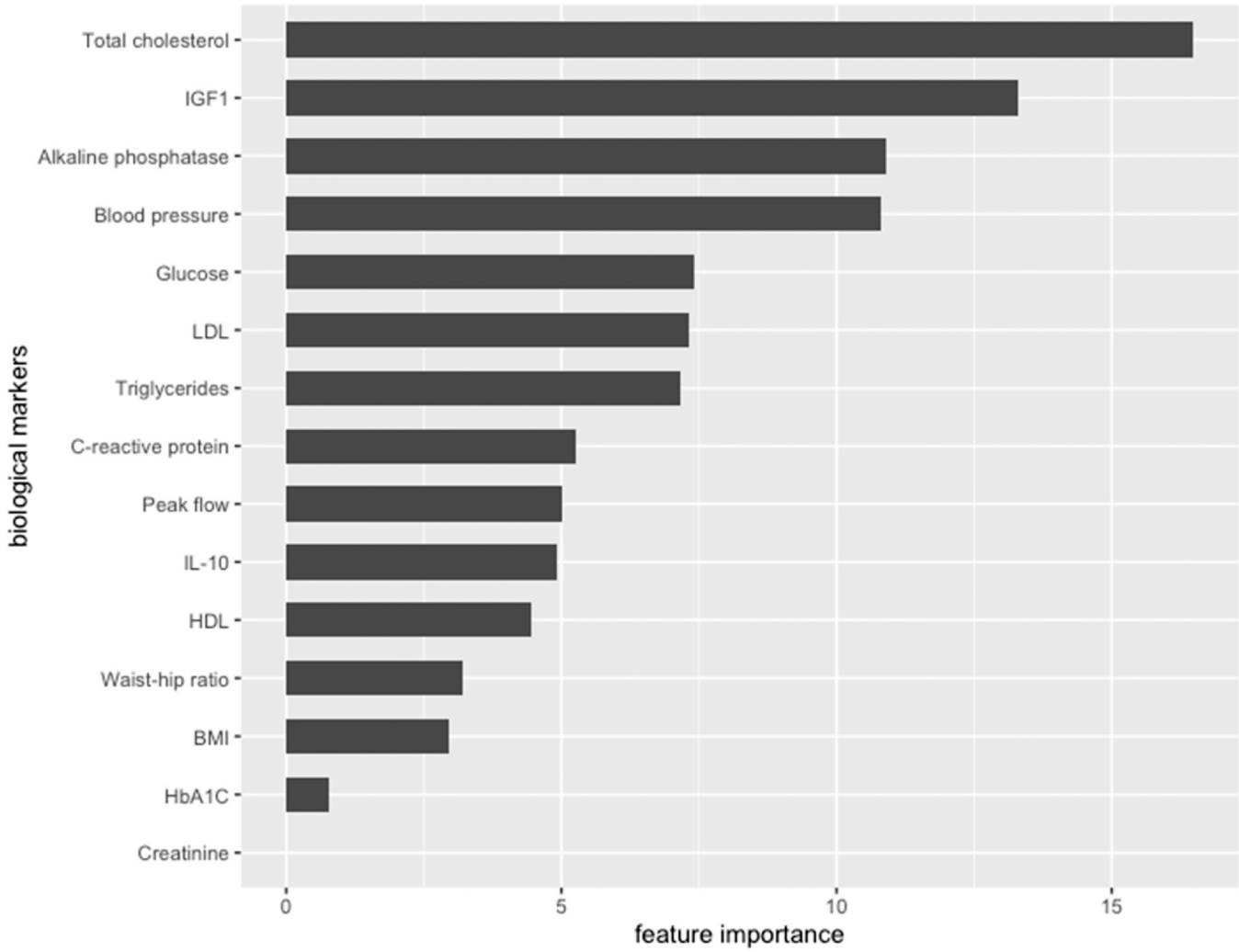

Fig. 2. Feature importance yielded by the machine learning models for the list of biological markers used to estimate biological age. The x-axis is the standardized unit of feature importance out of 100.

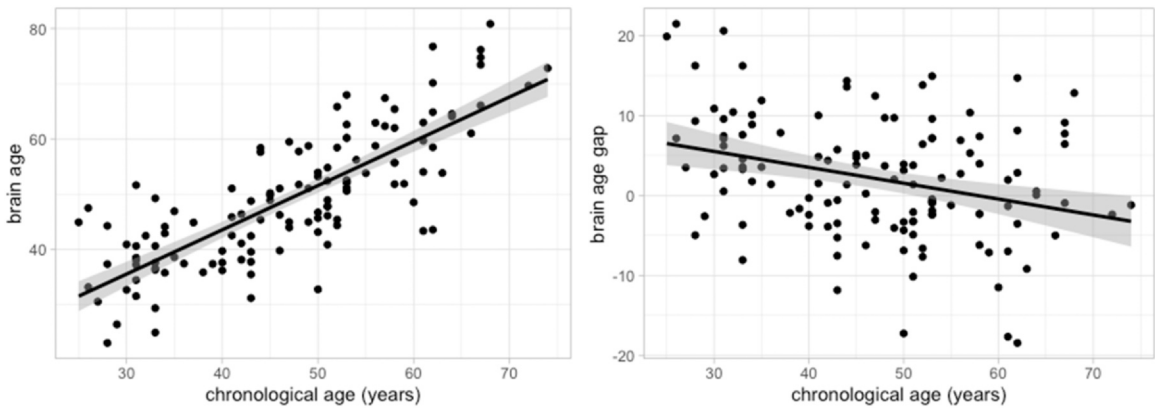

Fig. 3. Scatterplots of brain age/brain age gap (y-axis) and chronological age (x-axis) with fitted regression lines and 95% confidence bands for the test set.

3.4. Association with multimorbidity

As shown in Table 2, results from logistic regression found that a larger biological age gap was significantly associated with an increased risk of multimorbidity after controlling for sex, chronological age, race, and education (b = 0.06, $p = 0.007$, OR (Odds ratio) = 1.07, 95% CI (Confidence interval) [1.02, 1.12]). Among the covariates, higher chronological age (b = 0.13, $p < 0.001$, OR = 1.14, 95% CI [1.09, 1.19]) and being non-white (b = 0.45, $p = 0.022$, OR = 1.57, 95% CI [1.07, 2.30]) were significantly related to an increased risk of multimorbidity. Having a

Table 2
Results from the logistic regression models for examining the association between the residualized biological age gap and multimorbidity and for examining the moderating effect of sex

	Multimorbidity		Multimorbidity (with sex as a moderator)	
	b	*p* value	b	*p* value
Biological age gap	0.06	**0.007**	0.06	**0.011**
Sex (female)	0.03	0.870	0.04	0.822
Age	0.13	**< 0.001**	0.13	**< 0.001**
Race (non-white)	0.45	**0.022**	0.45	**0.022**
Education (some college)	−0.17	0.475	−0.17	0.478
Education (college or more)	−0.76	**< 0.001**	−0.76	**0.001**
Biological age gap * Sex	—	—	0.003	0.850

Specifically, we used bias-adjusted (i.e., residualized) biological age gap and included chronological age as a covariate. Multimorbidity is defined based on 13 different categories of chronic conditions.
Significance values are indicated as p value in the table.

college degree or more was negatively associated with the risk of multimorbidity (b = −0.76, $p < 0.001$, OR = 0.48, 95% CI [0.30, 0.73]).

Findings on the association between brain age gap and multimorbidity were summarized in Table 3. The brain age gap was not significantly associated with the risk of multimorbidity (b = −0.02, $p = 0.551$, OR = 0.98, 95% CI [0.92, 1.04]). Among the covariates, chronological age was positively associated with the risk of multimorbidity (b = 0.11, $p < 0.001$, OR = 1.11, 95% CI [1.07, 1.17]) while having a college degree or more was negatively related to the risk of multimorbidity (b = −1.36, $p = 0.021$, OR = 0.26, 95% CI [0.08, 0.79]).

3.5. Moderating effect of sex

The effect of the biological age gap on the risk of multimorbidity was not significantly moderated by sex (b = 0.003, $p = 0.850$, OR = 1.00, 95% CI [0.97, 1.03]). Including the interaction term between biological age gap and sex in the logistic regression did not change the pattern and significance of other variables (see the last 2 columns of Table 2). In contrast, the brain age gap by sex interaction term was statistically significant and was negatively associated with the risk of multimorbidity (b = −0.19, $p = 0.009$, OR = 0.83, 95% CI [0.71, 0.95]). This suggests that the effect of the brain age gap on the risk of multimorbidity depended on sex and was weaker among females compared to males. The inclusion of the interaction term did not change the pattern and significance of other variables in the logistic regression model (refer to the last 2 columns of Table 3).

3.6. Association with mental health multimorbidity

In the exploratory analyses, we found that the biological age gap was positively associated with the risk of mental health multimorbidity after controlling for sex, chronological age, race, and education (b = 0.07, $p = 0.046$, OR = 1.07, 95% CI [1.00, 1.15]). Among the covariates, having some college education was negatively related to the risk of mental health multimorbidity (b = −0.66, $p = 0.031$, OR = 0.52, 95% CI [0.28, 0.94]). Additionally, having a college degree or more was associated with a decreased risk of mental health multimorbidity (b = −1.17, $p < 0.001$, OR = 0.31, 95% CI [0.17, 0.57]).

Similarly, the brain age gap was found to be positively related to the risk of mental health multimorbidity after controlling for sex, chronological age, race, and education (b = 0.10, $p = 0.038$, OR = 1.10, 95% CI [1.01, 1.22]). All other covariates were not statistically significant. This is likely due to the fact that the sample size of the Neuroscience Project is much smaller compared to the Biomarker Project (see "Methods" section).

Table 3
Results from the logistic regression models for examining the association between the residualized brain age gap and multimorbidity and for examining the moderating effect of sex

	Multimorbidity		Multimorbidity (with sex as a moderator)	
	b	*p* value	b	*p* value
Brain age gap	−0.02	0.551	0.07	0.129
Sex (female)	0.27	0.554	0.59	0.236
Age	0.11	**< 0.001**	0.11	**< 0.001**
Race (non-white)	−0.03	0.951	−0.26	0.608
Education (some college)	−0.72	0.216	−1.04	0.088
Education (college or more)	−1.36	**0.021**	−1.76	**0.006**
Brain age gap * Sex	—	—	−0.19	**0.009**

Multimorbidity is quantified using 13 different categories of chronic conditions.
Significance values are indicated as p value in the table.

4. Discussion

In this study, we estimated biological age using machine learning models and a comprehensive set of biological markers collected from a large number of adults in the MIDUS Refresher sample. We showed that elevated biological age was associated with an increased risk of multimorbidity defined based on 13 different categories of chronic conditions. In addition, it is the first study to examine the relationship between accelerated brain age and multimorbidity. We also investigated whether the relationship between the surrogate aging biomarkers and multimorbidity was moderated by sex. Though the brain age gap was not associated with the risk of multimorbidity, the interaction between brain age gap and sex was significantly negatively related to the risk of multimorbidity, suggesting that the effect of the brain age gap on the risk of multimorbidity was weaker among females compared to males. Lastly, in our exploratory analyses, we found that elevated biological age and accelerated brain age were both associated with an increased risk of mental health multimorbidity. These findings are helpful to improve our understanding of the accumulation of physical and mental health conditions in an individual and sex-related differences in aging.

Though varied in the methods of estimating biological age and quantifying multimorbidity, our study obtained findings consistent with previous studies that elevated biological age was related to an increased risk of multimorbidity (Crimmins et al., 2021; Liu et al., 2018; Sayed et al., 2021). This points to the value of investigating biological aging as an underlying mechanism for multimorbidity and a shared pathway for different chronic conditions. Early detection of accelerated biological aging before the onset of chronic conditions holds the potential for disease prevention. Cole (2020) reported that advanced brain aging was associated with hypertension, diabetes, and stroke when testing with 14,701 individuals from the UK Biobank (Cole, 2020). However, in our study, brain age was not found to be significantly associated with multimorbidity. The relatively small sample size in the Neuroscience Project could limit the statistical power to detect the effect. As our study is the first attempt to explore the association between brain age and multimorbidity, future studies with larger sample sizes are needed to validate the findings.

We identified a set of biological measures, which were found to influence biological age that was positively associated with the risk of multimorbidity. Our top-ranked biological markers (i.e., feature importance ≥5) for estimating biological age, including total cholesterol, IGF1, alkaline phosphatase, glucose, C-reactive protein, and peak flow, have also been reported to be predictive of multimorbidity (Crimmins et al., 2021). Systolic blood pressure has been identified as a risk factor for death and disease (Liu et al., 2018; Port et al., 2000). In addition to total cholesterol, LDL and triglycerides were also found to be important for the quantification of biological aging (Belsky et al., 2015). These identified biological markers are modifiable factors, which may shed the light on new approaches to

improve the treatment of multimorbidity. This also points to the need of collecting these biological measures during routine medical visits, which may assist with the detection of at-risk individuals.

The negative association between brain age gap and chronological age has been thoroughly investigated in prior work published by our group (Liang et al., 2019; Niu et al., 2020, 2022) as well as other studies (Beheshti et al., 2019; Cole et al., 2017a, 2017b; Smith et al., 2019). The reason for this systematic bias can be explained by regression to the mean (Liang et al., 2019). However, this issue has not been formally reported in the literature of biological age estimation. Thus, we presented the pattern between biological age gap and chronological age using the MIDUS refresher sample and highlighted the importance of controlling for the confounding effect of chronological age. It is important to note that the bias adjustment step uses chronological age as an input to the model, making it circular for evaluating brain age or biological age prediction accuracy (Butler et al., 2021). Therefore, the evaluation metrics (e.g., correlation coefficient r, MAE) calculated from bias-adjusted brain or biological age gap can be artificially inflated and are no longer appropriate for evaluating the prediction performance of the brain or biological age estimation model. The purpose of the age-bias adjustment is to control for the confounding effect of chronological age when testing the brain or biological age gap as a predictor of health outcomes. In the case where the age dependency cannot be perfectly eliminated by the bias adjustment, including chronological age as a covariate in subsequent analysis of multimorbidity allows us to further control for the potential confounding effect of age. Additionally, we observe that the MAE obtained from a test set of healthy adults by the biological age prediction model was larger than the MAE calculated from a training set. This demonstrated the need of evaluating model performance using an independent test set as statistical models can easily overfit a training dataset. Because biological age can deviate from chronological age in disease groups, the correlation coefficient and MAE are the only meaningful metrics for evaluating model performance when examining healthy participants. This speaks to the value of training/calibrating biological age estimation model only using health participants. Presenting these methodological issues in our study is helpful to guide the design and analysis of future studies on biological age and brain age.

Furthermore, while brain or biological age-bias adjustment models are designed to mitigate age-related prediction bias, their effectiveness can be influenced by various factors, particularly when the models are trained on existing data and then applied to a different cohort. This generalizability may not always be perfect, as the underlying biological and demographic characteristics of the test data might differ from the training data. Thus, even with robust bias correction, there may be residual variations that are due to differences in the data sets, such as different MRI scanning parameters and biospecimen qualities. Further investigation is warranted to delve into the nuanced mechanisms driving the age-related dependency, enhancing our understanding of the intricate interplay between biological aging and data analysis techniques.

Our study found that the 2-way interaction between brain age and sex was negatively associated with the risk of multimorbidity. This suggests that the potential sex difference in how brain aging is related to the accumulation of multiple chronic conditions. Previous study on brain aging reported on average younger brain in women throughout adulthood compared to men of the same age (Goyal et al., 2019). Sex differences in MRI-based volume loss were also reported across the brain, with females having less volume loss over time than males (Armstrong et al., 2019). Studies have suggested that sex hormones (e.g., estrogen) can play important roles in brain aging and may have a neuroprotective effect in women (Green and Simpkins, 2000; Zarate et al., 2017). Predictors of brain age were also found to be sex-specific, highlighting the value of sex-specific analyses (Sanford et al., 2022). Further research is needed on examining sex-specific risk and protective factors that influence brain aging and disease accumulation.

Multimorbidity in mental health has been relatively under-investigated, despite its strength in capturing complex clinical representations of psychiatric disorders (Bhalla et al., 2018; Langan et al., 2013). Our study presents the first attempt to investigate how biological age and brain age are related to mental health multimorbidity. As hypothesized, we found that elevated biological age and accelerated brain age were associated with an increased risk of mental health multimorbidity. Our findings are consistent with previous studies that found accelerated brain aging in alcohol use (Amen et al., 2018; Cole, 2020), cannabis use (Amen et al., 2018), anxiety (Amen et al., 2018), and depression (Niu et al., 2022). In addition, accelerated biological aging has been reported in substance use (Bachi et al., 2017) and alcohol abuse (Piniewska-Róg et al., 2021). Some biological age indicators (e.g., lung function, telomere length) have also been found to be altered in depression and anxiety disorder (Han et al., 2019). It will be interesting to examine the relationship between these surrogate aging biomarkers and mental health multimorbidity using data with larger samples and more categories of mental health conditions.

Because of our interest in examining brain age estimated by neuroimaging data, we chose the MIDUS refresher sample in this study, and thus our findings are limited to cross-sectional association. Future research can benefit from longitudinal data to investigate whether biological age and brain age predict multimorbidity and mental health multimorbidity prospectively. The longitudinal nature of the MIDUS study will allow such future investigations. Furthermore, the estimated brain age was obtained based on structural MRI data. It will be interesting to test whether brain age estimated by multimodal neuroimaging data shows a stronger association with multimorbidity and mental health multimorbidity. In addition, our study defined the primary outcome as multimorbidity based on 13 different categories of chronic conditions and explored mental health multimorbidity based on measures available in the MIDUS refresher sample. Alternative ways of defining multimorbidity (e.g., whether the duration and severity of chronic conditions are considered) and physical-mental multimorbidity patterns need to be examined in future research. Integrating other lifestyle factors such as diet and physical activity in the relationship between aging and multimorbidity may merit future research.

Verification

Authors declare that data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging. All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

CRediT authorship contribution statement

Fengqing Zhang: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft preparation. **Hansoo Chang**: Conceptualization, Methodology, Writing – review & editing. **Stacey M. Schaefer**: Conceptualization, Methodology, Writing – review & editing. **Jiangtao Gou**: Conceptualization, Methodology, Writing – review & editing.

Disclosure statement

The authors declare no competing interests.

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